GLP2: an underestimated signal for improving glycaemic control and insulin sensitivity

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Abstract

Glucagon-like peptide 2 (GLP2) is a proglucagon-derived peptide produced by intestinal enteroendocrine L-cells and by a discrete population of neurons in the brainstem, which projects mainly to the hypothalamus. The main biological actions of GLP2 are related to the regulation of energy absorption and maintenance of mucosal morphology, function and integrity of the intestine; however, recent experimental data suggest that GLP2 exerts beneficial effects on glucose metabolism, especially in conditions related to increased uptake of energy, such as obesity, at least in the animal model. Indeed, mice lacking GLP2 receptor selectively in hypothalamic neurons that express proopiomelanocortin show impaired postprandial glucose tolerance and hepatic insulin resistance (by increased gluconeogenesis). Moreover, GLP2 acts as a beneficial factor for glucose metabolism in mice with high-fat diet-induced obesity. Thus, the aim of this review is to update and summarize current knowledge about the role of GLP2 in the control of glucose homeostasis and to discuss how this molecule could exert protective effects against the onset of related obesity type 2 diabetes.

Key Words

- ▶ GLP2
- ▶ obesity
- ▶ insulin resistance
- ▶ type 2 diabetes

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Introduction

Glucagon-like peptide 2 (GLP2) is a 33 amino acid proglucagon-derived peptide produced by a subset of enteroendocrine cells (L-cells) residing within the epithelium of the small and large intestine (Yusta *et al.* 2000). GLP2 is also produced in a discrete population of neurons in the brainstem, which projects mainly to the hypothalamus, brain area that plays a key role in control of food intake (Vrang *et al.* 2007). Prohormone convertase 1/3 (PC1/3) processes proglucagon in the gastrointestinal tract and in the brain, resulting in glucagon-like peptide-1 (GLP1), GLP2, intervening peptide-2, oxyntomodulin and glicentin (Ugleholdt *et al.* 2004). The studies on proglucagon-derived peptides have supplied two classes of glucose-lowering agents, the dipeptidyl peptidase

IV (DPP-IV) inhibitors and GLP1 receptor agonists, useful tools for the treatment of type 2 diabetes (T2D) (Drucker & Nauck 2006). However, the overwhelming interest attracted by GLP1 analogues as potent incretins has somewhat clouded the efforts to understand the importance of other proglucagon-derived peptides. In fact, unlike GLP1, initially, GLP2 was not reported to modulate insulin secretion or glucose homeostasis (Schmidt *et al.* 1985, Ørskov *et al.* 1988); however, recent experimental data suggest that GLP2 exerts beneficial effects on glucose metabolism, especially in conditions related to increased uptake of energy, such as obesity, at least in the animal model (Cani *et al.* 2009, Bahrami *et al.* 2010, Shi *et al.* 2013, Guan 2014, Baldassano *et al.* 2015).

Thus, the aim of this review is to update and summarize current knowledge about the role of GLP2 in the control of glucose homeostasis and to discuss how this molecule could exert protective effects against the onset of related obesity type 2 diabetes.

Overview on GLP2

GLP2 was first discovered as an intestinotrophic factor in 1996 (Drucker *et al.* 1996); however, today it is considered a pleiotropic hormone with a wide range of effects, mainly in the gastrointestinal tract. The main biological actions of GLP2 are related to the regulation of energy absorption and maintenance of mucosal morphology, function and integrity of the intestine (Drucker & Yusta 2014 for an extensive review).

GLP2 is released in response to stimulation by luminal nutrients, such as glucose, fatty acids and dietary fibre (Brubaker 2006). GLP2 is cleaved to inactive GLP2 (3–33) by DPP-IV; consequently, the half-life of intravenous GLP2 is very short, about 7 min in healthy humans (Hartmann *et al.* 2000). Indeed, GLP2 (3–33) may act as a weak agonist at pharmacological concentrations (Shin *et al.* 2005); however, it is able to act as a competitive antagonist of the GLP2 receptor (GLP2R) in rodents (Thulesen *et al.* 2002, Shin *et al.* 2005, Baldassano *et al.* 2009, 2013). Then DPP-IV-resistant GLP2 analogues, such as [Gly²]-GLP2 (teduglutide), exhibit greater bioactivity relative to native molecule, due to their longer circulating half-lives (Tavares *et al.* 2000, Baldassano & Amato 2014).

GLP2 promotes energy absorption within the gastrointestinal tract through non-specific and specific adaptation. In fact, it induces crypt cell proliferation and inhibition of apoptosis, resulting in an increase of villous height and in the expansion of the absorptive mucosal surface in the small and large intestine (Drucker *et al.* 1996, Tsai *et al.* 1997, Drucker & Yusta 2014). Moreover, GLP2 increases the uptake of sugars by augmenting the activity and the expression of transporters (Cheeseman 1997, Au *et al.* 2002, Ramsanahie *et al.* 2004, Sueyoshi *et al.* 2014) and by enhancing the expression of different enzymes involved in digestion (Brubaker *et al.* 1997a, Petersen *et al.* 2002). Evidence from studies on humans and animal models suggests that GLP2 also plays a role in lipid absorption. Indeed, GLP2 facilitates intestinal absorption of lipids (Meier *et al.* 2006, Hsieh *et al.* 2009) and enhances and regulates chylomicron secretion from the intestine (Hsieh *et al.* 2009, Hein *et al.* 2013, Dash *et al.* 2014).

The gastrointestinal responses to GLP2 are mediated via GLP2R, a member of the glucagon/secretin G protein-coupled receptor superfamily that is located on enteric (Bjerknes & Cheng 2001, Baldassano *et al.* 2009) and vagal nerves (Nelson *et al.* 2007), subepithelial myofibroblasts (Ørskov *et al.* 2005) and a subset of intestinal epithelial cells (Thulesen *et al.* 2000). Activation of GLP2 receptors regulates epithelial cell growth (Tsai *et al.* 1997, Bjerknes & Cheng 2001), reduces intestinal permeability, enhances the barrier function (Benjamin *et al.* 2000, Moran *et al.* 2012, Drucker & Yusta 2014), increases mesenteric blood flow (Guan *et al.* 2006, Stephens *et al.* 2006, Bremholm *et al.* 2009) and inhibits gastrointestinal motility, thus providing another mechanism to increase digestion and absorption of nutrient (Wøjdemann *et al.* 1998, McDonagh *et al.* 2007, Amato *et al.* 2009, 2010, Cinci *et al.* 2011).

GLP2R is also expressed in the central nervous system (CNS), specifically in key regions of the brain for energy balance, including the hypothalamus, hippocampus and brainstem (Tang-Christensen *et al.* 2000, Lovshin *et al.* 2004, Guan *et al.* 2012). As a neurotransmitter, GLP2 may mediate preproglucagonergic (PPG) neuron-induced synaptic transmission linking the hypothalamus and the brainstem and may act as a satiation signal in the control of feeding behaviour (Tang-Christensen *et al.* 2000). In fact, intracerebroventricular administrations of GLP2 reduce food intake in rodents (Tang-Christensen *et al.* 2000, Lovshin *et al.* 2001). In addition, knockout mice with GLP2R deletion selectively in hypothalamic arcuate nucleus neurons expressing proopiomelanocortin (POMC) display hyperphagic behaviour and late-onset obesity (Guan *et al.* 2012). These observations support the hypothesis of a physiological role in the regulation of food intake and body weight (Guan *et al.* 2012). Up to date, studies in humans have not demonstrated a decrease in food intake after peripheral GLP2 administration (Schmidt *et al.* 2000, Sørensen *et al.* 2003), even if recent data have shown that intraperitoneal injections of GLP2 or [Gly²]-GLP2 reduce food intake in mice, suggesting a role for GLP2 in the short-term regulation of the eating behaviour (Baldassano *et al.* 2012). This effect is related to a significant decrease in the rate of gastric emptying (Baldassano *et al.* 2012), and it is well known that gastric emptying is a critical process for the short-term control of food intake (Janssen *et al.* 2011, Rotondo *et al.* 2011a). Moreover, in mice, GLP2R deletion in POMC neurons accelerates the rate of gastric emptying accounting for the hyperphagic behaviour and supporting the hypothesis that CNS GLP2 is a key satiety signal for the physiological short-term control of feeding behaviour (Guan *et al.* 2012).

GLP2 and glycaemic control

Up to date, the GLP2 action on glucose homeostasis has been scarcely investigated, and the importance of GLP2R signalling is not clear yet (Guan 2014). GLP2R global deficiency is not critical for glucose homeostasis in normal or lean diabetic mice because it is not associated with changes in fasting glucose, glucose tolerance or plasma glucagon level (Bahrami *et al.* 2010). Also the observation that the chronic treatment with GLP2 (3–33), a GLP2R antagonist used to reveal the physiological actions of GLP2 (Shin *et al.* 2005, Nelson *et al.* 2008, Iakoubov *et al.* 2009, Baldassano *et al.* 2013), does not affect glycaemic parameters, glucose tolerance, insulin sensitivity or pancreas weight and β -cell mass in mice, has led to rule out a role for the endogenous GLP2 in glucose homeostasis in normal conditions (Baldassano *et al.* 2015). In addition, changes in glucagon levels or glycaemia have not been reported following acute or chronic GLP2 or teduglutide administration in patients with short bowel syndrome (Drucker & Yusta 2014).

We know for a long time that GLP2 does not exhibit any insulin-releasing properties (Schmidt *et al.* 1985, Ørskov *et al.* 1988). On the contrary, i.v. infusion of the peptide has been demonstrated to increase glucagon secretion in healthy, non-obese human subjects both in physiological and pharmacological plasma concentration (Sørensen *et al.* 2003, Meier *et al.* 2006) or in diabetic patients (Christensen *et al.* 2010, Lund *et al.* 2011). GLP2R has been localized to α -cells in both human and rat islets by means of immunohistochemistry as well as real-time PCR (de Heer *et al.* 2007). In contrast, full-length *Glp2r* mRNA transcripts have not been detected in RNA from murine islets, and GLP2 does not increase plasma glucagon levels in mice (Yusta *et al.* 2000, Bahrami *et al.* 2010). The discrepancy could be attributed to different species; however, caution should be taken in ruling out a glucagonotrophic role of GLP2 in mice. In fact, recent researches have demonstrated that GLP2R is more widely expressed than it was estimated (Angelone *et al.* 2012, El-Jamal *et al.* 2014). Therefore, it is necessary to explore the presence and localization of GLP2R in the pancreatic islets using validated GLP2R antibody and the opportune positive and negative controls to yield to conclusive results. Anyway, the GLP2 glucagonotrophic properties could suggest that the endogenous or exogenous peptide exacerbates the hyperglycaemia conditions related to diabetes. Nevertheless, the glucagon hypersecretion induced by oral glucose, which is typical of patients with T2D (Knop *et al.* 2007), is not a consequence of

exaggerated secretion or effect of GLP2 (Lund *et al.* 2011), and GLP2R global absence in genetically obese mice increases glucagon secretion and hyperglycaemia (Bahrami *et al.* 2010). Therefore, the importance of GLP2 in the control of glucagon secretion in different species and in diverse pathological conditions related to glucose impairment requires further elucidation.

New evidence obtained from GLP2R tissue-specific KO mice indicates that GLP2R in POMC neurons is essential for suppressing hepatic glucose production (Shi *et al.* 2013). Indeed, mice lacking GLP2R selectively in POMC neurons display impaired postprandial glucose tolerance and hepatic insulin resistance (by increased gluconeogenesis), suggesting a physiological significance of GLP2 neural action in glycaemic control. Moreover, intracerebroventricular infusion of GLP2 increases glucose tolerance and insulin sensitivity and suppresses basal hepatic glucose production through GLP2R activation in POMC neurons (Shi *et al.* 2013). Therefore, GLP2 has been proposed as a crucial neuroendocrine signal for glucose homeostasis (Guan 2014). It will be crucial to determine whether CNS GLP2R is a key contributor to glycaemic control and insulin sensitivity also in humans.

Moreover, GLP2 seems to act as a beneficial factor for glucose metabolism in obesity condition. Loss of GLP2R leads to increased glucagon secretion and α -cell mass, impaired intraperitoneal glucose tolerance and hyperglycaemia, reduced β -cell mass and decreased islet proliferation in genetic obese *ob/ob:Glp2r^{-/-}* mice. Then, the authors have suggested that GLP2R is required for the adaptation of the endocrine pancreas to metabolic stress (Bahrami *et al.* 2010). In mice fed a high-fat diet (HFD), endogenous GLP2 acts as a protective factor against the dysregulation of glucose metabolism that occurs in HFD-fed mice because GLP2 (3–33) chronic treatment exacerbates glucose metabolism disorders (Baldassano *et al.* 2015). It is well known that in C57BL/6J mouse model, a chronic exposure to HFD induces obesity and a progressive deterioration of metabolic control, characterized by hyperglycaemia, hyperinsulinaemia and peripheral and central insulin resistance (Surwit *et al.* 1988, Ahrén *et al.* 1997, Lee *et al.* 1995, Nuzzo *et al.* 2015). Mice after 10 weeks on HFD have greater mass gain compared with standard diet-fed mice, show hyperglycaemia, an impaired glycaemic response following intraperitoneal glucose load, high plasma insulin level after glucose load, increased pancreas weight and β cell expansion, but not insulin resistance. In HFD-fed mice, GLP2 (3–33) treatment for 4 weeks (from the sixth to the tenth week of diet) does not affect fasting

glycaemia; however, it significantly increases glucose intolerance, enhances both fasting and glucose-induced insulin concentrations and reduces sensitivity to insulin. Therefore, the reduction of GLP2R signalling accelerates the process leading to insulin resistance in HFD-fed mice (Baldassano *et al.* 2015). On the contrary, long-term exposure to GLP2 stable analogue improves the obesity-related glucose dysmetabolism in a concentration-dependent manner (Baldassano *et al.* 2016a). In fact, HFD-fed mice treated with pharmacological doses of Gly²-GLP2 for 4 weeks show a significant increase in glucose tolerance and exogenous insulin sensitivity and reduction in glucose-stimulated plasma insulin levels in comparison with pair-aged HFD-untreated animals, suggesting that the peptide is able to delay the onset of insulin resistance (Baldassano *et al.* 2016a). Indeed, a previous study has described the failure of GLP2 to modify diabetes onset in non-obese diabetic mouse, a model of type 1 diabetes (T1D) (Hadjiyanni *et al.* 2009). Therefore, due to different aetiology of T1D compared with T2D, it could be proposed that the protective role is associated exclusively with obesity conditions. An association between insulin resistance and GLP2 secretion has been found in a pilot study on obese human subjects (Geloneze *et al.* 2013). Hypothesis has been advanced that GLP2-increased secretion could be the cause of insulin resistance as GLP2 increases absorption of nutrients, especially fatty acids, a key factor for insulin resistance (Delarue & Magnan 2007) or for its glucagonotrophic action in healthy or diabetic subjects (Christensen *et al.* 2010, Lund *et al.* 2011). Glucagon is counter-regulatory to insulin action, increasing glucose output and inhibiting glucose uptake in the liver, and it has been linked to insulin resistance in obese subjects with normal or impaired glucose tolerance (Ahrén 2006, Weiss *et al.* 2011). However, this appears unlikely because bariatric surgery, particularly Roux-en-Y gastric bypass (RYGB) (Saeidi *et al.* 2013), which is the most effective therapy for obesity and T2D (Carlsson *et al.* 2012, Cummings 2012), increases blood GLP2 (by 200%) at postprandial status (le Roux *et al.* 2010). Bariatric surgery normalizes blood glucose concentrations in the majority of T2D humans independent of weight loss (Mingrone *et al.* 2012, Schauer *et al.* 2012) and can induce diabetes remission up to 6 years (Cohen *et al.* 2012). It is likely that the enhancement of nutrient flux into the distal small intestine after bariatric surgery triggers a signal leading to an antidiabetic effect that does not appear related to gastric inhibitory peptide

(GIP) or GLP1 (Breen *et al.* 2013). In fact, changes in circulating GLP1 are not consistent between models of diabetes that received duodenal-jejunal bypass (Breen *et al.* 2012). Moreover, bariatric surgery still improves glucose tolerance in GLP1 receptor-deficient HFD-fed mice (Wilson-Pérez *et al.* 2013). Therefore, GLP2 could be responsible for glycaemic improvement after bariatric surgery and can be considered as a key signal to drive intestinal reprogramming of glucose metabolism (Saeidi *et al.* 2013). In addition, animal studies have shown that GLP2 secretion from ileal tissue is decreased in diabetic conditions (Shan *et al.* 2013), and reduced numbers of L-cells have been detected in the intestinal tissue from HFD-fed mice compared with intestinal tissue from mice receiving a control diet (Kappe *et al.* 2014). Therefore, GLP2-decreased production might lead to T2D. Further studies are needed to clarify definitively whether and how GLP2 is involved in the improvement of glycaemic control after bariatric surgery.

We speculate that GLP2 may act as a protective factor against the deregulation of the glucose metabolism that occurs in obese conditions. Of note, in obese patients without T2D, GLP2 concentrations in plasma are higher (8500 pg/mL) (Valderas *et al.* 2014) than in healthy human subjects (851 ± 230 pg/mL) (Brubaker *et al.* 1997b). These GLP2 plasma high levels can be interpreted as a beneficial factor against T2D development. Also HFD-fed obese mice show GLP2 plasma levels higher than those in lean animals (Baldassano *et al.* 2013) and increased gut gene and protein expression of GLP2R (Rotondo *et al.* 2011b, Baldassano *et al.* 2013). Although nothing is known about the expression in human individuals, the positive correlation between GLP2 plasma levels and GLP2R mRNA and protein abundance indicates that *Glp2r* expression is not negatively affected by increased plasma GLP2 concentrations and could be interpreted as an adaptive signal involved in the regulation of glucose metabolism in conditions of obesity. In fact, as endogenous GLP2 causes a trophic effect on the mucosa of the small intestine with increased absorptive surface in HFD-fed obese mice (Baldassano *et al.* 2013), the hormone could aim to preserve and improve the glucose metabolic disorders induced by HFD. Although down-regulation after exposure to the receptor ligand is a common phenomenon, diverse studies report that exogenous GLP2 or inhibition of DDPIV (which doubles GLP2 levels) augments GLP2R expression in ileum and jejunum (Koopmann *et al.* 2008, Sueyoshi *et al.* 2014).

Mechanistic insight

The mechanisms underlying the beneficial effect of GLP2 on glucose metabolism remain to be established.

About glucose homeostasis in healthy mice, an elegant, recent work has pointed out that GLP2 can surely act in the CNS. In fact, GLP2 is able to modulate the excitability of POMC neurons in GLP2R- and PI3K-dependent manner (Shi *et al.* 2013). Hypothesis has been advanced that GLP2 activates POMC neurons to release α -melanocyte-stimulating hormone. This hormone activates melanocortin receptor 4-positive cholinergic neurons of vagal dorsal motor nucleus that enhance hepatic vagal output to suppress hepatic glucose production (Guan 2014). Alternatively, GLP2 may activate neurons in the nucleus of the solitary tract via afferent vagal inputs to modulate glucose metabolism. In fact, GLP2 activates vagal afferent pathways where GLP2R is expressed (Nelson *et al.* 2007). Although GLP2-modulated neural circuitries have not been fully defined, it has been speculated that GLP2R activation on vagal afferents may influence the preproglucagonergic neurons in the brainstem nucleus of solitary tract with consequent release of GLP2-activating hypothalamic neurons and vagal outputs from dorsal motor nucleus to regulate control of feeding behaviour and glucose homeostasis (Guan 2014). Anyway, according to these hypotheses, the glycaemic control by GLP2 involves fine-tuning vagal outputs. One problem is represented by the identification of the GLP2 source (endocrine L-cells or PPG neurons), responsible for

the control of glucose metabolism. Of note, to date, to our knowledge, there is no report on the passage of GLP2 across the blood–brain barrier (BBB); however, peripheral hormone could act in the hypothalamic arcuate nucleus where BBB is semipermeable.

The molecular mechanisms and the tissue targets underlying the beneficial effect of GLP2 on glucose metabolism in obese/HFD-fed mice have not been established yet (Baldassano *et al.* 2015, 2016a), but different speculations may be made (Fig. 1).

Because no changes in body weight or in the daily food intake have been observed in GLP2 (3–33)- or Gly²-GLP2-treated mice in comparison with untreated animals, an indirect effect mediated by an anti-obesity action has been ruled out (Baldassano *et al.* 2015, 2016a). It is well known that reduced adiposity could contribute to the preservation of insulin sensitivity because the adipose tissue has a considerable influence on systemic glucose homeostasis through secretion of adipocytokines (McArdle *et al.* 2013). Interestingly, *Glp2r* mRNA expression has been detected in mouse mesenteric adipose tissue (El-Jamal *et al.* 2014); however, its functional significance is still unknown. It is important to characterize whether GLP2 increases glucose incorporation in adipocytes when their response to insulin is impaired by obesity conditions. In fact, although there are conflicting reports regarding the alterations in the basal glucose uptake, most reports provide evidence that insulin-induced glucose incorporation is suppressed in the obese state (Talior *et al.* 2003, Sancho *et al.* 2006, Crowe *et al.* 2008), and as GLP2

Figure 1

Hypothetical targets and relative actions responsible for the beneficial effects of GLP2 on glucose dysmetabolism in HFD-fed obese mice. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-16-0035>.

rapidly increases intestinal hexose absorption (Cheeseman 1997, Au *et al.* 2002), it is not unlikely to hypothesize that it contributes to clearance of plasma glucose through an action on adipocytes. In addition or alternatively, GLP2 might influence and modulate the quality and quantity of secreted adipokines and the inflammatory state of adipose tissue.

Another hypothetical target for GLP2 could be represented by pancreas. Indeed, a direct action on β -cell function is improbable because the *Glp2r* mRNA transcripts have not been detected in pancreatic islets, but only in mouse whole pancreas (Bahrami *et al.* 2010) or rat and human pancreatic α -cells (de Heer *et al.* 2007). Despite exogenous GLP2 increases glucagon secretion (Meier *et al.* 2006, de Heer *et al.* 2007), elimination of GLP2R signalling in genetically obese mice leads to increased glucagon secretion and α -cell mass, which has been interpreted as due to increased proinflammatory signals (Bahrami *et al.* 2010), for example interleukin-6, in turn, involved in α -cell mass expansion (Ellingsgaard *et al.* 2008). Unluckily, glucagon levels were not measured in HFD-fed mice after GLP2 (3–33) or Gly²-GLP2 chronic treatment. Of note, GLP2 and GLP2R expression might change following HFD, as reported in gut tissue (Rotondo *et al.* 2011b, Baldassano *et al.* 2013). Moreover, recent studies on glucose-dependent insulinotropic polypeptide (GIP) and GLP1 have pointed out that both hormones are synthesized and secreted from islet α -cells under conditions of cellular stress imposed by β cytotoxic attack or increased insulin demand (Fujita *et al.* 2010, Donath & Burcekin 2013, Moffett *et al.* 2014). Increased expression of PC1/3 relative to PC2 in islet α -cells directs proglucagon processing away from glucagon towards GLP1 in these conditions (Wideman *et al.* 2007, Marchetti *et al.* 2012). As GLP1 and GLP2 are produced by the same convertase in equimolar amount (Janssen *et al.* 2013), it is likely to hypothesize that GLP2 pancreatic expression also changes in stress conditions, such as derangements of islet cell function associated with prolonged consumption of HFD. Therefore, before reaching any conclusions about a link between GLP2 and pancreas function, it is necessary to verify whether GLP2 and GLP2R expressions in pancreatic islets also change consistently with their beneficial role in glucose metabolism.

It is also possible that GLP2 acts directly on the liver to modulate hepatic function related to glucose or lipid metabolism. Mouse liver expresses GLP2R (El-Jamal *et al.* 2014); however, nothing is known about other species, including humans. Moreover, fatty liver is strongly associated with insulin resistance (Asrih &

Jornayvaz 2013), and non-alcoholic hepatic steatosis has been reported in HFD-fed mice (de Meijer *et al.* 2010, Fraulob *et al.* 2010). Indeed, in HFD-fed mice, chronic treatment with GLP2 (3–33) aggravates dyslipidaemia and hepatic lipid accumulation. It increases plasma triglyceride, cholesterol, ALT and AST levels, intrahepatic lipid concentration, reduces HDL and exacerbates the liver steatosis, suggesting that endogenous GLP2 may exert defensive role against lipid imbalance in obesity condition (Baldassano *et al.* 2016b). However, chronic administration of Gly²-GLP2 for 4 weeks is not associated with remarkable improvements in dyslipidaemia-related circulating parameters, and it does not prevent liver fat accumulation and the presence of microvesicular steatosis, suggesting that the level of insulin sensitivity in peptide-treated mice is not related to an apparently less severe tissue fat infiltration. Therefore, the beneficial effects of GLP2 chronic treatment on insulin sensitivity do not seem to be a consequence of an improvement in lipid metabolism (Baldassano *et al.* 2016a).

Another potential account for explaining endogenous or exogenous GLP2-protective effects against insulin resistance is related to GLP2 ability to reduce gut permeability and consequently the leakage of bacterial endotoxins into the portal blood circulation (Benjamin *et al.* 2000). Endotoxemia and low-grade inflammation have been associated with insulin resistance (Hotamisligil 2006, Cani *et al.* 2007), and activation of inflammatory pathways has emerged as an imperative link between T2D and obesity (Hameed *et al.* 2015). It is well accepted that GLP2 is involved in modulation of intestinal permeability (Moran *et al.* 2012). In diabetic rats, plasma lipopolysaccharide, zonulin 1 expression, insulin level and insulin-resistant index are closely related to GLP2 levels (Shan *et al.* 2013). On the basis of these observations, the authors advanced the very suggestive hypothesis that impaired GLP2 in prediabetic subjects may predispose these patients to T2D by increasing intestinal permeability and endotoxemia-related inflammation. However, prospective studies in humans are needed. They found that glutamine-induced GLP2 secretion is decreased in rats with streptozotocin-induced experimental diabetes (Shan *et al.* 2013). Impaired GLP1 secretion is a characteristic of the prediabetic situation such as impaired glucose tolerance and gestational diabetic mellitus (Lim & Brubaker 2006) and likely also GLP2 secretion from the same L-cells is impaired. However, the fasting plasma GLP2 concentrations reported in humans with glucose dysmetabolism (Gjesing *et al.* 2011) or patients with T2D (Aaboe *et al.* 2010) are lesser than those in healthy human subjects (Brubaker

et al. 1997b). Also obese mice exhibit an altered gut barrier (Brun *et al.* 2007), and increased endogenous production of GLP2 induced by prebiotic diet improves gut barrier function (Cani *et al.* 2009). In fact, the block of the GLP2R exacerbates inflammation, whereas therapy with GLP2 reduces systemic and hepatic inflammation in ob/ob mice (Cani *et al.* 2009). Therefore, it is conceivable that GLP2 by enhancing epithelial barrier function and limiting the activation of processes that drive inflammation (Moore *et al.* 2010) can delay and reduce the development of insulin resistance. However, this hypothesis needs to be verified.

Conclusion and perspective

New evidence underlines the importance of GLP2 in promoting the control and insulin sensitivity in animal model, particularly in conditions associated with obesity. Results from human studies so far remain inconsistent; therefore, future research should be addressed to clarify the role of GLP2 in preventing human insulin resistance and, in turn, the development of T2D. The potential mechanisms driving the beneficial effects of GLP2 receptor activation may be multiple and not fully elucidated. Greater attention should also be given to investigate on this hormone and its receptor as targets for treatment of obesity-associated diabetes in consideration of the chemical strategies for the design of multifunctional peptides.

Declaration of interest

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

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References

- Aaboe K, Knop FK, Vilsbøll T, Deacon CF, Holst JJ, Madsbad S & Krarup T 2010 Twelve weeks treatment with the DPP-4 inhibitor, sitagliptin, prevents degradation of peptide YY and improves glucose and non-glucose induced insulin secretion in patients with type 2 diabetes mellitus. *Diabetes Obesity and Metabolism* **12** 323–333. (doi:10.1111/j.1463-1326.2009.01167.x)
- Ahrén B 2006 Glucagon secretion in relation to insulin sensitivity in healthy subjects. *Diabetologia* **49** 117–122. (doi:10.1007/s00125-005-0056-8)
- Ahrén B, Simonsson E, Scheurink AJ, Mulder H, Myrén U & Sundler F 1997 Dissociated insulinotropic sensitivity to glucose and carbachol in high-fat diet-induced insulin resistance in C57BL/6j mice. *Metabolism* **46** 97–106. (doi:10.1016/S0026-0495(97)90175-X)
- Amato A, Baldassano S, Serio R & Mulè F 2009 Glucagon-like peptide-2 relaxes mouse stomach through vasoactive intestinal peptide release. *American Journal of Physiology: Gastrointestinal and Liver Physiology* **296** G678–G684. (doi:10.1152/ajpgi.90587.2008)
- Amato A, Rotondo A, Cinci L, Baldassano S, Vannucchi MG & Mulè F 2010 Role of cholinergic neurons in the motor effects of glucagon-like peptide-2 in mouse colon. *American Journal of Physiology: Gastrointestinal and Liver Physiology* **299** G1038–G1044. (doi:10.1152/ajpgi.00282.2010)
- Angelone T, Filice E, Quintieri AM, Imbrogno S, Amodio N, Pasqua T, Pellegrino D, Mulè F & Cerra MC 2012 Receptor identification and physiological characterization of glucagon-like peptide-2 in the rat heart. *Nutrition, Metabolism, and Cardiovascular Diseases* **22** 486–494. (doi:10.1016/j.numecd.2010.07.014)
- Asrih M & Jornayvaz FR 2013 Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance. *Journal of Endocrinology* **218** R25–R36. (doi:10.1530/JOE-13-0201)
- Au A, Gupta A, Schembri P & Cheeseman CI 2002 Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. *Biochemical Journal* **367** 247–254. (doi:10.1042/bj20020393)
- Bahrani J, Longuet C, Baggio LL, Li K & Drucker DJ 2010 The glucagon-like peptide-2 receptor modulates islet adaptation to metabolic stress in the ob/ob mouse. *Gastroenterology* **139** 857–868. (doi:10.1053/j.gastro.2010.05.006)
- Baldassano S & Amato A 2014 GLP-2: what do we know? What are we going to discover? *Regulatory Peptides* **194–195** 6–10. (doi:10.1016/j.regpep.2014.09.002)
- Baldassano S, Liu S, Qu MH, Mulè F & Wood JD 2009 Glucagon-like peptide-2 modulates neurally evoked mucosal chloride secretion in guinea pig small intestine in vitro. *American Journal of Physiology: Gastrointestinal and Liver Physiology* **297** G800–G805. (doi:10.1152/ajpgi.00170.2009)
- Baldassano S, Bellanca AL, Serio R & Mulè F 2012 Food intake in lean and obese mice after peripheral administration of glucagon-like peptide 2. *Journal of Endocrinology* **213** 277–284. (doi:10.1530/JOE-12-0092)
- Baldassano S, Amato A, Cappello F, Rappa F & Mulè F 2013 Glucagon-like peptide-2 and mouse intestinal adaptation to a high-fat diet. *Journal of Endocrinology* **217** 11–20. (doi:10.1530/JOE-12-0500)
- Baldassano S, Rappa F, Amato A, Cappello F & Mulè F 2015 GLP-2 as beneficial factor in the glucose homeostasis in mice fed a high fat diet. *Journal of Cellular Physiology* **230** 3029–3036. (doi:10.1002/jcp.25039)
- Baldassano S, Amato A, Caldara GF & Mulè F 2016a Glucagon-like peptide-2 treatment improves glucose dysmetabolism in mice fed a high fat diet. *Endocrine* [in press]. (doi:10.1007/s12020-016-0871-3)
- Baldassano S, Amato A, Rappa F, Cappello F & Mulè F 2016b Influence of endogenous glucagon like peptide-2 on lipid disorders in mice fed a high fat diet. *Endocrine Research* [in press]. (doi:10.3109/07435800.2016.1141950)
- Benjamin MA, McKay DM, Yang PC, Cameron H & Perdue MH 2000 Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. *Gut* **47** 112–119. (doi:10.1136/gut.47.1.112)
- Bjerknes M & Cheng H 2001 Modulation of specific intestinal epithelial progenitors by enteric neurons. *PNAS* **98** 12497–12502. (doi:10.1073/pnas.211278098)
- Breen DM, Rasmussen BA, Kokorovic A, Wang R, Cheung GW & Lam TK 2012 Jejunal nutrient sensing is required for duodenal-jejunal bypass surgery to rapidly lower glucose concentrations in uncontrolled diabetes. *Nature Medicine* **18** 950–955. (doi:10.1038/nm.2745)
- Breen DM, Rasmussen BA, Côté CD, Jackson VM & Lam TK 2013 Nutrient-sensing mechanisms in the gut as therapeutic targets for diabetes. *Diabetes* **62** 3005–3013. (doi:10.2337/db13-0523)

- Bremholm L, Hornum M, Henriksen BM, Larsen S & Holst JJ 2009 Glucagon-like peptide-2 increases mesenteric blood flow in humans. *Scandinavian Journal of Gastroenterology* **44** 314–319. (doi:10.1080/00365520802538195)
- Brubaker PL 2006 The glucagon-like peptides: pleiotropic regulators of nutrient homeostasis. *Annals of the New York Academy of Sciences* **1070** 10–26. (doi:10.1196/annals.1317.006)
- Brubaker PL, Izzo A, Hill M & Drucker DJ 1997a Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. *American Journal of Physiology* **272** E1050–E1058.
- Brubaker PL, Crivici A, Ehrlich P, Tsai CH & Drucker DJ 1997b Circulating and tissue forms of the intestinal growth factor, glucagon-like peptide-2. *Endocrinology* **138** 4837–4843.
- Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G & Martinez D 2007 Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *American Journal of Physiology: Gastrointestinal and Liver Physiology* **292** G518–G525. (doi:10.1152/ajpgi.00024.2006)
- Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, et al. 2007 Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56** 1761–1772. (doi:10.2337/db06-1491)
- Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, et al. 2009 Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58** 1091–1103. (doi:10.1136/gut.2008.165886)
- Carlsson LM, Peltonen M, Ahlin S, Anveden A, Bouchard C, Carlsson B, Jacobson P, Lonroth H, Maglio C, Naslund I, et al. 2012 Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. *New England Journal of Medicine* **367** 695–704. (doi:10.1056/NEJMoa1112082)
- Cheeseman CI 1997 Upregulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo. *American Journal of Physiology* **273** R1965–R1971.
- Christensen M, Knop FK, Vilsbøll T, Aaboe K, Holst JJ, Madsbad S & Krarup T 2010 Glucagon-like peptide-2, but not glucose-dependent insulinotropic polypeptide, stimulates glucagon release in patients with type 1 diabetes. *Regulatory Peptides* **163** 96–101. (doi:10.1016/j.regpep.2010.05.004)
- Cinci L, Faussone-Pellegrini MS, Rotondo A, Mulè F & Vannucchi MG 2011 GLP-2 receptor expression in excitatory and inhibitory enteric neurons and its role in mouse duodenum contractility. *Neurogastroenterology and Motility* **23** e383–e392. (doi:10.1111/j.1365-2982.2011.01750.x)
- Cohen RV, Pinheiro JC, Schiavon CA, Salles JE, Wajchenberg BL & Cummings DE 2012 Effects of gastric bypass surgery in patients with type 2 diabetes and only mild obesity. *Diabetes Care* **35** 1420–1428. (doi:10.2337/dc11-2289)
- Crowe S, Turpin SM, Ke F, Kemp BE & Watt MJ 2008 Metabolic remodeling in adipocytes promotes ciliary neurotrophic factor-mediated fat loss in obesity. *Endocrinology* **149** 2546–2556. (doi:10.1210/en.2007-1447)
- Cummings DE 2012 Metabolic surgery for type 2 diabetes. *Nature Medicine* **18** 656–658. (doi:10.1038/nm.2773)
- Dash S, Xiao C, Morgantini C, Connelly PW, Patterson WB & Lewis GF 2014 Glucagon-like peptide 2 regulates release of chylomicron from the intestine. *Gastroenterology* **2** 1–10. (doi:10.1053/j.gastro.2014.08.037)
- de Heer J, Pedersen J, Ørskov C & Holst JJ 2007 The alpha cell expresses glucagon-like peptide-2 receptors and glucagon-like peptide-2 stimulates glucagon secretion from the rat pancreas. *Diabetologia* **50** 2135–2142. (doi:10.1007/s00125-007-0761-6)
- de Meijer VE, Le HD, Meisel JA, Akhavan Sharif MR, Pan A, Nosé V & Puder M 2010 Dietary fat intake promotes the development of hepatic steatosis independently from excess caloric consumption in a murine model. *Metabolism* **59** 1092–1105. (doi:10.1016/j.metabol.2009.11.006)
- Delarue J & Magnan C 2007 Free fatty acids and insulin resistance. *Current Opinion in Clinical Nutrition and Metabolic Care* **10** 142–148. (doi:10.1097/MCO.0b013e328042ba90)
- Donath MY & Burcelin R 2013 GLP-1 effects on islets: hormonal, neuronal, or paracrine? *Diabetes Care* **36** S145–S148. (doi:10.2337/dcS13-2015)
- Drucker DJ & Nauck MA 2006 The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* **368** 1696–1705. (doi:10.1016/S0140-6736(06)69705-5)
- Drucker DJ & Yusta B 2014 Physiology and pharmacology of the enteroendocrine hormone glucagon-like peptide-2. *Annual Review of Physiology* **76** 561–583. (doi:10.1146/annurev-physiol-021113-170317)
- Drucker DJ, Erlich P, Asa SL & Brubaker PL 1996 Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *PNAS* **93** 7911–7916. (doi:10.1073/pnas.93.15.7911)
- El-Jamal N, Erdual E, Neunlist M, Koriche D, Dubuquoy C, Maggiotto F, Chevalier J, Berrebi D, Dubuquoy L, Boulanger E, et al. 2014 Glucagon-like peptide-2: broad receptor expression, limited therapeutic effect on intestinal inflammation and novel role in liver regeneration. *American Journal of Physiology: Gastrointestinal and Liver Physiology* **307** G274–G285. (doi:10.1152/ajpgi.00389.2012)
- Ellingsgaard H, Ehres JA, Hammar EB, Van Lommel L, Quintens R, Martens G, Kerr-Conte J, Pattou F, Berney T, Pipeleers D, et al. 2008 Interleukin-6 regulates pancreatic alpha-cell mass expansion. *PNAS* **105** 13163–13168. (doi:10.1073/pnas.0801059105)
- Fraulob JC, Ogg-Diamantino R, Fernandes-Santos C, Aguila MB & Mandarim-de-Lacerda CA 2010 A mouse model of metabolic syndrome: insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFPD) in C57BL/6 mice fed a high fat diet. *Journal of Clinical Biochemistry and Nutrition* **46** 212–223. (doi:10.3164/jcbn.09-83)
- Fujita Y, Wideman RD, Asadi A, Yang GK, Baker R, Webber T, Zhang T, Wang R, Ao Z, Warnock GL, et al. 2010 Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion. *Gastroenterology* **138** 1966–1975. (doi:10.1053/j.gastro.2010.01.049)
- Geloneze B, Lima MM, Pareja JC, Barreto MR & Magro DO 2013 Association of insulin resistance and GLP-2 secretion in obesity: a pilot study. *Arquivos Brasileiros de Endocrinologia & Metabologia* **57** 632–635. (doi:10.1590/S0004-27302013000800008)
- Gjesing AP, Vestmar MA, Jørgensen T, Henri M, Holst JJ, Witte DR, Hansen T & Pedersen O 2011 The effect of PCSK1 variants on waist, waist-hip ratio and glucose metabolism is modified by sex and glucose tolerance status. *PLoS ONE* **6** e23907. (doi:10.1371/journal.pone.0023907)
- Guan X 2014 The CNS glucagon-like peptide-2 receptor in the control of energy balance and glucose homeostasis. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **307** R585–R596. (doi:10.1152/ajpregu.00096.2014)
- Guan X, Karpén HE, Stephens J, Bukowski JT, Niu S, Zhang G, Stoll B, Finegold M, Holst JJ, Hadsell DL, et al. 2006 GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive peptides and mediates increased blood flow. *Gastroenterology* **130** 150–164. (doi:10.1053/j.gastro.2005.11.005)
- Guan X, Shi X, Li X, Chang B, Wang Y, Li DP & Chan L 2012 GLP-2 receptor in POMC neurons suppresses feeding behavior and gastric motility. *American Journal of Physiology: Endocrinology and Metabolism* **303** E853–E864. (doi:10.1152/ajpendo.00245.2012)
- Hadjiyanni I, Li KK & Drucker DJ 2009 Glucagon-like peptide-2 reduces intestinal permeability but does not modify the onset of type 1 diabetes in the nonobese diabetic mouse. *Endocrinology* **150** 592–599. (doi:10.1210/en.2008-1228)

- Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K & Ganai BA 2015 Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. *World Journal of Diabetes* **6** 598–612. (doi:10.4239/wjd.v6.i4.598)
- Hartmann B, Harr MB, Jeppesen PB, Wojdemann M, Deacon CF, Mortensen PB & Holst JJ 2000 In vivo and in vitro degradation of glucagon like peptide-2 in humans. *Journal of Clinical Endocrinology & Metabolism* **85** 2884–2888. (doi:10.1210/jcem.85.8.6717)
- Hein GJ, Baker C, Hsieh J, Farr S & Adeli K 2013 GLP-1 and GLP-2 as yin and yang of intestinal lipoprotein production: evidence for predominance of GLP-2-stimulated postprandial lipemia in normal and insulin-resistant states. *Diabetes* **62** 373–381. (doi:10.2337/db12-0202)
- Hotamisligil GS 2006 Inflammation and metabolic disorders. *Nature* **444** 860–867. (doi:10.1038/nature05485)
- Hsieh J, Longuet C, Maida A, Bahrami J, Xu E, Baker CL, Brubaker PL, Drucker DJ & Adeli K 2009 Glucagon-like peptide-2 increases intestinal lipid absorption and chylomicron production via CD36. *Gastroenterology* **137** 997–1005. (doi:10.1053/j.gastro.2009.05.051)
- Iakoubov R, Lauffer LM, Trivedi S, Kim YI & Brubaker PL 2009 Carcinogenic effects of exogenous and endogenous glucagon-like peptide-2 in azoxymethane-treated mice. *Endocrinology* **150** 4033–4043. (doi:10.1210/en.2009-0295)
- Janssen P, Vanden Berghe P, Verschueren S, Lehmann A, Depoortere I & Tack J 2011 The role of gastric motility in the control of food intake. *Alimentary Pharmacology & Therapeutics* **33** 880–894. (doi:10.1111/j.1365-2036.2011.04609.x)
- Janssen P, Rotondo A, Mulè F & Tack J 2013 Review article: a comparison of glucagon-like peptides 1 and 2. *Alimentary Pharmacology & Therapeutics* **37** 18–36. (doi:10.1111/apt.12092)
- Kappe C, Zhang Q, Nyström T & Sjöholm A 2014 Effects of high-fat diet and the anti-diabetic drug metformin on circulating GLP-1 and the relative number of intestinal L-cells. *Diabetologia & Metabolic Syndrome* **6** 70. (doi:10.1186/1758-5996-6-70)
- Knop FK, Vilsbøll T, Madsbad S, Holst JJ & Krarup T 2007 Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. *Diabetologia* **50** 797–805. (doi:10.1007/s00125-006-0566-z)
- Koopmann MC, Nelson DW, Murali SG, Liu X, Brownfield MS, Holst JJ & Ney DM 2008 Exogenous glucagon-like peptide-2 (GLP-2) augments GLP-2 receptor mRNA and maintains proglucagon mRNA levels in resected rats. *Journal of Parenteral and Enteral Nutrition* **32** 254–265. (doi:10.1177/0148607108316198)
- le Roux CW, Borg C, Wallis K, Vincent RP, Bueter M, Goodlad R, Ghatei MA, Patel A, Bloom SR & Aylwin SJ 2010 Gut hypertrophy after gastric bypass is associated with increased glucagon-like peptide 2 and intestinal crypt cell proliferation. *Annals of Surgery* **252** 50–56. (doi:10.1097/SLA.0b013e3181d3d21f)
- Lee SK, Opara EC, Surwit RS, Feinglos MN & Akwari OE 1995 Defective glucose-stimulated insulin release from perfused islets of C57BL/6J mice. *Pancreas* **11** 206–211. (doi:10.1097/00006676-199508000-00016)
- Lim GE & Brubaker PL 2006 Glucagon-like peptide 1 secretion by the L cell. *Diabetes* **55** S70–S77.
- Lovshin J, Estall J, Yusta B, Brown TJ & Drucker J 2001 Glucagon-like peptide (GLP)-2 action in the murine central nervous system is enhanced by elimination of GLP-1 receptor signaling. *Journal of Biological Chemistry* **276** 21489–21499. (doi:10.1074/jbc.M009382200)
- Lovshin JA, Huang Q, Seaberg R, Brubaker PL & Drucker DJ 2004 Extrahypothalamic expression of the glucagon-like peptide-2 receptor is coupled to reduction of glutamate-induced cell death in cultured hippocampal cells. *Endocrinology* **145** 3495–3506. (doi:10.1210/en.2004-0100)
- Lund A, Vilsbøll T, Bagger JJ, Holst JJ & Knop FK 2011 The separate and combined impact of the intestinal secretion in type 2 diabetes hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes. *American Journal of Physiology: Endocrinology and Metabolism* **300** E1038–E1046. (doi:10.1152/ajpendo.00665.2010)
- Marchetti P, Lupi R, Bugliani M, Kirkpatrick CL, Sebastiani G, Grieco FA, Del Guerra S, D'Aleo V, Piro S, Marselli L, et al. 2012 A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. *Diabetologia* **55** 3262–3272. (doi:10.1007/s00125-012-2716-9)
- McArdle MA, Finucane OM, Connaughton RM, McMorris AM & Roche HM 2013 Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. *Frontiers in Endocrinology* **4** 52. (doi:10.3389/fendo.2013.00052)
- McDonagh SC, Lee J, Izzo A & Brubaker PL 2007 Role of glial cell-line derived neurotrophic factor family receptor $\alpha 2$ in the actions of the glucagon-like peptides on the murine intestine. *American Journal of Physiology: Gastrointestinal and Liver Physiology* **293** G461–G468. (doi:10.1152/ajpgi.00424.2006)
- Meier JJ, Nauck MA, Pott A, Heinze K, Goetze O, Bulut K, Schmidt WE, Gallwitz B & Holst JJ 2006 Glucagon-like peptide 2 stimulates glucagon secretion, enhances lipid absorption, and inhibits gastric acid secretion in humans. *Gastroenterology* **130** 44–54. (doi:10.1053/j.gastro.2005.10.004)
- Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaiconelli A, Leccesi L, Nanni G, Pomp A, Castagneto M, Ghirlanda G, et al. 2012 Bariatric surgery versus conventional medical therapy for type 2 diabetes. *New England Journal of Medicine* **366** 1577–1585. (doi:10.1056/NEJMoa1200111)
- Moffett RC, Vasu S, Thorens B, Drucker DJ & Flatt PR 2014 Incretin receptor null mice reveal key role of GLP-1 but not GIP in pancreatic beta cell adaptation to pregnancy. *PLoS ONE* **9** e96863. (doi:10.1371/journal.pone.0096863)
- Moore BA, Pepper N, Pirone A, Bassiri A, Sague S, Palmer JM, Johnson DL, Nesspor T, Kliwinski C & Hornby PJ 2010 GLP-2 receptor agonism ameliorates inflammation and gastrointestinal stasis in murine postoperative ileus. *Journal of Pharmacology and Experimental Therapeutics* **333** 574–583. (doi:10.1124/jpet.109.161497)
- Moran GW, O'Neill C & McLaughlin JT 2012 GLP-2 enhances barrier formation and attenuates TNF α -induced changes in a Caco-2 cell model of the intestinal barrier. *Regulatory Peptides* **178** 95–101. (doi:10.1016/j.regpep.2012.07.002)
- Nelson DW, Sharp JW, Brownfield MS, Raybould HE & Ney DM 2007 Localization and activation of GLP-2 receptors on vagal afferents in the rat. *Endocrinology* **148** 1954–1962. (doi:10.1210/en.2006-1232)
- Nelson DW, Murali SG, Liu X, Koopmann MC, Holst JJ & Ney DM 2008 Insulin-like growth factor I and glucagon-like peptide-2 responses to fasting followed by controlled or ad libitum refeeding in rats. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **294** R1175–R1184. (doi:10.1152/ajpregu.00238.2007)
- Nuzzo D, Picone P, Baldassano S, Caruana L, Messina E, Marino Gammazza A, Cappello F, Mulè F & Di Carlo M 2015 Insulin resistance as common molecular denominator linking obesity to Alzheimer's disease. *Current Alzheimer Research* **12** 723–735. (doi:10.2174/1567205012666150710115506)
- Ørskov C, Holst JJ & Nielsen OV 1988 Effect of truncated glucagon-like peptide-1 [proglucagon-(78–107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* **123** 2009–2013. (doi:10.1210/endo-123-4-2009)
- Ørskov C, Hartmann B, Poulsen SS, Thulesen J, Hare KJ & Holst JJ 2005 GLP-2 stimulates colonic growth via KGF, released by subepithelial myofibroblasts with GLP-2 receptors. *Regulatory Peptides* **124** 105–112. (doi:10.1016/j.regpep.2004.07.009)
- Petersen YM, Elnif J, Schmidt M & Sangild PT 2002 Glucagon-like peptide 2 enhances maltase-glucoamylase and sucrase-isomaltase gene expression and activity in parenterally fed premature neonatal piglets. *Pediatric Research* **52** 498–503. (doi:10.1203/00006450-200210000-00007)

- Ramsanahie AP, Berger UV, Zinner MJ, Whang EE, Rhoads DB & Ashley SW 2004 Effect of glucagon-like peptide-2 (GLP-2) on diurnal SGLT1 expression. *Digestive Diseases and Sciences* **49** 1731–1737. (doi:10.1007/s10620-004-9561-8)
- Rotondo A, Amato A, Lentini L, Baldassano S & Mulè F 2011a Glucagon-like peptide-1 relaxes gastric antrum through nitric oxide in mice. *Peptides* **32** 60–64. (doi:10.1016/j.peptides.2010.09.028)
- Rotondo A, Amato A, Baldassano S, Lentini L & Mulè F 2011b Gastric relaxation induced by glucagon-like peptide-2 in mice fed a high-fat diet or fasted. *Peptides* **32** 1587–1592. (doi:10.1016/j.peptides.2011.06.031)
- Saeidi N, Meoli L, Nestoridi E, Gupta NK, Kvas S, Kucharczyk J, Bonab AA, Fischman AJ, Yarmush ML & Stylopoulos N 2013 Reprogramming of intestinal glucose metabolism and glycemic control in rats after gastric bypass. *Science* **341** 406–410. (doi:10.1126/science.1235103)
- Sancho V, Trigo MV, Martin-Duce A, Gonz Lez N, Acitores A, Arnés L, Valverde I, Malaisse WJ & Villanueva-Peñacarrillo ML 2006 Effect of GLP-1 on D-glucose transport, lipolysis and lipogenesis in adipocytes of obese subjects. *International Journal of Molecular Medicine* **17** 1133–1137. (doi:10.3892/ijmm.17.6.1133)
- Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, Thomas S, Abood B, Nissen SE & Bhatt DL 2012 Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *New England Journal of Medicine* **366** 1567–1576. (doi:10.1056/NEJMoa1200225)
- Schmidt WE, Siegel EG & Creutzfeldt W 1985 Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. *Diabetologia* **28** 704–707. (doi:10.1007/BF00291980)
- Schmidt PT, Hartmann B, Bregenholt S, Hoist JJ & Claesson MH 2000 Deficiency of the intestinal growth factor, glucagon-like peptide 2, in the colon of SCID mice with inflammatory bowel disease induced by transplantation of CD4+ T cells. *Scandinavian Journal of Gastroenterology* **35** 522–527.
- Shan CY, Yang JH, Kong Y, Wang XY, Zheng MY, Xu YG, Wang Y, Ren HZ, Chang BC & Chen LM 2013 Alteration of the intestinal barrier and GLP2 secretion in Berberine-treated type 2 diabetic rats. *Journal of Endocrinology* **218** 255–262. (doi:10.1530/JOE-13-0184)
- Shi X, Zhou F, Li X, Chang B, Li D, Wang Y, Tong Q, Xu Y, Fukuda M, Zhao JJ, et al. 2013 Central GLP-2 enhances hepatic insulin sensitivity via activating PI3K signaling in POMC neurons. *Cell Metabolism* **18** 86–98. (doi:10.1016/j.cmet.2013.06.014)
- Shin ED, Estall JL, Izzo A, Drucker DJ & Brubaker PL 2005 Mucosal adaptation to enteral nutrients is dependent on the physiologic actions of glucagon-like peptide-2 in mice. *Gastroenterology* **128** 1340–1353. (doi:10.1053/j.gastro.2005.02.033)
- Sørensen LB, Flint A, Raben A, Hartmann B, Holst JJ & Astrup A 2003 No effect of physiological concentrations of glucagon-like peptide-2 on appetite and energy intake in normal weight subjects. *International Journal of Obesity and Related Metabolic Disorders* **27** 450–456. (doi:10.1038/sj.ijo.0802247)
- Stephens J, Stoll B, Cottrell J, Chang X, Helmrath M & Burrin DG 2006 Glucagon-like peptide-2 acutely increases proximal small intestinal blood flow in TPN-fed neonatal piglets. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **290** R283–R289. (doi:10.1152/ajpgi.00275.2005)
- Sueyoshi R, Woods Ignatoski KM, Okawada M, Hartmann B, Holst J & Teitelbaum DH 2014 Stimulation of intestinal growth and function with DPP4 inhibition in a mouse short bowel syndrome model. *American Journal of Physiology: Gastrointestinal and Liver Physiology* **307** G410–G419. (doi:10.1152/ajpgi.00363.2013)
- Surwit RS, Kuhn CM, Cochrane C, McCubbin JA & Feinglos MN 1988 Diet-induced type II diabetes in C57BL/6j mice. *Diabetes* **37** 1163–1167. (doi:10.2337/diab.37.9.1163)
- Talior I, Yarkoni M, Bashan N & Eldar-Finkelman H 2003 Increased glucose uptake promotes oxidative stress and PKC- δ activation in adipocytes of obese, insulin-resistant mice. *American Journal of Physiology: Endocrinology and Metabolism* **285** E295–E302. (doi:10.1152/ajpendo.00044.2003)
- Tang-Christensen M, Larsen PJ, Thulesen J, Romer J & Vrang N 2000 The proglucagon-derived peptide, glucagon-like peptide-2, is a neurotransmitter involved in the regulation of food intake. *Nature Medicine* **6** 802–807. (doi:10.1038/77535)
- Tavares W, Drucker DJ & Brubaker PL 2000 Enzymatic- and renal-dependent catabolism of the intestinotropic hormone glucagon-like peptide-2 in rats. *American Journal of Physiology: Endocrinology and Metabolism* **278** E134–E139.
- Thulesen J, Hartmann B, Ørskov C, Jeppesen PB, Holst JJ & Poulsen SS 2000 Potential targets for glucagon-like peptide 2 (GLP-2) in the rat: distribution and binding of i.v. injected 125 I-GLP-2. *Peptides* **21** 1511–1517. (doi:10.1016/S0196-9781(00)00305-3)
- Thulesen J, Knudsen LB, Hartmann B, Hastrup S, Kissow H, Jeppesen PB, Ørskov C, Holst JJ & Poulsen SS 2002 The truncated metabolite GLP-2 (3-33) interacts with the GLP-2 receptor as a partial agonist. *Regulatory Peptides* **103** 9–15. (doi:10.1016/S0167-0115(01)00316-0)
- Tsai CH, Hill M, Asa SL, Brubaker PL & Drucker DJ 1997 Intestinal growth-promoting properties of glucagon-like peptide-2 in mice. *American Journal of Physiology: Endocrinology and Metabolism* **273** E77–E84.
- Ugleholdt R, Zhu X, Deacon CF, Ørskov C, Steiner DF & Holst JJ 2004 Impaired intestinal proglucagon processing in mice lack in prohormone convertase 1. *Endocrinology* **145** 1349–1355. (doi:10.1210/en.2003-0801)
- Valderas JP, Padilla O, Solari S, Escalona M & González G 2014 Feeding and bone turnover in gastric bypass. *Journal of Clinical Endocrinology and Metabolism* **99** 491–497. (doi:10.1210/jc.2013-1308)
- Vrang N, Hansen M, Larsen PJ & Tang-Christensen M 2007 Characterization of brainstem preproglucagon projections to the paraventricular and dorsomedial hypothalamic nuclei. *Brain Research* **1149** 118–126. (doi:10.1016/j.brainres.2007.02.043)
- Weiss R, D'Adamo E, Santoro N, Hershkop K & Caprio S 2011 Basal alpha-cell up-regulation in obese insulin-resistant adolescents. *Journal of Clinical Endocrinology and Metabolism* **96** 91–97. (doi:10.1210/jc.2010-1275)
- Wideman RD, Covey SD, Webb GC, Drucker DJ & Kieffer TJ 2007 A switch from prohormone convertase (PC)-2 to PC1/3 expression in transplanted alpha-cells is accompanied by differential processing of proglucagon and improved glucose homeostasis in mice. *Diabetes* **56** 2744–2752. (doi:10.2337/db07-0563)
- Wilson-Pérez HE, Chambers AP, Ryan KK, Li B, Sandoval DA, Stoffers D, Drucker DJ, Pérez-Tilve D & Seeley RJ 2013 Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like peptide-1 receptor deficiency. *Diabetes* **62** 2380–2385. (doi:10.2337/db12-1498)
- Wøjdemann M, Wettergren A, Hartmann B & Holst JJ 1998 Glucagon-like peptide-2 inhibits centrally induced antral motility in pigs. *Scandinavian Journal of Gastroenterology* **33** 828–832.
- Yusta B, Huang L, Munroe D, Wolff G, Fantaski R, Sharma S, Demchyshyn L, Asa SL & Drucker DJ 2000 Enterendocrine localization of GLP-2 receptor expression in humans and rodents. *Gastroenterology* **119** 744–755. (doi:10.1053/gast.2000.16489)

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