MOLECULAR
METABOLISM

Action and therapeutic potential of oxyntomodulin[*]

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ABSTRACT

Oxyntomodulin (OXM) is a peptide hormone released from the gut in post-prandial state that activates both the glucagon-like peptide-1 receptor (GLP1R) and the glucagon receptor (GCGR) resulting in superior body weight lowering to selective GLP1R agonists. OXM reduces food intake and increases energy expenditure in humans. While activation of the GCGR increases glucose production posing a hyperglycemic risk, the simultaneous activation of the GLP1R counteracts this effect. Acute OXM infusion improves glucose tolerance in T2DM patients making dual agonists of the GCGR and GLP1R new promising treatments for diabetes and obesity with the potential for weight loss and glucose lowering superior to that of GLP1R agonists.

1. INTRODUCTION

T2DM therapies such as metformin, sulfonylureas (SU), glinides and insulin result in progressive deterioration of glycemic control associated with β-cell decline [1] and weight gain [2]. Incretin-based therapies such as glucagon-like peptide-1 receptor (GLP1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors lower blood glucose primarily by promoting glucose dependent insulin secretion and by inhibiting glucagon secretion [3]. The glucagonostatic effect of GLP1R activation is likely mediated by a local action of somatostatin released by δ cells that inhibits glucagon secretion by pancreatic α cells [4,5]. The delay of gastric emptying by GLP-1 has been involved in the postprandial regulation of glucose absorption [6,7]. However, Nauck et al. [8] demonstrated in healthy volunteers that GLP-1-induced delay in gastric emptying is subject to rapid tachyphylaxis. As a consequence, postprandial glucose control by GLP-1 is attenuated during its chronic administration [8]. Thus far, these therapies are providing durable glycemic control with low risk of hypogly-cemia, improved insulin resistance, pancreatic function and modest body weight-loss at tolerated doses [9]. GLP1R agonism has been shown to lower plasma glucose in advanced type 2 diabetes long after sulfonylurea secondary failure [10]. GLP1R activation may delay or prevent secondary SU failure [11]. With such promising results, the next generation of diabetes drugs will likely focus on combined activation of more than one receptor ideally resulting in beneficial cardio-renal effects.

2. OXYNTOMODULIN

Oxyntomodulin (OXM) is a 37-amino acid peptide hormone secreted from the gut together with GLP-1 following nutrient ingestion [12–18].

OXM is mainly produced in gut endocrine L-cells by processing of the preproglucagon precursor by prohormone convertase 1/3 [12–15,19,20] (Figure 1). While the existence of gut 'glucagon-like substances' was reported in gut extracts since 1948 [21], it was discovered in 1981 that one moiety with 'glucagon-like immunoreactivity' [22] was formed by the sequence of glucagon and a C-terminal octapeptide extension (IP-1, intervening peptide, Figure 1). This moiety was named oxyntomodulin for its ability to modulate gastric acid secretion in gastric oxyntic glands [23–26].

3. RECEPTORS AND SIGNALING IN VITRO

OXM is reported to be a full agonist in cell lines over expressing the human GLP1R and GCGR-mediated cAMP accumulation although with reduced affinity compared to GLP-1 and glucagon [27–32]. It was found to be a partial agonist in recruiting β-arrestin and G-protein-coupled receptor kinase 2 to the GLP1R. OXM has been proposed as a GLP1R-biased agonist relative to GLP-1 as it has less preference towards cAMP signaling relative to phosphorylation of ERK1/2, but similar preference for cAMP relative to Ca^{2+} [31]. If these findings translate *in vivo*, the GLP1R-mediated effects of OXM could differ from that of GLP-1 [32]. Administration of OXM increased c-fos in the hypothalamus, but not in the brainstem [33], and OXM and GLP-1 result in differential neuronal activation in the hypothalamus [34,35]. Consistent with these data, peripheral administration of glucagon or GLP-1 or co-administration of glucagon and GLP-1 activates similar appetite regulating centers in the brainstem and amygdala [36]. It is unclear if these differences are due to engagement of additional receptors and/or the reported difference on

GLP1R signaling by OXM or simply a different brain penetration and affinity at the receptors.

4. EFFECTS OF OXYNTOMODULIN ON BODY WEIGHT AND GLUCOSE METABOLISM

OXM causes weight loss in humans [37,38] and rodents [30,39,40]. Overweight and obese subjects receiving subcutaneous administration three times daily of OXM (400 nmol pre-prandially) over a 4-week period resulted in an average weight loss of 2.3 kg [38]. The endogenous levels of 'OXM-like immunoreactivity' (OLI) (see review in [41]) increased ∼10-fold (972 ± 165 pmol/l) 30 min after the self-injection. These levels are comparable to those detected in patients following gastric bypass surgery [42] and in several conditions and procedures associated with anorexia such as tropical malabsorption [43] and small intestinal resection [44]. Moreover, in preclinical species, chronic treatment with OXM results in superior weight loss and comparable glucose lowering to a GLP1R-selective peptide [45–47]. OXM improves glucose metabolism during a glucose tolerance test in mice [48,49]. The acute improvement of glucose metabolism by OXM was recently confirmed in humans in a randomized, double-blind, placebo-controlled, crossover trial, evaluating the effect of OXM in a graded glucose infusion (GGI) performed in T2DM volunteers [50]. OXM was infused (3 pmol/kg/min) to match the plasma concentrations achieved in post-prandial state in the chronic weight loss
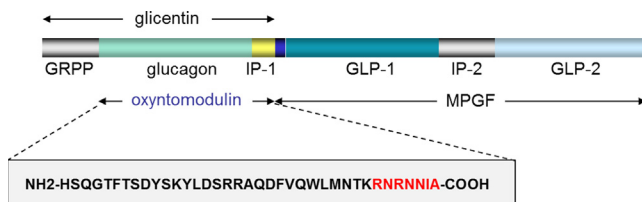

Figure 1: Preproglucagon. Preproglucagon is proteolytically cleaved in a tissue-specific manner. Post-translational processing in the gut and brain by prohormone convertases results in the secretion of GLP-1 and GLP-2, while the glucagon sequence remains in a larger peptide, glicentin or glicentin-related pancreatic peptide (GRPP) and oxyntomodulin. MPGF, major proglucagon fragment.

study performed on overweight and obese volunteers [38]. At matched glucose infusion rates, OXM significantly increased insulin secretion and glucose lowering versus placebo [50].

5. MECHANISMS INVOLVED IN THE EFFECTS OF OXM ON ENERGY AND GLUCOSE METABOLISM

OXM was shown to lower food intake and reduce body weight as well as increase core temperature compared to a pair-fed group suggesting that OXM increases energy expenditure [39,40]. This effect was later confirmed by indirect calorimetry [30]. The acute anorectic effect of OXM was then demonstrated in humans where continuous infusion reduced ad libitum energy intake in the absence of nausea or effects on food palatability [37]. Later Wynne et al. [38] showed that the reduction in food intake was seen and retained during chronic administration in overweight and obese healthy volunteers (Figure 2). In a similar population, four-day subcutaneous self-administration of pre-prandial oxyntomodulin increased energy expenditure while reducing energy intake [51]. Therefore the body weight-lowering effects of OXM likely involve suppression of food intake and an increase in energy expenditure (Figure 2) [30,38,51].

In addition to the established glucose-dependent insulin secretion action of OXM on the pancreas (Figure 2) [52], another mechanism that potentially plays a role on energy intake and glucose metabolism is gastric emptying. Intravenous infusion of OXM reduced gastric emptying in humans (Figure 2) [53]. Further studies are required to confirm these findings and to understand if reduction in gastric emptying is involved in the acute and long-term metabolic effects of OXM.

6. RECEPTORS INVOLVED IN THE EFFECT OF OXYNTOMODULIN

6.1. Body weight
OXM inhibits food intake and stimulates energy expenditure in rodents and in human subjects [37–40]. However, the receptors involved in the
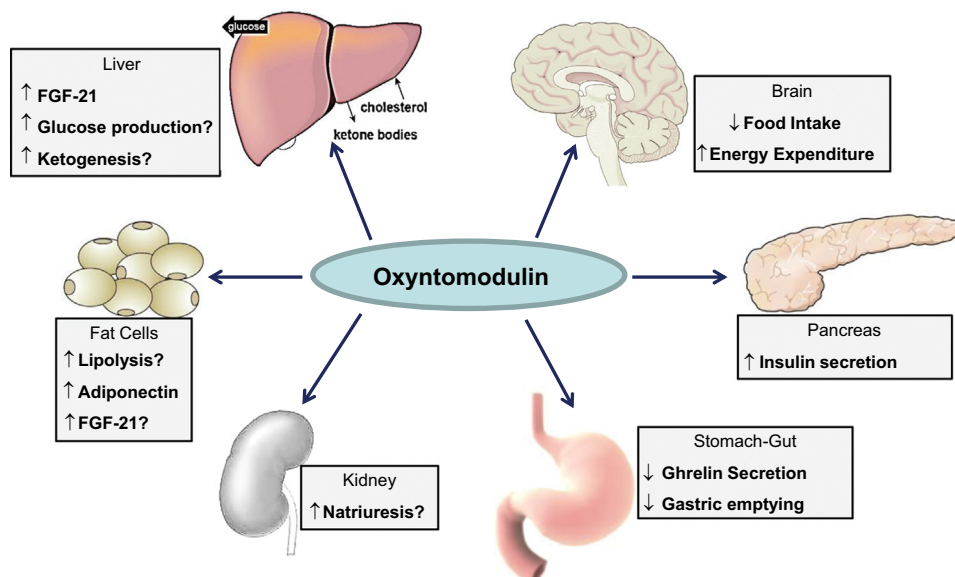

Figure 2: Effects of oxyntomodulin in humans.

body-lowering action of OXM are not fully delineated. The anorectic effects of central administrations of OXM are abolished by co-administration of the GLP1R antagonist, exendin(9–39), and are not observed in $Glp1r^{-/-}$ mice, suggesting that the acute central effect of OXM on food intake is mediated by the GLP1R [30,54,55]. Other effects of OXM, including stimulation of heart rate and energy expenditure, appear to be independent of GLP1R in vivo [30,54]. As OXM agonizes GLP1R and GCGR in vitro [27–32] and because increases in heart rate and energy expenditure were reported following GCGR activation [56], the differential effect of OXM vs. GLP1 could be mediated by activation of the GCGR.

Recently, two publications [45,46] expanded the initial findings on the mechanism of action of OXM and demonstrated that OXM has glycogenolytic properties in perfused mice liver [46]. Using an equipotent GLP1R agonist peptide obtained with a mutation of glutamine to glutamate (OXMQ3E) in position 3 that dials out activity on GCGR, researchers were able to demonstrate that OXM, not OXMQ3E, stimulated ketogenesis in wild-type mice and in $Glp1r^{-/-}$ mice, but not in $Gcgr^{-/-}$ mice [45]. These data show that OXM functionally activates the GCGR ex-vivo and in vivo [45,46]. Chronic treatment with OXM resulted in superior body weight lowering and comparable glucose-lowering to equimolar amounts of OXMQ3E [46]. Moreover, OXM significantly decreased body weight gain in lean $Glp1r^{-/-}$ mice and chronic blockade of the GCGR with a small molecule GCGR antagonist (Cpd A) during OXM infusion reduced the ability of OXM to decrease body weight [46]. These data, using genetic and pharmacological approaches, suggest that the body-weight lowering action of OXM involves GLP1R and GCGR activation [45,46]. Nevertheless these data are not conclusive as in the same study cpd A alone in feed resulted in significant body-weight reduction thereby limiting the interpretation of each receptor's contribution to the body weight-lowering effect of OXM [46].

6.2. Glucose metabolism

OXM acutely improves glucose metabolism in mice [48,49]. However, consistent with the known stimulatory effect of glucagon on hepatic glucose production, it was demonstrated that GLP1R-agonism exerts better glucose lowering than OXM at equimolar doses in a glucose challenge [45]. During a mouse hyperglycemic clamp, OXM infusion reduced the exogenous glucose required to maintain the hyperglycemic levels in $Glp1r^{-/-}$ vs. $Gcgr^{-/-}$ and wild-type mice despite increased plasma insulin levels. These data demonstrated that activation of GCGR contributes to the insulinotropic effect of OXM and partially attenuates its beneficial effects on glucose metabolism [45]. These findings are consistent with increased glucose production observed in mice treated with OXM during a hyperinsulinemic-euglycemic clamp [49]. A recent report [57] demonstrated in rats that intracerebroventricular glucagon administration improves whole-body glucose metabolism. If these data translate to higher species, it is possible that central GCGR signaling may contribute to the improvement of glucose metabolism in animals treated with OXM.

Although previous data have shown that the glucagon receptor is involved in the body weight and glucose lowering action of OXM in addition to GLP1R, OXM may activate additional receptors. Oxyntomodulin was shown to inhibit pancreatic secretion through the nervous system in rats [58]. While glucagon can depress pancreatic exocrine secretions in normal animals and humans, it is still unclear how glucagon exerts this effect [59]. As previously mentioned, the differential neuronal activation observed following OXM, GLP-1 and glucagon

administration seems to support a dissociation between the effects of oxyntomodulin and those of GLP-1 and glucagon [33–36]. Intravenous administrations of OXM and glucagon have also been reported to increase intestinal glucose uptake in rats [60]. While in vivo data may be confounded by differential levels of circulating insulin, OXM was described as a more potent regulator than glucagon in stimulating intestinal glucose absorption in the isolated small intestine despite being 1–2 orders of magnitude less potent at the GCGR than glucagon [61]. Because GLP-1 does not stimulate glucose absorption and an increase in hexose transport has been previously described for glucagon-like peptide-2 (GLP-2) and glucose-dependent insulinotropic peptide (GIP) [62,63], OXM could engage additional G-protein-coupled receptors (GPCR) of the secretin like (class B) family such as GLP-2 and GIP receptors [64,65]. Treatment of BHK-GIPR or BHK-GLP2R rat cells with OXM had no effect on cAMP production, whereas BHK cells that express the rat GLP-1 or glucagon receptors exhibited significant increases in cAMP accumulation in response to treatment with OXM [30]. Taken together, these data suggest that OXM may engage additional receptors but that additional downstream pathways aside from cAMP may be involved [31]. OXM also delayed gastric emptying in humans [53] but not in mice [48] adding a layer of complexity in interpreting receptors activated by OXM. Further investigation is required on these data. It is possible that differences interspecies may further complicate the interpretation of the mechanisms involved in the effects of OXM.

7. POTENTIAL MEDIATORS OF THE METABOLIC EFFECTS OF OXM

While the anorectic effect of GLP-1 in humans seems to involve an intact vagus nerve [66], it is unclear how glucagon receptor activation suppresses food intake. The anorectic effect of glucagon is abolished following selective hepatic vagotomy in rats suggesting that glucagon acts in the liver to produce a satiety signal that is transmitted to the brain by the vagus nerve [67]. Recently, Tan and colleagues reported an inhibitory effect on plasma levels of the anorexigenic hormone ghrelin in humans during short-term infusion of GLP-1 and glucagon [68]. While part of this effect could be mediated by insulin stimulation, central administration of OXM suppresses circulating ghrelin-like immunoreactivity in rodents [69] and intravenous infusion of OXM at a dose that did not stimulate insulin secretion lowered ghrelin in humans [37]. Thus, it appears that while GLP-1 suppresses plasma ghrelin in humans via insulin secretion in the late postprandial period [70], OXM and GLP-1/glucagon co-administration exert a ghrelin-lowering effect independent of insulin (Figure 2). Ghrelin stimulates appetite in humans [71,72], but it is unclear whether ghrelin suppression contributes to the anorectic effect of OXM in humans as no changes in plasma ghrelin were detected during a 28-day weight loss study performed in overweight and obese volunteers [38].

In the aforementioned study, during infusion of GLP-1 and glucagon in healthy overweight and obese volunteers [68] GLP-1 blunted the hyperglycemic effect of glucagon compared to the individuals receiving glucagon alone. An initial rise in glucose levels diminished over time [68]. The authors suggested that the hyperglycemic effect of glucagon may be completely counteracted by the activation of GLP1R in a longer-term study because of the synergistic insulinotropic action combined with a potential exhaustion of the liver glycogen stores [68]. In this study GLP-1 was infused at 8 pmol/Kg/min resulting in ∼4-fold increase in plasma levels of total GLP-1 over baseline (15–23 pmol/l vs. 90–103 pmol/l) while glucagon was infused at 50 mg/kg/min resulting in

∼25-fold increase in levels vs. baseline (8–11 pmol/l vs. 239–260 pmol/l). It is possible that the infusion of GLP-1 and glucagon does not reflect the relative percent of activation of GLP1R and GCGR *in vivo* achieved by OXM but rather a relative higher percent activation of GCGR vs. GLP1R. The reduced ability of GLP1R activation to counteract the hyperglycemic effect of glucagon was demonstrated in mice with a set of peptides with increased GCGR affinity relative to GLP1R activation [73].

Another study that potentially shed light on the mechanism of action of OXM demonstrated that selective activation of GCGR increased liver FGF-21 mRNA and FGF-21 plasma concentrations [74,75]. This observation is consistent with increased FGF-21 liver expression observed with an oxyntomodulin analog peptide (GLP1R/GCGR dual agonist), but not with a GLP1R-selective peptide [29]. The authors subsequently found in human obese healthy volunteers that a significant elevation of plasma FGF-21 (Figure 2) was observed after a glucagon challenge (1 mg intramuscularly). Because glucagon receptor activation failed to induce weight loss in mice with genetic deletion of FGF-21, it was suggested that FGF-21 mediates at least part of the effects of glucagon on body weight [74]. Stimulation of plasma FGF-21 by glucagon was also reported in rodents and humans with Type 1 diabetes [76]. Chronic administration of the FGF-21 analog LY2405319 reduced body weight, triglycerides and improved glucose metabolism in diabetic non-human primates and humans [77,78] in association with dose-dependent elevation of plasma adiponectin [78]. Recently adiponectin was suggested as a mediator of the glucose lowering and insulin sensitizing effects of FGF-21 in mice possibly via regulation of PPARγ [79–81]. Therefore, it is possible that FGF-21 and adiponectin are involved in the metabolic effects of oxyntomodulin. While the studies were performed at pharmacological doses of glucagon, a critical physiological relevance of glucagon in the regulation of body weight and lipid metabolism was established by Engel and colleagues [82]. They found after a 12-week treatment of T2DM patients with GCGR small molecule antagonist dose-dependent elevations in body weight, cholesterol and triglycerides [82]. Thus, oxyntomodulin or simultaneous activation of GLP1R and GCGR should increase plasma concentrations of FGF-21 and adiponectin in humans (Figure 2). In addition, GLP1R/GCGR dual agonism may be involved in the control of expression and release of many protein factors from adipose tissue through FGF-21 control of PPARγ, not just adiponectin. In support of these data, during a 4-week weight loss study performed with OXM [38] adiponectin was significantly increased in the treatment group. Because adiponectin is reduced in obesity and T2DM, GLP1R/GCGR dual agonism may increase or restore the adiponectin deficiency observed in mice and humans as seen following administration of the FGF-21 analog, LY2405319 [66]. Despite the anorectic effects during chronic dosing of OXM in humans [38], there is insufficient evidence to establish whether the chronic body-lowering effect of OXM involves increase in energy expenditure and if FGF-21, adiponectin or other downstream mediators contribute to these effects in humans.

Ghrelin has been shown to inhibit insulin secretion in rodents and humans [83], so ghrelin suppression observed during simultaneous infusion of GLP-1 and glucagon [56], and acute infusion of OXM [37] may be jointly involved with FGF-21 [74] and adiponectin [77–79] in the acute beneficial effects on glucose metabolism [45,48–50].

A potential mechanism that could also be involved in the anti-obesity effects of oxyntomodulin is the direct effect of glucagon on brown adipose tissue associated with the adrenergic GLP1R-dependent stimulation of adipose tissue thermogenesis [84].

In conclusion, OXM acutely improves energy and glucose metabolism in rodents and humans. Despite different affinity for the respective receptors, different pharmacokinetics and tissue distribution, the acute glucoregulatory actions of OXM in mice [45] and human T2DM [50] appear to resemble the results of the simultaneous infusion of glucagon and GLP-1 [68]. A potential limitation that affects the interpretation of the human results is that no data were disclosed supporting engagement of the glucagon receptor *in vivo* during the weight loss study [38] and the GGI study [50]. Further studies in humans are required to confirm these findings.

8. POTENTIAL ADVERSE EVENTS AND SAFETY CONCERNS

8.1. Gastrointestinal (GI) adverse effects

Nausea, vomiting and diarrhea are commonly reported for GLP1R agonists. They are dose-limiting and in some patients responsible for treatment discontinuation [85]. Delay in gastric emptying may contribute to the GI events reported but the mechanisms underlying these adverse effects are still unclear. The GI tolerability with GLP1R agonists diminish over a period of 4–6 weeks [86]. The nausea associated with pharmacological doses of glucagon is well recognized [87]. Transient mild nausea was reported in 3% of oxyntomodulin self-administered subcutaneous injections for 4 weeks during the body weight loss study conducted in healthy overweight and obese volunteers [38].

8.2. Pancreatitis and pancreatic cancer with incretin-based therapies

A few cases of acute pancreatitis have been reported during treatment with exenatide and other GLP-1 receptor agonists [88]. Acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis has been reported in patients prescribed incretin-based agents. The majority of reports are from postmarketing surveillance and it is therefore difficult to determine a true incidence of pancreatitis associated with incretin therapy [88–90]. Of note, glucagon can depress human pancreatic exocrine secretion [91,92]. There are no biomarkers that can presage pancreatitis in the context of routine clinical surveillance. At the present time, it is unknown whether incretin therapies play a causative role in pancreatitis or whether reported cases reflect the ∼2–3 fold increased risk of acute pancreatitis in T2DM [93]. Recently a report showed in pancreases of patients treated with incretin therapies, increased pancreatic mass as well as a proliferation of exocrine cells [94]. This paper triggered several commentaries [95–97] highlighting several issues with the study. Despite the issues raised by investigators, the report will likely prompt reanalysis of clinical data available with these agents and additional post-mortem studies with well-matched cohorts. These results will also likely impact any potential new therapies involving GLP1R activation.

8.3. C-cell hyperplasia and medullary carcinoma of the thyroid

An increased incidence of C-cell hyperplasia and medullary thyroid cancer was reported in rats and mice following continuous GLP1R activation [98]. This effect was demonstrated to be rodent-specific because GLP1R was not detected in monkey and human thyroid and no C-cell hyperplasia was observed in monkeys treated for over 20 months with GLP1R agonists [98]. A meta-analysis of sequential calcitonin measurements in over 5000 patients treated with liraglutide indicated that calcitonin levels remained low throughout the trials, which were from 26 weeks to 2 years duration [99]. Recently GLP-1 receptor immunoreactivity has been reported in five of 15 of the examined cases in about 35% of the total C cells assessed in normal human thyroid tissue [100]. Because glucagon stimulates calcitonin secretion by thyroid C cells [101] long-term consequences of sustained GLP-1 receptor and

by extension GLP1R/GCGR dual activation remain unknown and require further investigation.

8.4. Fat free mass

Chronic glucagon receptor activation can theoretically contribute to whole-body protein catabolism. Glucagon increases liver gluconeogenesis which utilizes amino acids as a substrate and participates in the control of blood amino acid levels [102]. It has not been determined whether glucagon receptor activation plays a role in muscle protein catabolism. Excessive loss of skeletal muscle mass was not observed in rodent studies with a GLP1R/GCGR dual agonist peptide equipotent at the GLP1R and GCGR [29,103]. However we cannot exclude that long-term GCGR agonism does not cause catabolism of muscle protein. Glucagonomas are associated with muscle wasting and hypoaminoacidemia and whole-body protein breakdown was demonstrated to be enhanced in patients with glucagonoma compared with healthy control subjects [104]. Glucagon has also been implicated in negative nitrogen balance associated with stress, e.g. post-surgery, trauma, burns, and acute infections. All of the aforementioned are complex factors other than glucagon (e.g. other hormones and cytokines) that could be contributing to negative nitrogen balance [105]. Glucagon administration has also been demonstrated to produce protein catabolism in human subjects in the setting of experimentally-induced insulin deficiency [106]. However insulin is a major anabolic hormone and insulin action is not expected to be absent in patients treated with GLP1R/GCGR dual agonism. Nevertheless, the potential for GLP1R/GCGR treatment to cause skeletal muscle loss needs to be appropriately assessed in long term studies.

8.5. Cardiovascular function

Glucagon and GLP-1 have positive inotropic and chronotropic action on the heart [56,107–110]. GLP1R agonists cause a 1–3 mmHg decrease in systolic and diastolic blood pressure, and a 2–4 bpm increase in heart rate [111–120]. Acute administration of 1 mg of glucagon to normal volunteers resulted in increases in heart rate and mean arterial pressure and decreases in systemic vascular resistance [121]. Glucagon causes natriuresis with changes in kidney sodium handling. Enhanced proximal tubular sodium reabsorption and a higher prevalence of hypertension have been associated with the Gly40Ser polymorphism of the glucagon receptor gene resulting in a mutated receptor less responsive to glucagon [122,123]. Consistently, a trend towards blood pressure increase was observed during pharmacological GCGR antagonism in T2DM patients in a 4-week Phase IIa proof-of-concept study [124]. No effects of oxyntomodulin on pulse rate were observed in human studies [37,38,51].

8.5.1. Serum lipids

Serum lipids are another important cardiovascular consideration. Liraglutide is associated with a small increase in HDL-C and modest decreases in LDL-C and triglycerides [111–117]. There is preclinical and clinical evidence that glucagon decreases serum triglyceride, choles-

terol, and VLDL cholesterol levels [121]. While initial results suggest a potential cardioprotective effect of GLP-1 based therapies [125] prospective outcome trials are required to confirm these positive findings together with potential risks associated with the increased heart rate in diabetic patients.

A body of evidence is beginning to emerge on the long-term CV safety profile of incretin-based therapies. The results of the first trial with saxagliptin met the FDA criteria for non-inferiority over placebo, but did not provide evidence of cardiovascular risk reduction [126].

In summary, these observations suggest the need to carefully assess the impact of GLP1R/GCGR dual agonist treatments on heart rate, systolic and diastolic blood pressure and lipids. Subtle changes in those parameters could alter what is generally viewed as a positive overall cardiac profile of GLP1R-selective agonists.

8.6. Considerations

While there is a long list of potential safety concerns associated with pharmacological activation of GLP1R or GCGR, it is important to consider that because of the synergistic metabolic effects of GLP1R and GCGR activation, GLP1R/GCGR dual agonists are expected to require lower plasma concentrations and, therefore, lower receptor activation to elicit its effect on body weight and glucose lowering as demonstrated in rodents and monkeys [29,103,127]. It is difficult to predict whether the documented tolerability, GI issues, the increase in heart rate seen at pharmacological doses of GLP-1 and glucagon and other effects seen with GLP1R agonists or glucagon administration will be observed to the same extent and degree in GLP1R/GCGR dual agonism.

9. THERAPEUTIC POTENTIAL

Gastric bypass is the only approach that produces lasting improvement in glucose metabolism and weight reduction [128]. Among the changes consistently observed following roux-en-Y gastric bypass are exaggerated postprandial increase in OXM, glucagon, PYY and GLP-1 [128,129]. Hence, administration of endogenous gut peptides or more metabolically-stable analog represents a potential long-term therapeutic approach to obesity and diabetes. With the promising results seen with GLP1R agonists [130], the next generations of diabetes drugs will likely focus on the alternate delivery for injectables as illustrated by recent experience with GLP-1 analogs and insulin [131–134] and the combined activation of more than one receptor [135]. Among these, oxyntomodulin is a promising weight-loss and glucose-lowering therapy and appears well-tolerated in human studies reported to date [37,38,50,51]. The clinical utility of OXM is limited, mainly because of its short circulating half-life [52]. Because glucagon and GLP-1 share ~50% amino acid sequence identity (Figure 3), several groups have recently developed GLP1R/GCGR dual agonist peptides that are resistant to peptidase degradation [29,103,127,136,137]. Two independent papers reported for the first time the use of GLP1R/GCGR dual agonists as being of enhanced efficacy relative to pure GLP1R agonists in the treatment of rodent obesity, with simultaneous improvement in glycemic control [29,103].

Peptides	1	10	20	30	40	
GLP-1	HAE GTFT SDV SSYL EG QAAK EF I AWL V K GR					
Glucagon	HSQ GTFT SDY SKYL DS RR AQDFVQWLMNT					
GIP	Y AE GTFI SDY SIAM DK IH QQDFVNWL L AQKGKKNDWKHNITQ					

Figure 3: Amino acids sequence alignment of GLP-1, glucagon and glucose-dependent insulinotropic peptide (GIP).

The hyperglycemic risk posed by GCGR has triggered questions about the appropriate ideal ratio of receptor activation. When a PEGylated GLP1R/GCGR dual agonist was given to $Glp1r^{-/-}$ mice, the decrease in body weight was no longer associated with improvement in glucose metabolism [103]. Using a spectrum of peptides with different relative receptor selectivity, it was demonstrated that a dual agonist peptide with comparable functional potencies at the GLP1R and GCGR (similar ratio to native OXM) maximizes the weight loss and mitigated the hyperglycemic risk associated with GCGR activation [73]. Recently the translation of these observations was reported to obese, non-diabetic rhesus monkeys, which were treated with a protease-resistant GLP1R/GCGR dual agonist [127]. Daily administration of a lipidated dual agonist at a nearly seven-fold lower dose (3 μg/kg s.c.) to liraglutide (GLP1R-selective agonist, 20 μg/kg s.c.) caused superior weight loss. The same group evaluated the impact on glycemic control and the potential diabetogenic risk of GCGR activation in diabetic rhesus monkeys demonstrating reductions in fasting glucose and improved glucose tolerance in the absence of changes in body weight [127]. These results demonstrate successful translation of the superior pharmacology of GLP1R/GCGR dual agonists from rodents to non-human primates deepening our belief that this approach constitutes a promising new mechanism in the treatment of T2DM.

10. CONCLUSION

Activation of GLP1R and GCGR with chimeric peptides and in the future orally available GLP1R/GCGR dual agonists is a conceivable option to achieve improved therapeutic goals. It is also conceivable to stimulate endogenous secretion of oxyntomodulin from intestinal L-cells and leveraging the similarity of glucagon-based peptides to activate GLP1R, GCGR and GIP receptors (Figure 3) [138,139] and other family B GPCRs. Nevertheless, it will be critical to deepen our understanding of the mechanisms of action and how structurally related peptides like GLP-1 and glucagon interact with their respective receptors [140,141]. Important considerations for the success of future GLP-1 based therapies will not only be efficacy but also a good safety profile and compliance. Future injectable therapies will need to significantly lower glucose and exceed the benefits of current GLP1R-selective agonists in addition to possessing superior efficacy on body weight and lipid metabolism. Bariatric surgery lowers body weight and often normalizes HbA1c in T2D patients [128,129] showing that although other mechanisms besides glucagon–peptides may be involved, the enhanced therapeutic goal is achievable. Observing a cardiovascular protective effect on outcome trials would also be ideal. Safety concerns attributed to GLP-1-based therapies such as risk of pancreatitis will need to be explored and may constitute a major differentiation factor. For example, for GLP1R/GCGR dual agonists, glucagon has been shown to depress human pancreatic exocrine secretion and was proposed as a possible treatment for acute pancreatitis [91,92]. GIPR activation does not appear to reduce gastric emptying in healthy humans although it may be involved in distal bowel motility [142]. Therefore other combinations like GLP1R/GIPR dual agonists or GLP1R/GCGR/GIPR triple agonists could possibly offer enhanced efficacy with a superior tolerability profile.

Identifying new transformational approaches to controlling T2D that free patients from frequent self-injections resulting in enhanced therapeutic compliance and control is critical. An example is the approach of Intarcia Therapeutics which optimizes GLP-1 therapy with a once- or twice-yearly dosing of ITCA 650, a device that allows continued subcutaneous delivery of exenatide. A potential limitation of this approach is the ambulatory surgery required for the implant and replacement of this device [143]. Nevertheless, it may help enhance adherence not only in diabetic patients but in diabetic patients with neurodegenerative diseases [144]. Despite recent innovations for injectables, the oral route of administration remains the most widely accepted [145] and will need to be considered together with the safety and efficacy profile for the success of new GLP-1 based therapies. Future uses of these drugs might also include the treatment of obesity [146] as well as adjunctive treatment of type 1 diabetes mellitus in combination with insulin. Addition of liraglutide was shown to reduce the insulin dose required to maintain glucose control in T1D patients [147] potentially lowering the risk of hypoglycemia. The fear of iatrogenic hypoglycemia remains a major obstacle for appropriate glucose control in patients with both Type 1 and advanced Type 2 diabetes mellitus where the counter-regulatory response is impaired especially following recurrent hypoglycemic episodes [148,149]. Sustained GCGR activation in conjunction with GLP1R activation may further reduce the hypoglycemic risk and weight gain during intensive insulin therapy and, although more research is needed, the potential exists to uncover alternate and viable treatment options for individuals living with diabetes.

CONFLICT OF INTEREST

The author is an employee of Janssen R&D, pharmaceutical companies of Johnson & Johnson.

REFERENCES

[1] UKPDS, 1995. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. Diabetes 44:1249–1258, http://dx.doi.org/10.2337/diabetes.44.11.1249.

[2] Bray, G.A., 2004. Medical consequences of obesity. Journal of Clinical Endocrinology and Metabolism 89:2583–2589, http://dx.doi.org/10.1210/jc.2004-0535.

[3] Holst, J.J., 2000. Gut hormones as pharmaceuticals. From enteroglucagon to GLP-1 and GLP-2. Regulatory Peptides 93:45–51, http://dx.doi.org/10.1016/S0167-0115(00)00185-3.

[4] Hansen, L., Hartmann, B., Bisgaard, T., Mineo, H., Jørgensen, P.N., Holst, J.J., 2000. Somatostatin restrains the secretion of glucagon-like peptide-1 and -2 from isolated perfused porcine ileum. American Journal of Physiology – Endocrinology and Metabolism 278 (6):E1010–E1018.

[5] Nauck, M.A., Kleine, N., Orskov, C., Holst, J.J., Willms, B., Creutzfeldt, W., 1993. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 36:741–744.

[6] Meier, J.J., Gallwitz, B., Salmen, S., Goetze, O., Holst, J.J., Schmidt, W.E., et al., 2003. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. Journal of Clinical Endocrinology and Metabolism 88:2719–2725.

[7] Meier, J.J., Kemmeries, G., Holst, J.J., Nauck, M.A., 2005. Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. Diabetes 54:2212–2218, http://dx.doi.org/10.2337/diabetes.54.7.2212.

[8] Nauck, M.A., Kemmeries, G., Holst, J.J., Meier, J.J., 2011. Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes 60:1561–1565, http://dx.doi.org/10.2337/db10-0474.

[9] Amori, R.E., Lau, J., Pittas, A.G., 2007. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. Journal of the American Medical Association 298:194–206, http://dx.doi.org/10.1001/jama.298.2.194.

[10] Nauck, M.A., Sauerwald, A., Ritzel, R., Holst, J.J., Schmiegel, W., 1998. Influence of glucagon-like peptide-1 on fasting glycemia in type 2 diabetic patients treated with insulin after sulfonylurea secondary failure. Diabetes Care 21:1925–1931.

[11] Kim, J.Y., Lim, D.M., Park, H.S., Moon, C.I., Choi, K.J., Lee, S.K., et al., 2012. Exendin-4 protects against sulfonylurea-induced β-cell apoptosis. Journal of Pharmacological Science 118:65–74.

[12] Drucker, D.J., 2005. Biologic actions and therapeutic potential of the glucagon-like peptides. Nature Clinical Practice. Endocrinology and Metabolism 1:22–31, http://dx.doi.org/10.1038/ncpendmet0017.

[13] Ghatei, M.A., Uttenthal, L.O., Christofides, N.D., Bryant, M.G., Bloom, S.R., 1983. Molecular forms of human enteroglucagon in tissue and plasma: plasma responses to nutrient stimuli in health and in disorders of the upper gastrointestinal tract. Journal of Clinical Endocrinology and Metabolism 57:488–495, http://dx.doi.org/10.1210/jcem-57-3-488.

[14] Holst, J.J., 1997. Enteroglucagon. Annual Review of Physiology 59:257–271, http://dx.doi.org/10.1146/annurev.physiol.59.1.257.

[15] Le Quellec, A., Kervran, A., Blache, P., Ciurana, A.J., Bataille, D., 1992. Oxyntomodulin-like immunoreactivity: diurnal profile of a new potential enterogastrone. Journal of Clinical Endocrinology and Metabolism 74:1405–1409, http://dx.doi.org/10.1210/jc.74.6.1405.

[16] Furuta, M., Zhou, A., Webb, G., Carroll, R., Ravazzola, M., Orci, L., Steiner, D.F., 2001. Severe defect in proglucagon processing in islet A-cells of prohormone convertase 2 null mice. Journal of Biological Chemistry 276:27197–27202, http://dx.doi.org/10.1074/jbc.M103362200.

[17] Kieffer, T.J., Habener, J.F., 1999. The glucagon-like peptides. Endocrine Reviews 20:876–913, http://dx.doi.org/10.1210/er.20.6.876.

[18] Rouille, Y., Westermark, G., Martin, S.K., Steiner, D.F., 1994. Proglucagon is processed to glucagon by prohormone convertase PC2 in a TC1-6 cells. Proceedings of the National Academy of Sciences 91:3242–3246, http://dx.doi.org/10.1073/pnas.91.8.3242.

[19] Brubaker, P.L., 2012. A beautiful cell (or two or three?). Endocrinology 153:2945–2948, http://dx.doi.org/10.1210/en.2012-1549.

[20] Habib, A.M., Richards, P., Cairns, L.S., Rogers, G.J., Bannon, C.A., Parker, et al., 2012. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. Endocrinology 153:3054–3065, http://dx.doi.org/10.1210/en.2011-2170.

[21] Sutherland, E.W., De Duve, C., 1948. Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas. Journal of Biological Chemistry 175:663–674.

[22] Unger, R.H., Eisentraut, A.M., McCall, M.S., Keller, S., Lanz, H.C., Madison, L.L., 1959. Glucagon antibodies and their use for immunoassay for glucagon. Proceedings of the Society for Experimental Biology and Medicine 102:621–623.

[23] Bataille, D., Gespach, C., Coudray, A.M., Rosselin, G., 1981. 'Enteroglucagon': a specific effect on gastric glands isolated from the rat fundus. Evidence foran 'oxyntomodulin' action. Bioscience Reports 1:151–155, http://dx.doi.org/10.1007/BF01117012.

[24] Bataille, D., Gespach, C., Tatemoto, K., Marie, J.C., Coudray, A.M., Rosselin, G., et al., 1981. Bioactive enteroglucagon (oxyntomodulin): present knowledge on its chemical structure and its biological activities. Peptides 2 (Suppl. 2): S41–S44, http://dx.doi.org/10.1016/0196-9781(81)90008-5.

[25] Bataille, D., Tatemoto, K., Coudray, A.M., Rosselin, G., Mutt, V., 1981. Bioactive 'enteroglucagon' (oxyntomodulin): evidence for a C-terminal extension of the glucagon molecule. Comptes Rendus des Séances de l'Académie des Sciences. Série III 293:323–328.

[26] Bataille, D., Tatemoto, K., Gespach, C., Jornvall, H., Rosselin, G., Mutt, V., 1982. Isolation of glucagon-37 (bioactive enteroglucagon/oxyntomodulin) from porcine jejuno-ileum. Characterization of the peptide. FEBS Letters 146:79–86, http://dx.doi.org/10.1016/0014-5793(82)80709-6.

[27] Baldissera, F.G., Holst, J.J., Knuhtsen, S., Hilsted, L., Nielsen, O.V., 1988. Oxyntomodulin (glicentin-(33–69)): pharmacokinetics, binding to liver cell membranes, effects on isolated perfused pig pancreas, and secretion from isolated perfused lower small intestine of pigs. Regulatory Peptides 21:151–166, http://dx.doi.org/10.1016/0167-0115(88)90099-7.

[28] Gros, L., Thorens, B., Bataille, D., Kervran, A., 1993. Glucagon-like peptide-1-(7–36) amide, oxyntomodulin, and glucagon interact with a common receptor in a somatostatin-secreting cell line. Endocrinology 133:631–638, http://dx.doi.org/10.1210/en.133.2.631.

[29] Pocai, A., Carrington, P.E., Adams, J.R., Wright, M., Eiermann, G., Zhu, L., et al., 2009. Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. Diabetes 58:2258–2266, http://dx.doi.org/10.2337/db09-0278.

[30] Baggio, L.L., Huang, Q., Brown, T.J., Drucker, D.J., 2004. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. Gastroenterology 127:546–558, http://dx.doi.org/10.1053/j.gastro.2004.04.063.

[31] Jorgensen, R., Kubale, V., Vrecl, M., Schwartz, T.W., Elling, C.E., 2007. Oxyntomodulin differentially affects glucagon–like peptide–1 receptor b-arrestin recruitment and signaling through Gα(s). Journal of Pharmacology and Experimental Therapeutics 322:148–154, http://dx.doi.org/10.1124/jpet.107.120006.

[32] Schepp, W., Dehne, K., Riedel, T., Schmidtler, J., Schaffer, K., Classen, M., 1996. Oxyntomodulin: a cAMP-dependent stimulus of rat parietal cell function via the receptor for glucagon-like peptide-1 (7–36)NH2. Digestion 57:398–405, http://dx.doi.org/10.1159/000201367.

[33] Dakin, C.L., Small, C.J., Batterham, R.L., Neary, N.M., Cohen, M.A., Patterson, M., Ghatei, M.A., Bloom, S.R., 2004. Peripheral oxyntomodulin reduces food intake and body weight gain in rats. Endocrinology 145:2687–2695, http://dx.doi.org/10.1210/en.2003-1338.

[34] Chaudhri, O.B., Parkinson, J.R., Kuo, Y.T., Druce, M.R., Herlihy, A.H., Bell, J.D., et al., 2006. Differential hypothalamic neuronal activation following peripheral injection of GLP-1 and oxyntomodulin in mice detected by manganese-enhanced magnetic resonance imaging. Biochemical and Biophysical Research Communications 350:298–306, http://dx.doi.org/10.1016/j.bbrc.2006.09.033.

[35] Parkinson, J.R., Chaudhri, O.B., Kuo, Y.T., Field, B.C., Herlihy, A.H., Dhillo, W.S., Ghatei, M.A., et al., 2009. Differential patterns of neuronal activation in the brainstem and hypothalamus following peripheral injection of GLP-1, oxynto-modulin and lithium chloride in mice detected by manganese-enhanced magnetic resonance imaging (MEMRI). Neurolmage 44:1022–1031, http://dx.doi.org/10.1016/j.neuroimage.2008.09.047.

[36] Parker, J.A., McCullough, K.A., Field, B.C., Minnion, J.S., Martin, N.M., Ghatei, M.A., et al., 2013. Glucagon and GLP-1 inhibit food intake and increase c-fos expression in similar appetite regulating centres in the brainstem and amygdala. International Journal of Obesity (London) 37:1391–1398, http://dx.doi.org/10.1038/ijo.2012.227.

[37] Cohen, M.A., Ellis, S.M., Le Roux, C.W., Batterham, R.L., Park, A., Patterson, M., et al., 2003. Oxyntomodulin suppresses appetite and reduces food intake in humans. Journal of Clinical Endocrinology and Metabolism 88:4696–4701.

[38] Wynne, K., Park, A.J., Small, C.J., Patterson, M., Ellis, S.M., Murphy, K.G., et al., 2005. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. Diabetes 54:2390–2395, http://dx.doi.org/10.2337/diabetes.54.8.2390.

[39] Dakin, C.L., Gunn, I., Small, C.J., Edwards, C.M., Hay, D.L., Smith, D.M., et al., 2001. Oxyntomodulin inhibits food intake in the rat. Endocrinology 142:4244–4250, http://dx.doi.org/10.1210/en.142.10.4244.

[40] Dakin, C.L., Small, C.J., Park, A.J., Seth, A., Ghatei, M.A., Bloom, S.R., 2002. Repeated ICV administration of oxyntomodulin causes a greater reduction in

body weight gain than in pair-fed rats. American Journal of Physiology – Endocrinology and Metabolism 283:E1173–E1177.

[41] Pocai, A., 2012. Unraveling oxyntomodulin, GLP1's enigmatic brother. Journal of Endocrinology 215:335–346, http://dx.doi.org/10.1530/JOE-12-0368.

[42] Holst, J.J., Sorensen, T.I., Andersen, A.N., Stadil, F., Andersen, B., Lauritsen, K. B., et al., 1979. Plasma enteroglucagon after jejunoileal bypass with 3:1 or 1:3 jejunoileal ratio. Scandinavian Journal of Gastroenterology 14:205–207, http://dx.doi.org/10.3109/00365527909179871.

[43] Besterman, H.S., Cook, G.C., Sarson, D.L., Christofides, N.D., Bryant, M.G., Gregor, M., et al., 1979. Gut hormones in tropical malabsorption. British Medical Journal 2:1252–1255, http://dx.doi.org/10.1136/bmj.2.6200.1252.

[44] Besterman, H.S., Adrian, T.E., Mallinson, C.N., Christofides, N.D., Sarson, D.L., Pera, A., et al., 1982. Gut hormone release after intestinal resection. Gut 23:854–861, http://dx.doi.org/10.1136/gut.23.10.854.

[45] Du, X., Kosinski, J.R., Lao, J., Shen, X., Petrov, A., Chicchi, G.G., et al., 2012. Differential effects of oxyntomodulin and GLP-1 on glucose metabolism. American Journal of Physiology – Endocrinology and Metabolism 303: E265–E271, http://dx.doi.org/10.1152/ajpendo.00142.2012.

[46] Kosinski, J.R., Hubert, J., Carrington, P.E., Chicchi, G.G., Mu, J., Miller, C., et al., 2012. The glucagon receptor is involved in mediating the body weight lowering effects of oxyntomodulin. Obesity 20:1566–1571, http://dx.doi.org/10.1038/oby.2012.67.

[47] Santoprete, A., Capito, E., Carrington, P.E., Pocai, A., Finotto, M., Langella, A., et al., 2011. DPP-IV-resistant, long-acting oxyntomodulin derivatives. Journal of Peptide Science 17:270–280, http://dx.doi.org/10.1002/psc.1328.

[48] Maida, A., Lovshin, J.A., Baggio, L.L., Drucker, D.J., 2008. The glucagon-like peptide-1 receptor agonist oxyntomodulin enhances β-cell function but does not inhibit gastric emptying in mice. Endocrinology 149:5670–5678, http://dx.doi.org/10.1210/en.2008-0336.

[49] Parlevliet, E.T., Heijboer, A.C., Schroder-van der Elst, J.P., Havekes, L.M., Romijn, J.A., Pijl, H., et al., 2008. Oxyntomodulin ameliorates glucose intolerance in mice fed a high-fat diet. American Journal of Physiology – Endocrinology and Metabolism 294:E142–E147, http://dx.doi.org/10.1152/ajpendo.00576.2007.

[50] Shankar, S.S., Shankar, R.R., Mixson, L., Pramanik, B.S., Stoch, A., Steinberg, H.O., et al., 2013. Oxyntomodulin has significant acute glucoregulatory effects comparable to liraglutide in subjects with type 2 diabetes. Diabetes 62 (Suppl. 1):A48, http://dx.doi.org/10.2337/db13-1-387.

[51] Wynne, K., Park, A.J., Small, C.J., Meeran, K., Ghatei, M.A., Frost, G.S., et al., 2006. Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomized controlled trial. International Journal of Obesity 30:1729–1736, http://dx.doi.org/10.1038/sj.ijo.0803344.

[52] Schjoldager, B.T., Baldissera, F.G., Mortensen, P.E., Holst, J.J., Christiansen, J., 1988. Oxyntomodulin: a potential hormone from the distal gut. Pharmacokinetics and effects on gastric acid and insulin secretion in man. European Journal of Clinical Investigation 18:499–503, http://dx.doi.org/10.1111/j.1365-2362.1988.tb01046.x.

[53] Schjoldager, B., Mortensen, P.E., Myhre, J., Christiansen, J., Holst, J.J., 1989. Oxyntomodulin from distal gut. Role in regulation of gastric and pancreatic functions. Digestive Diseases and Sciences 34 (9):1411–1419.

[54] Sowden, G.L., Drucker, D.J., Weinshenker, D., Swoap, S.J., 2007. Oxyntomodulin increases intrinsic heart rate in mice independent of the glucagon-like peptide-1 receptor. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 292:R962–R970.

[55] Wynne, K., Field, B.C., Bloom, S.R., 2010. The mechanism of action for oxyntomodulin in the regulation of obesity. Current Opinion in Investigational Drugs 11:1151–1157. (Review).

[56] Habegger, K.M., Heppner, K.M., Geary, N., Bartness, T.J., DiMarchi, R., Tschop, M.H., 2010. The metabolic actions of glucagon revisited. Nature Reviews. Endocrinology 6:689–697, http://dx.doi.org/10.1038/nrendo.2010.187.

[57] Mighiu, P.I., Yue, J.T., Filippi, B.M., Abraham, M.A., Chari, M., Lam, C.K., et al., 2013. Hypothalamic glucagon signaling inhibits hepatic glucose production. Nature Medicine 19:766–772, http://dx.doi.org/10.1038/nm.3115.

[58] Anini, Y., Jarrousse, C., Chariot, J., Nagain, C., Yanaihara, N., Sasaki, K., Bernad, N., Le Nguyen, D., Bataille, D., Rozé, C., 2000. Oxyntomodulin inhibits pancreatic secretion through the nervous system in rats. Pancreas 20:348–360.

[59] Schapiro, H., Ludewig, R.M., 1978. The effect of glucagon on the exocrine pancreas. American Journal of Gastroenterology 70:274–281. (Review).

[60] Collie, N.L., Zhu, Z., Jordan, S., ReeveJr, 1997. Oxyntomodulin stimulates intestinal glucose uptake in rats. Gastroenterology 112:1961–1970.

[61] Stümpel, F., Scholtka, B., Hunger, A., Jungermann, K., 1998. Enteric glucagon 37 rather than pancreatic glucagon 29 stimulates glucose absorption in rat intestine. Gastroenterology 115:1163–1171.

[62] Cheeseman, C.I., Tsang, R., 1996. The effect of GIP and glucagon-like peptides on intestinal basolateral membrane hexose transport. American Journal of Physiology 271:G477–G482.

[63] Cheeseman, C.I., 1997. Upregulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo. American Journal of Physiology 273: R1965–R1971.

[64] Lagerström, M.C., Schiöth, H.B., 2008. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nature Reviews Drug Discovery 7:339–357, http://dx.doi.org/10.1038/nrd2518. (Review).

[65] Siu, F.Y., He, M., de Graaf, C., Han, G.W., Yang, D., Zhang, Z., et al., 2013. Structure of the human glucagon class B G-protein-coupled receptor. Nature 499:444–449, http://dx.doi.org/10.1038/nature12393.

[66] Plamboeck, A., Veedfald, S., Deacon, C.F., Hartmann, B., Wettergren, A., Svendsen, L.B., et al., 2013. The effect of exogenous GLP-1 on food intake is lost in male truncally vagotomized subjects with pyloroplasty. American Journal of Physiology – Gastrointestinal and Liver Physiology 304:G1117–G1127, http://dx.doi.org/10.1152/ajpgi.00035.2013.

[67] Geary, N., Le Sauter, J., Noh, U., 1993. Glucagon acts in the liver to control spontaneous meal size in rats. American Journal of Physiology 264: R116–R122.

[68] Tan, T.M., Field, B.C., McCullough, K.A., Troke, R.C., Chambers, E.S., Salem, V., et al., 2013. Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. Diabetes 62:1131–1138, http://dx.doi.org/10.2337/db12-0797.

[69] Patterson, M., Murphy, K.G., Patel, S.R., Patel, N.A., Greenwood, H.C., Cooke, J.H., et al., 2009. Hypothalamic injection of oxyntomodulin suppresses circulating ghrelin-like immunoreactivity. Endocrinology 150:3513–3520, http://dx.doi.org/10.1210/en.2008-0796.

[70] Hagemann, D., Holst, J.J., Gethmann, A., Banasch, M., Schmidt, W.E., Meier, J.J., 2007. Glucagon-like peptide 1 (GLP-1) suppresses ghrelin levels in humans via increased insulin secretion. Regulatory Peptides 143:64–68.

[71] Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., et al., 2001. Ghrelin enhances appetite and increases food intake in humans. Journal of Clinical Endocrinology and Metabolism 86:5992.

[72] Neary, N.M., Small, C.J., Wren, A.M., Lee, J.L., Druce, M.R., Palmieri, C., et al., 2004. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. Journal of Clinical Endocrinology and Metabolism 89:2832–2836.

[73] Day, J.W., Smiley, D., Carrington, P.E., Eiermann, G., Chicchi, G., Erion, M.D., et al., 2012. Optimization of co-agonism at GLP-1 and glucagon receptors to safely maximize weight reduction in DIO-rodents. Peptide Science 98:443–450, http://dx.doi.org/10.1002/bip.22072.

[74] Habegger, K.M., Stemmer, K., Cheng, C., Müller, T.D., Heppner, K.M., Ottaway, N., et al., 2013. Fibroblast growth factor 21 mediates specific glucagon actions. Diabetes 62:1453–1463, http://dx.doi.org/10.2337/db12-1116.

[75] Berglund, E.D., Unger, R.H., 2013. Role of fibroblast growth factor 21 in biology of glucagon. Diabetes 62:1376, http://dx.doi.org/10.2337/db12-1840.

[76] Arafat, A.M., Kaczmarek, P., Skrzypski, M., Pruszy◆ska-Oszmalek, E., Kołodziejski, P., Szczepankiewicz, D., et al., 2013. Glucagon increases circulating fibroblast growth factor 21 independently of endogenous insulin levels: a novel mechanism of glucagon-stimulated lipolysis? Diabetologia 56:588–597, http://dx.doi.org/10.1007/s00125-012-2803-y.

[77] Adams, A.C., Halstead, C.A., Hansen, B.C., Irizarry, A.R., Martin, J.A., Myers, S.R., et al., 2013. LY2405319, an engineered FGF21 variant, improves the metabolic status of diabetic monkeys. PLoS One 8:e65763.

[78] Gaich, G., Chien, J.Y., Fu, H., Glass, L.C., Deeg, M.A., Holland, W.L., et al., 2013. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metabolism 18:333–340, http://dx.doi.org/10.1016/j.cmet.2013.08.005.

[79] Lin, Z., Tian, H., Lam, K.S., Lin, S., Hoo, R.C., Konishi, M., et al., 2013. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. Cell Metabolism 17:779–789, http://dx.doi.org/10.1016/j.cmet.2013.04.005.

[80] Dutchak, P.A., Katafuchi, T., Bookout, A.L., Choi, J.H., Yu, R.T., Mangelsdorf, D.J., et al., 2012. Fibroblast growth factor-21 regulates PPARγ activity and the antidiabetic actions of thiazolidinediones. Cell 148:556–567, http://dx.doi.org/10.1016/j.cell.2011.11.062.

[81] Adams, A.C., Coskun, T., Cheng, C.C., Farrell, O., Dubois, L.S., Kharitonenkov, S.L., A., 2013. Fibroblast growth factor 21 is not required for the antidiabetic actions of the thiazoladinediones. Molecular Metabolism 2:205–214, http://dx.doi.org/10.1016/j.molmet.2013.05.005.

[82] Engel, S.S., Xu, L., Andryuk, P.J., Davies, M.J., Amatruda, J., Kaufman, K., et al., 2011. Efficacy and tolerability of MK-0893, a glucagon receptor antagonist (GRA), in patients with type 2 diabetes (T2DM). Diabetes 60 (Suppl. 1):A85, http://dx.doi.org/10.2337/db11-1-378.

[83] Pradhan, G., Samson, S.L., Sun, Y., 2013. Ghrelin: much more than a hunger hormone. Current Opinion in Clinical Nutrition & Metabolic Care 16:619–624, http://dx.doi.org/10.1097/MCO.0b013e328365b9be.

[84] Lockie, S.H., Heppner, K.M., Chaudhary, N., Chabenne, J.R., Morgan, D.A., Veyrat-Durebex, C., et al., 2012. Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. Diabetes 61:2753–2762, http://dx.doi.org/10.2337/db11-1556.

[85] Drucker, D.J., Buse, J.B., Taylor, K., Kendall, D.M., Trautmann, M., Zhuang, D., et al., 2008. DURATION-1 Study Group. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. Lancet 372:1240–1250, http://dx.doi.org/10.1016/S0140-6736(08)61206-4.

[86] Orskov, C., Poulsen, S.S., Møller, M., Holst, J.J., 1996. Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. Diabetes 45:832–835.

[87] Waldhäusl, W., Haydl, H., Nowotny, P., 1976. ACTH and cortisol responses to glucagon stimulation. Journal of Clinical Endocrinology and Metabolism 43:675–678.

[88] Elashoff, M., Matveyenko, A.V., Gier, B., Elashoff, R., Butler, P.C., 2011. Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. Gastroenterology 141:150–156, http://dx.doi.org/10.1053/j.gastro.2011.02.018.

[89] Nauck, M.A., 2013. A critical analysis of the clinical use of incretin-based therapies: the benefits by far outweigh the potential risks. Diabetes Care 36:2126–2132, http://dx.doi.org/10.2337/dc12-2504.

[90] Butler, P.C., Elashoff, M., Elashoff, R., Gale, E.A., 2013. A critical analysis of the clinical use of incretin-based therapies: are the GLP-1 therapies safe? Diabetes Care 36:2118–2125, http://dx.doi.org/10.2337/dc12-2713.

[91] Eckhauser, F.E., Knol, J.A., Inman, M.G., Strodel, W.E., 1985. Efficacy of pharmacologic glucagon in acute experimental pancreatitis. Archives of Surgery 120:355–360.

[92] Harada, H., Kochi, F., Hanafusa, E., Kobayashi, T., Oka, H., Kimura, I., 1985. Studies on the effect of glucagon on human pancreatic secretion by analysis of endoscopically obtained pure pancreatic juice. Gastroenterologia Japonica 20:28–36.

[93] Girman, C.J., Kou, T.D., Cai, B., Alexander, C.M., O'Neill, E.A., Williams-Herman, D.E., et al., 2010. Patients with type 2 diabetes mellitus have higher risk for acute pancreatitis compared with those without diabetes. Diabetes, Obesity and Metabolism 12:766–771.

[94] Butler, A.E., Campbell-Thompson, M., Gurlo, T., Dawson, D.W., Atkinson, M., Butler, P.C., 2013. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. Diabetes 62:2595–2604, http://dx.doi.org/10.2337/db12-1686.

[95] Kahn, S.E., 2013. Incretin therapy and islet pathology: a time for caution. Diabetes 62:2178–2180, http://dx.doi.org/10.2337/db13-0520.

[96] Heine, R.J., Fu, H., Kendall, D.M., Moller, D.E., 2013. Comment on: Butler et al. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. Diabetes 62:e16–e17, http://dx.doi.org/10.2337/db13-0690.

[97] Engel, S.S., Golm, G.T., Lauring, B., 2013. Comment on: Butler et al. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. Diabetes 62:e18, http://dx.doi.org/10.2337/db13-0525.

[98] Bjerre Knudsen, L., Madsen, L.W., Andersen, S., Almholt, K., de Boer, A.S., Drucker, D.J., et al., 2010. Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology 151:1473–1486, http://dx.doi.org/10.1210/en.2009-1272.

[99] Hegedüs, L., Moses, A.C., Zdravkovic, M., Le Thi, T., Daniels, G.H., 2011. GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide. Journal of Clinical Endocrinology and Metabolism 96:853–860.

[100] Gier, B., Butler, P.C., Lai, C.K., Kirakossian, D., DeNicola, M.M., Yeh, M.W., 2012. Glucagon like peptide-1 receptor expression in the human thyroid gland. Journal of Clinical Endocrinology and Metabolism 97:121–131, http://dx.doi.org/10.1210/jc.2011-2407.

[101] Crespel, A., De Boisvilliers, F., Gros, L., Kervran, A., 1996. Effects of glucagon and glucagon-like peptide-1-(7-36) amide on C cells from rat thyroid and medullary thyroid carcinoma CA-77 cell line. Endocrinology 137:3674–3680.

[102] Boden, G., Rezvani, I., Owen, O.E., 1984. Effects of glucagon on plasma amino acids. Journal of Clinical Investigation 73:785–793.

[103] Day, J.W., Ottaway, N., Patterson, J.T., Gelfanov, V., Smiley, D., Gidda, J., et al., 2009. A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. Nature Chemical Biology 5:749–757, http://dx.doi.org/10.1038/nchembio.209.

[104] Barazzoni, R., Zanetti, M., Tiengo, A., Tessari, P., 1999. Protein metabolism in glucagonoma. Diabetologia 42:326–329.

[105] Yabe, D., Rokutan, M., Miura, Y., Komoto, I., Usui, R., Kuwata, H., et al., 2013. Enhanced glucagon-like peptide-1 secretion in a patient with glucagonoma: implications for glucagon-like peptide-1 secretion from pancreatic α cells in vivo. Diabetes Research and Clinical Practice 102:e1–e4, http://dx.doi.org/10.1016/j.diabres.2013.08.005.

[106] Nair, K.S., Halliday, D., Matthews, D.E., Welle, S.L., 1987. Hyperglucagonemia during insulin deficiency accelerates protein catabolism. American Journal of Physiology 253:E208–E213.

[107] Buse, M.G., Biggers, J.F., Drier, C., Buse, J.F., 1973. The effect of epinephrine, glucagon, and the nutritional state on the oxidation of branched chain amino acids and pyruvate by isolated hearts and diaphragms of the rat. Journal of Biological Chemistry 248:697–706.

[108] Farah, A., Tuttle, R., 1960. Studies on the pharmacology of glucagon. Journal of Pharmacology and Experimental Therapeutics 129:49–55.

[109] Gonzalez-Munoz, C., Nieto-Ceron, S., Cabezas-Herrera, J., Hernandez-Cascales, J., 2008. Glucagon increases contractility in ventricle but not in atrium of the rat heart. European Journal of Pharmacology 587:243–247, http://dx.doi.org/10.1016/j.ejphar.2008.04.001.

[110] Grieve, D.J., Cassidy, R.S., Green, B.D., 2009. Emerging cardiovascular actions of the incretin hormone glucagon-like peptide-1: potential therapeutic benefits beyond glycaemic control? British Journal of Pharmacology 157:1340–1351, http://dx.doi.org/10.1111/j.1476-5381.2009.00376.x.

[111] Marre, M., Shaw, J., Brändle, M., Bebakar, W.M., Kamaruddin, N.A., Strand, J., et al., 2009. LEAD-1 SU study group. Liraglutide, a once-daily human GLP-1 analog, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU). Diabetic Medicine 26:268–278.

[112] Nauck, M., Frid, A., Hermansen, K., Shah, N.S., Tankova, T., Mitha, I.H., et al., 2009. LEAD-2 Study Group. Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study. Diabetes Care 32:84–90.

[113] Garber, A., Henry, R., Ratner, R., Garcia-Hernandez, P.A., Rodriguez-Pattzi, H., Olvera-Alvarez, I., et al., 2009. LEAD-3 (Mono) Study Group. Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. Lancet 373:473–481.

[114] Zinman, B., Gerich, J., Buse, J.B., Lewin, A., Schwartz, S., Raskin, P., et al., 2009. LEAD-4 Study Investigators. Efficacy and safety of the human glucagon-like peptide-1 analog liraglutide in combination with metformin and thiazolidinedione in patients with type 2 diabetes (LEAD-4 Met + TZD). Diabetes Care 32:1224–1230.

[115] Russell-Jones, D., Vaag, A., Schmitz, O., Sethi, B.K., Lalic, N., Antic, S., et al., 2009. Liraglutide Effect and Action in Diabetes 5 (LEAD-5) met + SU Study Group. Liraglutide vs. insulin glargine and placebo in combination with metformin and sulfonylurea therapy in type 2 diabetes mellitus (LEAD-5 met + SU): a randomised controlled trial. Diabetologia 52:2046–2055.

[116] Buse, J.B., Rosenstock, J., Sesti, G., Schmidt, W.E., Montanya, E., Brett, J.H., et al., 2009. LEAD-6 Study Group. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet 374:39–47.

[117] Pratley, R.E., Nauck, M., Bailey, T., Montanya, E., Cuddihy, R., Filetti, S., et al., 2010. Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial. Lancet 375:1447–1456.

[118] Gill, A., Hoogwerf, B.J., Burger, J., Bruce, S., Macconell, L., Yan, P., et al., 2010. Effect of exenatide on heart rate and blood pressure in subjects with type 2 diabetes mellitus: a double-blind, placebo-controlled, randomized pilot study. Cardiovascular Diabetology 9:6.

[119] Okerson, T., Yan, P., Stonehouse, A., Brodows, R., 2010. Effects of exenatide on systolic blood pressure in subjects with type 2 diabetes. American Journal of Hypertension 23:334–339.

[120] Umpierrez, G.E., Blevins, T., Rosenstock, J., Cheng, C., Anderson, J.H., Bastyr, E.J., 3rd, 2011. for the EGO Study Group, 2011. The effects of LY2189265, a long-acting glucagon-like peptide-1 analog, in a randomized, placebo-controlled, double-blind study of overweight/obese patients with type 2 diabetes: the EGO study. Diabetes, Obesity and Metabolism 13:418–425.

[121] Parmley, W.W., Glick, G., Sonneblick, E.H., 1968. Cardiovascular effects of glucagon in man. New England Journal of Medicine 279:12–17.

[122] Barbato, A., Russo, P., Venezia, A., Strazzullo, V., Siani, A., Cappuccio, F.P., 2003. Analysis of Gly40Ser polymorphism of the glucagon receptor (GCGR) gene in different ethnic groups. Journal of Human Hypertension 17:577–579, http://dx.doi.org/10.1038/sj.jhh.1001591.

[123] Strazzullo, P., Iacone, R., Siani, A., Barba, G., Russo, O., Russo, P., et al., 2001. Altered renal sodium handling and hypertension in men carrying the glucagon receptor gene (Gly40Ser) variant. Journal of Molecular Medicine 79:574–580, http://dx.doi.org/10.1007/s001090100257.

[124] Ruddy, M., Luncenford, J., Li, S., Cilissen, C., Stoch, A., Wagner, J., et al., 2011. Inhibition of glucagon-induced hyperglycemia predicts glucose lowering efficacy antagonist, MK-0893, in type 2 diabetes (T2DM). Diabetes 60 (Suppl. 1):A85, http://dx.doi.org/10.2337/ db11-1-378.

[125] Panchapakesan, U., Mather, A., Pollock, C., 2013. Role of GLP-1 and DPP-4 in diabetic nephropathy and cardiovascular disease. Clinical Science 124:17–26, http://dx.doi.org/10.1042/CS20120167.

[126] Scirica, B.M., Bhatt, D.L., Braunwald, E., Steg, P.G., Davidson, J., Hirshberg, B., 2013. The SAVOR-TIMI 53 Steering Committee and Investigators: saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. New England Journal of Medicine 369:1317–1326, http://dx.doi.org/10.1053/NEJMoa1307684.

[127] Lao, J., Hansen, B.C., DiMarchi, R., Pocai, A., 2013. Effect of GLP1R/GCGR dual agonist in monkeys. Diabetes 62 (Suppl. 1):A257, http://dx.doi.org/10.2337/db13-859-1394.

[128] Holst, J.J., 2013. Enteroendocrine secretion of gut hormones in diabetes, obesity and after bariatric surgery. Current Opinion in Pharmacology 13:00187-2, http://dx.doi.org/10.1016/j.coph.2013.09.014. (pii: S1471-4892).

[129] Chandarana, K., Batterham, R.L., 2012. Shedding pounds after going under the knife: metabolic insights from cutting the gut. Nature Medicine 18:668–669, http://dx.doi.org/10.1038/nm.2748.

[130] Garber, A.J., 2012. Novel GLP-1 receptor agonists for diabetes. Expert Opinion on Investigational Drugs 21:45–57, http://dx.doi.org/10.1517/13543784.2012.638282. (Review).

[131] Kaspar, A.A., Reichert, J.M., 2013. Future directions for peptide therapeutics development. Drug Discovery Today 18:807–817, http://dx.doi.org/10.1016/j.drudis.2013.05.011.

[132] Lee, K.C., Chae, S.Y., Kim, T.H., Lee, S., Lee, E.S., Youn, Y.S., 2009. Intrapulmonary potential of polyethylene glycol-modified glucagon-like peptide-1s as a type 2 anti-diabetic agent. Regulatory Peptides 152:101–107, http://dx.doi.org/10.1016/j.regpep.2008.09.008.

[133] Owens, D.R., 2002. New horizons – alternative routes for insulin therapy. Nature Reviews. Drug Discovery 1:529–540, http://dx.doi.org/10.1038/nrd836.

[134] Sloop, K.W., Willard, F.S., Brenner, M.B., Ficorilli, J., Valasek, K., Showalter, A.D., et al., 2010. Novel small molecule glucagon-like peptide-1 receptor agonist stimulates insulin secretion in rodents and from human islets. Diabetes 59:3099–3107, http://dx.doi.org/10.2337/db10-0689.

[135] Finan, B., Ma, T., Ottaway, N., Müller, T.D., Habegger, K.M., Heppner, K.M., et al., 2013. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. Science Translational Medicine 5:209ra151, http://dx.doi.org/10.1126/scitranslmed.3007218.

[136] Kerr, B.D., Flatt, P.R., Gault, V.A., 2010. D-Ser2)Oxm(mPEG-PAL): a novel chemically modified analog of oxyntomodulin with antihyperglycaemic, insulinotropic and anorexigenic actions. Biochemical Pharmacology 80:1727–1735, http://dx.doi.org/10.1016/j.bcp.2010.08.010.

[137] Liu, Y.L., Ford, H.E., Druce, M.R., Minnion, J.S., Field, B.C., Shillito, J.C., et al., 2010. Subcutaneous oxyntomodulin analog administration reduces body

weight in lean and obese rodents. International Journal of Obesity 34:1715–1725, http://dx.doi.org/10.1038/ijo.2010.110.

[138] Bhat, V.K., Kerr, B.D., Flatt, P.R., Gault, V.A., 2013. A novel GIP-oxyntomodulin hybrid peptide acting through GIP, glucagon and GLP-1 receptors exhibits weight reducing and anti-diabetic properties. Biochemical Pharmacology 85:1655–1662, http://dx.doi.org/10.1016/j.bcp.2013.03.009.

[139] Bhat, V.K., Kerr, B.D., Vasu, S., Flatt, P.R., Gault, V.A., 2013. A DPP-IV-resistant triple-acting agonist of GIP, GLP-1 and glucagon receptors with potent glucose-lowering and insulinotropic actions in high-fat-fed mice. Diabetologia 56:1417–1424, http://dx.doi.org/10.1007/s00125-013-2892-2.

[140] Roed, S.N., Orgaard, A., Jorgensen, R., De Meyts, P., 2012. Receptor oligomerization in family B1 of G-protein-coupled receptors: focus on BRET investigations and the link between GPCR oligomerization and binding cooperativity. Frontiers in Endocrinology 3:62, http://dx.doi.org/10.3389/fendo.2012.00062.

[141] Schelshorn, D., Joly, F., Mutel, S., Hampe, C., Breton, B., Mutel, V., Lutjens, R., 2012. Lateral allosterism in the glucagon receptor family: glucagon-like peptide 1 induces G-protein-coupled receptor heteromer formation. Molecular Pharmacology 81:309–318, http://dx.doi.org/10.1124/mol.111.074757.

[142] Meier, J.J., Goetze, O., Anstipp, J., Hagemann, D., Holst, J.J., Schmidt, W.E., et al., 2004. Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. American Journal of Physiology – Endocrinology and Metabolism 286: E621–E625.

[143] Henry, R.R., Rosenstock, J., Logan, D.K., Alessi, T.R., Luskey, K., Baron, M.A., 2013. Randomized trial of continuous subcutaneous delivery of exenatide by ITCA 650 versus twice-daily exenatide injections in metformin-treated type 2 diabetes. Diabetes Care 36:2559–2565, http://dx.doi.org/10.2337/dc12-2410.

[144] Vignini, A., Giulietti, A., Nanetti, L., Raffaelli, F., Giusti, L., Mazzanti, L., Provinciali, L., 2013. Alzheimer's disease and diabetes: new insights and unifying therapies. Current Diabetes Review 9:218–227. (Review).

[145] Rekha, M.R., Sharma, C.P., 2013. Oral delivery of therapeutic protein/peptide for diabetes–future perspectives. International Journal of Pharmaceutics 440:48–62, http://dx.doi.org/10.1016/j.ijpharm.2012.03.056.

[146] Astrup, A., Carraro, R., Finer, N., Harper, A., Kunesova, M., Lean, M.E., et al., 2012. Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. International Journal of Obesity 36:843–854, http://dx.doi.org/10.1038/ijo.2011.158.

[147] Kielgast, U., Krarup, T., Holst, J.J., Madsbad, S., 2011. Four weeks of treatment with liraglutide reduces insulin dose without loss of glycemic control in type 1 diabetic patients with and without residual beta-cell function. Diabetes Care 34:1463–1468, http://dx.doi.org/10.2337/dc11-0096.

[148] Amiel, S.A., Tamborlane, W.V., Simonson, D.C., Sherwin, R.S., 1987. Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. New England Journal of Medicine 316:1376–1383, http://dx.doi.org/10.1056/nejm198705283162205.

[149] Cryer, P.E., 2008. The barrier of hypoglycemia in diabetes. Diabetes 57:3169–3176, http://dx.doi.org/10.2337/db08-1084.