Targeting the gastrointestinal tract to treat type 2 diabetes

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Abstract
The rising global rates of type 2 diabetes and obesity present a significant economic and social burden, underscoring the importance for effective and safe therapeutic options. The success of glucagon-like-peptide-1 receptor agonists in the treatment of type 2 diabetes, along with the potent glucose-lowering effects of bariatric surgery, highlight the gastrointestinal tract as a potential target for diabetes treatment. Furthermore, recent evidence suggests that the gut plays a prominent role in the ability of metformin to lower glucose levels. As such, the current review highlights some of the current and potential pathways in the gut that could be targeted to improve glucose homeostasis, such as changes in nutrient sensing, gut peptides, gut microbiota and bile acids. A better understanding of these pathways will lay the groundwork for novel gut-targeted antidiabetic therapies, some of which have already shown initial promise.

Introduction
The incidence of type 2 diabetes has more than doubled since 1980, with over 382 million affected individuals worldwide, in conjunction with an increase in obesity rates and the spread of a western lifestyle (Scully 2012). Given that type 2 diabetes has many comorbidities, such as hypertension, dyslipidaemia and cardiovascular disease, which contribute to the ever-rising economic burden, it is of utmost importance to develop successful therapeutic options. Chronic hyperglycaemia is a hallmark characteristic of type 2 diabetes and is, therefore, a main target for diabetes treatment. As such, metformin remains the most prescribed drug for type 2 diabetes due to its potent antihyperglycaemic effect, largely from a reduction in hepatic glucose production (Rojas & Gomes 2013). Although its mechanism of action still remains largely debated, recent evidence suggests a major role of the gastrointestinal tract in mediating metformin’s glucose-lowering effect (Duca et al. 2015, Buse et al. 2016). Interestingly, this is not the only evidence for a therapeutic role of the gut in diabetes treatment. Over the past decade, incretin-based therapies including glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-IV (DPP-IV) inhibitors have demonstrated powerful glucose-lowering efficacy, and are now commonly prescribed, usually in conjunction with metformin (Deacon & Lebovitz 2016, Madsbad 2016). Furthermore, despite being prescribed for the treatment of morbid obesity, metabolic/bariatric surgery results in rapid and sustained remission of diabetes, and is potentially more effective than conventional therapy (Mingrone et al. 2012, Mingrone et al. 2015).

The success of these treatments has expanded the classical view of the gastrointestinal (GI) tract from a ‘digestion and absorption’ organ to a major contributor to metabolic homeostasis. The GI tract exhibits crucial negative feedback signals, of both
hormonal and neural origin, in response to incoming nutrients, preventing nutrient excess by suppressing food intake and endogenous nutrient production (Cote et al. 2014). The current review aims to highlight the current and potential therapeutic role of the GI tract in treating type 2 diabetes.

**Gut peptide signalling in regulating glucose metabolism**

Ingestion of nutrients leads to complex and integrative negative feedback mechanisms, which originate from the gut and contribute to the control of food intake, glucose metabolism, energy expenditure and thermogenesis, among other potential metabolic pathways (Bauer et al. 2016). In the case of glucose metabolism, postprandial gut-derived signals can lower hepatic glucose production, increase insulin production and secretion, reduce glucagon levels and alter glucose uptake. These signals are thought to originate from preabsorptive nutrients being sensed on the apical surface of enterendocrine cells (EECs) (Reimann et al. 2008), given the ‘open-type’ structure of these specialized epithelial endocrine cells. However, it is still unclear whether nutrient sensors of EECs are predominantly located on the apical side, with the possibility of basolateral nutrient sensing having been recently proposed (Christensen et al. 2015). Nonetheless, EECs can secrete gut peptides on their basolateral side in response to direct nutrient stimulation via binding to nutrient receptors localized on EECs, by intracellular metabolism and through neuroendocrine mechanisms (see Psichas et al. 2015a for extensive review). For example, fatty acids are potent secretogogues for both GLP-1 and CCK, potentially via activation of free fatty acid receptors (FFAR, FFAR1, FFAR4, activated by medium- to long-chain free fatty acids (Briscoe et al. 2003, Hirasawa et al. 2005), and FFAR2 and FFAR3, activated by short-chain fatty acids (Tohurston et al. 2012, Psichas et al. 2015b)) localized on EECs, although the mechanisms of action appear to be more complex than originally thought. In the case of lipids, while Ffar knockout (KO) animals have severely impaired release of both GLP-1 (Edfalk et al. 2008) and CCK (Liu et al. 2011b) in response to a triglyceride challenge, it was recently demonstrated that LCFA activate the FFAR1-G_{q} signalling pathway to induce only a modest release of GLP-1 (Hauge et al. 2015). However, both oleoylthanolamide and 2-monoyacylglycerols, which are derived from triglycerides, activate a GPR119-G_{q} signalling cascade (Overton et al. 2006, Hansen et al. 2012b), leading to the hypothesis that triglyceride-induced gut peptide signalling is likely due to a combined effect of direct LCFA-FFAR1-G_{q} signalling and activation of the G_{s} signalling pathway in EECs (Hauge et al. 2015). In addition to a direct action on FFARs, fatty acids are also taken up by the intestine, and mice lacking absorptive proteins such as CD36 or FAT4 results in impaired gut peptide release (Poreba et al. 2012, Sundaresan et al. 2013). This may be due to intracellular metabolism and activation of PKC-ζ or PKC-δ to induce GLP-1 or CCK release, respectively (Iakoubov et al. 2007, Breen et al. 2011), or via alteration of cellular respiration and stimulation of glycolysis (Clara et al. 2016). The same complexity in nutrient-induced gut peptide stimulation is observed for carbohydrates. For example, carbohydrates can be sensed by the T1R2/T1R3 sweet taste receptor found in the gut (Jang et al. 2007), although the physiological relevance of sweet taste receptor activation on gut peptide signalling in humans remains debated (Parker et al. 2009). Conversely, recent work suggests that GLP-1 release occurs via uptake of glucose coupled with Na^{+} through the sodium/glucose cotransporter member 1 (SGLT1), inducing small currents triggered by increased Na^{+} which leads to membrane depolarization and voltage-gated Ca^{2+} entry, ultimately resulting in GLP-1 secretion (Gribble et al. 2003, Kuhre et al. 2015). Less is known about intestinal protein sensing, with GPR93, CaSR and PepT1 all being suggested to mediate protein-induced gut peptide release (Nemoz-Gaillard et al. 1998, Darcel et al. 2005, Liou et al. 2011a,c).

In the traditional view, the proximal intestine contains I-cells, which secrete CCK, and GIP-releasing K-cells, while L-cells cosecrete PY and GLP-1 and are located mainly in the distal intestine (Little et al. 2006). However, recent work has challenged these classical views, as individual enteroendocrine cells have been shown to express a variety of gut peptides (Egerod et al. 2012, Habib et al. 2012, Svendsen et al. 2015, Grunddal et al. 2016), while the proximal small intestine has been shown to secrete significant amounts of GLP-1 (Theodorakis et al. 2006). Once released, gut peptides can act locally on afferent neurons innervating the GI tract that signal to the caudal brainstem or enteric neurons, and/or they can enter the circulation to act centrally, or on peripheral targets, to regulate glucose metabolism (Cote et al. 2014). For example, GLP-1 receptors (GLP-1Rs) are located on vagal afferents that innervate the gut in close proximity to L-cells (Richards et al. 2014), indicating a possible paracrine gut–brain axis for mediating its glycaemic effects. However, GLP-1Rs are also located on neurons innervating the portal vein (Vahl et al. 2007), on β cells of the pancreas (Pyke et al. 2014), and in the central...
nervous system (Shimizu et al. 1987, Heppner et al. 2015), all possible targets for GLP-1 action (described in more detail below). Nonetheless, most studies demonstrate that vagal neural transmission is necessary for nutrient-induced gut feedback as anaesthetics, neurotoxins or vagotomy abolishes nutrient-induced reductions in food intake (Schwartz 2011), and in the case of lipid-induced CCK release, the lowering of hepatic glucose production (Wang et al. 2008a).

In response to lipids, CCK is released from EECs in a process dependent on intracellular esterification of long-chain fatty acids to long-chain fatty acyl-CoA via acyl-CoA synthase-3 (Sundaresan et al. 2013) and upon PKC-δ stimulation (Breen et al. 2011, Kokorovic et al. 2011). Released CCK activates the CCK receptor (CCK1-R) on vagal afferents innervating the small intestine (Raybould et al. 1988), which leads to PKA activation and vagal afferent firing (Rasmussen et al. 2012). Vagal afferent activation enhances N-Methyl-d-aspartate (NMDA) receptor-mediated neuronal transmission in the nucleus of the solitary tract (NTS) to lower glucose production via the hepatic vagal branch (Rasmussen et al. 2012). Interestingly, the ability of intestinal lipids and CCK to reduce glucose production is diminished in rats fed a 3-day high-fat diet (Wang et al. 2008a, Cheung et al. 2009), highlighting the pathophysiological relevance of this pathway. Although preliminary studies demonstrate that preabsorptive lipids may not potently inhibit hepatic glucose production in humans (Xiao et al. 2015), this lipid-CCK pathway requires much more extensive and thorough testing. Furthermore, 8 weeks of treatment with the bile acid sequestrant, coleselvelam, improves glycaemic control in humans with impaired glucose tolerance through a mechanism dependent on prevention of bile acid absorption and increased local CCK secretion (Marina et al. 2012), suggesting this aforementioned CCK gut–brain–liver axis could be of therapeutic relevance. Additionally, cotreatment of a CCK receptor agonist with a GLP-1R agonist has demonstrated initial therapeutic promise (Irwin et al. 2015; see section below).

GLP-1 has been widely studied for its incretin effect, where it stimulates an increase in insulin secretion at the level of the pancreas (Kreymann et al. 1987, Mojsov et al. 1987). Interestingly, recent evidence suggests that GLP-1 may additionally exert its effect via activation of visceral afferent neurons (Yamamoto et al. 2003), challenging the conventional model of GLP-1 action. GLP-1 is rapidly degraded by dipeptidyl peptidase-IV (DPP-IV) in the portal vein and liver, leaving only 10–15% of secreted GLP-1 for entry into the systemic circulation (Holst & Deacon 2005). As a result, studies hypothesize that effects on insulin release, as well as other glucoregulatory effects of GLP-1, such as decreased glucose production, increased glucose utilization and regulation of counter-regulatory hormones, are mediated at least in part by a gut–brain–periphery axis (Burcelin et al. 2001). Complicating this model further, GLP-1 can also act centrally to regulate food intake (Tang-Christensen et al. 1996), energy expenditure (Lockie et al. 2012), GI function (Seeley et al. 2000) and importantly, glucose homeostasis (Knauf et al. 2005, Gustavson et al. 2008). However, in contrast to these studies, others suggest that the glucoregulatory effects of GLP-1 are primarily mediated by pancreatic GLP-1R activation (Lamont et al. 2012, Smith et al. 2014). Nonetheless, GLP-1 plays an important role in the regulation of metabolism and glucose homeostasis, and as a result, some of the latest drugs to come onto the market have aimed to exploit the GLP-1R signalling pathway. Two main drug classes have emerged, degradation-resistant GLP-1R agonists and DPP-IV inhibitors.

**Incretin-based drug**

**GLP-1R agonists**

GLP-1R agonists commonly fall into two categories based on their duration of receptor activation: short-acting compounds, which deliver short-lived GLP-1R activation, and long-acting compounds, which activate their receptor continuously at their recommended dose (Madsbad 2016). Short-acting compounds include exenatide (Byetta), which was the first GLP-1R agonist approved for clinical use, and lixisenatide (Lyxumia), which has subsequently been approved for use in Europe, but not in the USA. Exenatide exhibits approximately 50% amino acid identity with human GLP-1 and has an affinity for the GLP-1R that is equivalent to native GLP-1. It contains a glycine residue at position 2, which provides resistance to degradation by DPP-IV and an increased circulating half-life (Furman 2012). Exenatide is the most widely studied of the GLP-1R agonists, with over 7 years of continuous clinical follow-up data. Early clinical trials examining the efficacy of exenatide showed that twice-daily 10μg injections effectively lowered both fasting and postprandial glucose concentrations in diabetic individuals after 30 weeks of treatment (Buse et al. 2004, DeFronzo et al. 2005, Kendall et al. 2005).

The success of exenatide led to the development of new long-acting GLP-1R agonists with pharmacokinetic properties designed for once-daily or once-weekly
administration. Liraglutide (marketed as Victoza) is a modified form of GLP-1 that contains a Ser34Arg substitution and has a C16 palmitoyl fatty acid side chain at Lys26, which allow binding to serum albumin and provide resistance to DPP-IV degradation (Lovshin & Drucker 2009). Liraglutide thus exhibits a prolonged half-life with stable plasma levels for up to 13 h after subcutaneous injection. Liraglutide administration (1.8 mg once daily) results in 24-h glucose control when prescribed as monotherapy or in combination therapy with oral glucose-lowering agents (Buse et al. 2009, Garber et al. 2009, Marre et al. 2009, Nauck et al. 2009, Russell-Jones et al. 2009). Other long-acting GLP-1R agonists include the once-weekly formulations of exenatide (Bydureon), albiglutide (Eperzan and Tanzeum) and dulaglutide (Trulicity) (Madsbad 2016). Differences in the duration of action largely account for differences in glucose control between GLP-1R agonists. For example, delayed gastric emptying is more strongly associated with short-acting GLP-1R agonists, resulting in greater effects on postprandial plasma glucose when compared with long-acting agonists (Drucker et al. 2008, Ji et al. 2013, Kapitza et al. 2013, Meier et al. 2015). On the other hand, the longer half-lives of long-acting GLP-1R agonists allow a greater improvement in 24-h glucose control, including fasting plasma glucose, when compared with short-acting agonists (Drucker et al. 2008, Buse et al. 2009, Blevins et al. 2011, Kapitza et al. 2013).

Despite the popularity of GLP-1R agonists, considerable mystery surrounds the main site of action for GLP-1R agonist antidiabetic effects. Exenatide readily crosses the blood–brain barrier, even more efficiently than native GLP-1 (Kastin & Akerstrom 2003), and it has been shown to activate brain regions involved in food reward and glucose homeostasis when administered subcutaneously (Daniele et al. 2015). The effects of exenatide on food intake may be mediated by its central action, as exenatide-induced reductions in energy intake in humans have been associated with increased hypothalamic connectivity (Schlogl et al. 2013), and intracerebroventricular injection of the GLP-1R antagonist, exendin-9, blocks the inhibitory effects of exenatide on energy intake in rodents (Kanoski et al. 2011). However, the effects of exenatide on glucose regulation do not appear to be dependent on central GLP-1R activation (Lamont et al. 2012), and evidence suggests that exenatide may exert its effects on glycaemia through direct action on the pancreas (Smith et al. 2014). There has also been evidence that the anorectic effects of exenatide are mediated, at least in part, by the activation of GLP-1R expressed on peripheral vagal afferents. However, studies suggest that although the early effects of exenatide require vagal afferent signalling, the later effects do not (Kanoski et al. 2011, Labouesse et al. 2012). Similar to exenatide, the main site of action of liraglutide remains unknown. Interestingly, liraglutide has been shown to improve insulin sensitivity in humans, as assessed by the hyperinsulinaemic euglycaemic clamp (Jinnouchi et al. 2015), indicating that in addition to its effects on insulin secretion, liraglutide also exhibits beneficial extrapancreatic effects on glycaemia. Liraglutide also passes the blood–brain barrier and it has been shown to bind to neurons within the arcuate nucleus and other sites within the hypothalamus (Secher et al. 2014). Evidence suggests that the anorectic effects of liraglutide are mediated via GLP-1Rs expressed both centrally and on vagal afferent neurons (Kanoski et al. 2011, Secher et al. 2014). However, whether liraglutide mediates its glucose-lowering effects through a manner similar to its anorectic effects requires attention. Some studies suggest that liraglutide-induced improvements in glucose tolerance do not require central or vagal GLP-1R (Sisley et al. 2014) and that its effects on glycaemia are via direct action on the pancreas (Smith et al. 2014). Thus, although the effects of liraglutide on glycaemia appear to be primarily dependent on the activation of pancreatic GLP-1R, central and vagal GLP-1R signalling should not be overlooked given their importance in lowering food intake and body weight, which is a primary treatment strategy for type 2 diabetes. A better understanding of the exact mechanisms for the glucose-lowering effects of GLP-1R agonists could result in more targeted drug designs to exploit the specific pathways.

DPP-IV inhibitors

DPP-IV inhibitors, also referred to as ‘incretin enhancers’, lower blood glucose levels through a prolongation of the action of GLP-1, and to a lower extent, GIP (a second incretin hormone produced in the small intestine) (Hansotia et al. 2004). Typically, DPP-IV inhibitors reduce DPP-IV activity by about 80%, which corresponds to a twofold increase in biologically active GLP-1 (Heine et al. 2005). This is associated with an increase in insulin and decrease in glucagon secretion and reduced fasting and postprandial glucose levels in individuals with diabetes (Heine et al. 2005).

Sitagliptin was the first DPP-IV inhibitor approved for use in 2006. It is a nonpeptide heterocyclic compound with rapid onset and a long duration of action, which...
facilitates once-daily dosing (Lovshin & Drucker 2009). Vildagliptin and saxagliptin were approved for use soon after sitagliptin; these compounds are cyanopyrrolidines with a slow onset and prolonged action upon binding to DPP-IV (Lovshin & Drucker 2009). The shorter half-life of vildagliptin requires twice-daily dosing (Lovshin & Drucker 2009). However, saxagliptin is suitable for once-daily dosing as a result of the presence of the active metabolite BMS-510849, which also inhibits DPP-IV (Trujillo & Nuffer 2014). The most recent DPP-IV inhibitors to reach the market are linagliptin (a methylxanthine) and alogliptin (a heterocyclic aminopiperidine), which are also administered once daily due to their relatively longer half-lives (Trujillo & Nuffer 2014, Handelsman et al. 2015). Each DPP-IV inhibitor elevates GLP-1 and improves glycaemia to a similar degree (Trujillo & Nuffer 2014, Handelsman et al. 2015). Once DPP-IV is maximally inhibited, glycated haemoglobin (HbA1c) reductions plateau; therefore, improvements are consistent across this drug class and there is no basis for differentiation regarding efficacy (Lovshin & Drucker 2009).

Despite the success of DPP-IV inhibitors, the cellular site that is responsible for their glucoregulatory effects has yet to be determined. Indeed, it is likely that increased endogenous GLP-1 could reach the pancreas and brain to exert the aforementioned effects. Studies in rodents indicate that a dose of sitagliptin that is sufficient to inhibit intestinal, but not systemic, DPP-IV activity is sufficient for improving glucose tolerance and insulin levels. This effect is associated with increased activity of the vagal nerve, suggesting that DPP-IV inhibitors may regulate glycaemia predominantly through local inhibition of intestinal DPP-IV activity and activation of neuronal GLP-1Rs (Waget et al. 2011).

**Cocktail therapy**

Despite the early beneficial effects of gut peptide-based therapies, the signalling pathways involved are redundant and the body can adjust. Therefore, it follows that the design of multitarget peptides capable of modulating more than one hormonal pathway could have distinct therapeutic benefits for the treatment of type 2 diabetes. Combination therapy with long-acting GIP and GLP-1 mimetics has been considered in preclinical studies with some success (Irwin & Flatt 2009); however, issues of separate drug formulation and dosing limits the therapeutic success. As such, a single hybrid peptide, MAR701, has been developed that can directly activate both GIP receptor and GLP-1R and appears to have beneficial effects in rodents (Finan et al. 2013). Further studies have investigated the effects of GLP-1R agonism combined with either glucagon receptor agonism (Pan et al. 2006, Day et al. 2009, 2012) or antagonism (Pocai et al. 2009). Although contradictory in nature, these contrasting regimens utilize both the beneficial glucose-lowering effects of GLP-1, combined with either inhibition of glucagon-mediated gluconeogenesis and glycogenolysis (Sinclair & Drucker 2005), and activation of glucagon pathways involved in energy turnover and weight loss (Pocai et al. 2009). Another modified hybrid peptide, ZP3022, involves a combined GLP-1-gastrin agonist (Fosgerau et al. 2013), which activates GLP-1R and CCK2-R and improves glycaemic control in db/db mice through enhancement of β cell mass (Fosgerau et al. 2013). Perhaps a more appealing peptide would be one that targets the GLP-1R and CCK1-R given the involvement of the CCK1-R in the activation of the gut-brain-liver axis. Indeed, combined administration of long-acting GLP-1R and CCK1-R agonists has shown pronounced synergistic metabolic benefits in rodent models of type 2 diabetes, including improved glycaemic control and loss of body weight (Irwin et al. 2013, Trevaskis et al. 2015). As such, a novel CCK/GLP-1 hybrid peptide based on the chemical structures of the CCK1-R agonist, (pGlu-Gln)-CCK-8, and exenatide has recently been described and shown to have significant therapeutic potential in high-fat-fed mice (Irwin et al. 2015). This molecule clearly warrants further study as a potential new treatment option for type 2 diabetes.

Considering the evident therapeutic efficacy of dual target peptide therapies, single compounds with the ability to activate three or more regulatory peptides could potentially provide even greater metabolic benefits. As a result, modified peptides with the ability to activate glucagon, GLP-1 and GIP receptors have been developed and have been shown to produce dramatic improvements in glucose homeostasis and overall metabolic control in high-fat-fed mice (Bhat et al. 2013a,b, Finan et al. 2015). Despite the clear potential of these tri-agonists, issues regarding the ratio of GIP, GLP-1 and glucagon receptor activation still requires investigation. As such, a recent study has reported the distinct beneficial effects of a balanced glucagon, GLP-1 and GIP receptor tri-agonist for the correction of obesity and diabetes in high-fat-fed mice (Finan et al. 2015). There is, therefore, a clear and attractive rationale for further testing of multitarget peptides for the treatment of type 2 diabetes in humans. In addition, given the recent findings that EECs coexpress a variety of gut peptides (Egerod et al. 2012, Habib et al. 2012, Svendsen et al. 2015, Grunddal et al. 2016), it may be
possible to develop a drug that promotes the cosecretion of multiple gut peptides from EECs. For example, infusion of bombesin, the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, or peptone stimulates the cosecretion of GLP-1, PYY, neurotensin and CCK (Svendsen et al. 2015), and interestingly, neurotensin acts synergistically with GLP-1 to regulate metabolism (Grunnald et al. 2016). This suggests that stimulating the release of an endogenous gut peptide ‘cocktail’, similar to engineering poly-agonists that mimic these peptides (Day et al. 2009, 2012, Finan et al. 2013, 2015), could be a useful alternative approach for improving metabolic control in type 2 diabetes.

The potential treatment of diabetes via mimicry of gut peptide signalling is bolstered by the success of bariatric surgery. Indeed, bariatric surgery has demonstrated great efficacy in normalizing blood glucose levels and ameliorating diabetes in obese populations, which has been suggested to be due in part to improvements in intestinal nutrient sensing and subsequent modulation of the secretion and biological action of numerous gut-derived peptides (see below). The following section aims to not only describe the various surgical procedures demonstrated to improve glucose regulation, but to introduce some of the major hypothesized mechanisms, in addition to intestinal nutrient sensing, underlying the success of bariatric surgery and to highlight the current therapeutic strategies directly targeting these mechanisms.

**Bariatric surgery**

The long-term success of bariatric surgery to reverse diabetes in obese patients underscores the need for identifying the mechanisms of action. Bariatric surgery encompasses many surgical procedures that are either restrictive in nature, by altering the stomach size or nutrient flux into the stomach, or involve the rerouting of the intestinal tract (Fig. 1). Roux-en-y gastric bypass surgery (RYGB) is one of the most commonly performed bariatric surgical procedures and involves a reduction in the size of the stomach, by creating a gastric pouch out of the upper portion of the stomach, and rerouting the intestinal tract by connecting the proximal jejunum to the stomach and thus excluding the duodenum (Ward & Prachand 2009). RYGB induces substantial effects on diabetes remission (Buchwald et al. 2004) and produces metabolic benefits that are maintained for over 10 years (Karlsson et al. 2007). However, the biliopancreatic diversion with duodenal switch (BPD-DS) procedure, which involves a pylorus-preserving vertical sleeve gastrectomy (VSG) (as opposed to the original BPD procedure, which involved a distal gastrectomy sacrificing the pylorus (Scopinaro et al. 1979)) and creation of a Roux limb, a long biliopancreatic limb and a short common channel, achieves diabetes resolution rates that are significantly better than RYGB (90% vs 70%) (Buchwald et al. 2004, 2009). However, despite its long-term metabolic success (Buchwald et al. 2004, 2009), the technical difficulty and meticulous patient surveillance have limited the use of this surgical technique to about 17% of all bariatric surgeries (Buchwald et al. 2009), although it is the metabolic surgery of choice for some surgeons (Marceau et al. 2015). Interestingly, VSG alone has substantial weight loss effects and appears to induce rapid and long-term diabetes resolution in obese type 2 diabetics (Bayham et al. 2012, Madsbad et al. 2014), which has been attributed to much more than simply restriction (see below or Seeley et al. 2015 for review).

To evaluate the relative contribution of gastric restriction vs rearrangement of the intestinal tract to the antidiabetic effects of bariatric surgery, an experimental procedure entitled duodenal-jejunal bypass (DJB) was developed. This procedure involves repositioning the intestinal tract without restriction or exclusion of the stomach. Although DJB does not elicit the same weight loss effects as RYGB or BPD, this procedure has been shown to produce glucose-lowering effects in nonobese rodents (Rubino et al. 2004), and in a small subset of nonobese or mild-obese humans with type 2 diabetes, independent of weight loss (Cohen et al. 2007, 2012, Lee et al. 2010, Geloneze et al. 2012). Moreover, in Asia, a novel surgery similar to a BPD-DS has been developed that involves a DJB with VSG, and has demonstrated initial success in the treatment of diabetes (Kasama et al. 2009, Lee et al. 2014). Lastly, another experimental metabolic surgery still in early human trials is ileal interposition (with or without VSG), which involves resection of 10–20 cm of the distal ileum and its transposition into the proximal jejunum. This procedure results in weight loss, reduced food intake and improved glycemic regulation in both rodents and humans (Wang et al. 2008b, Gagner 2011, Zhang et al. 2011, Grueneberger et al. 2013, Grueneberger et al. 2014, Ramzy et al. 2014).

Surgical intervention remains one of the most successful treatment options for the remission of diabetes. However, it is important that the best metabolic procedure is selected with benefits vs risks assessed for...
each individual patient (Castagneto Gissey et al. 2016). Despite the relative risks associated with procedures like RYGB and BPD-DS, a better understanding of these surgeries may continue to lead to less invasive and risky surgical procedures that equal in effectiveness. Moreover, noninvasive devices mimicking bariatric surgery could provide substantial benefits, such as the duodenal endoluminal sleeve, which involves inserting a flexible tube that inhibits the interaction of nutrients with the duodenum and has been shown to improve glucose regulation (Habegger et al. 2014). Ultimately, elucidation of the mechanisms underpinning the resolution of diabetes following bariatric surgery could lead to the discovery of novel pharmacological bariatric mimetics that could one day replace the need for bariatric surgery altogether. Therefore, the following sections describe some of the more developed hypotheses regarding the success of bariatric surgery, mainly the role of intestinal gut peptide signalling, gut microbiota and bile acids, and highlight the therapeutic potential of directly targeting these pathways.

Gut peptide and nutrient sensing

Given that many patients have exhibited postsurgical changes in gut hormone secretion (Rodieux et al. 2008), many studies have investigated whether changes in nutrient sensing mediate the weight loss and glucose-lowering effects of this procedure. One of the leading candidates for the success of bariatric surgery is altered gut peptide signalling, mainly GLP-1 (Salehi et al. 2011, Jimenez et al. 2013). Indeed, after RYGB, there is an increase in the number of gut peptide-expressing EECs that could one day replace the need for bariatric surgery altogether. Therefore, the following sections describe some of the more developed hypotheses regarding the success of bariatric surgery, mainly the role of intestinal gut peptide signalling, gut microbiota and bile acids, and highlight the therapeutic potential of directly targeting these pathways.
Postprandial GLP-1 levels are increased as early as 2 days postsurgery (le Roux et al. 2007) and have persisted as long as 10 years postsurgery (Dar et al. 2012). Improvements in glucose tolerance following RYGB or VSG in rats are abolished with exendin-9 administration (Chambers et al. 2011), all suggesting a role for GLP-1 in the glucose-lowering success of RYGB. Some studies argue against this (Clements et al. 2004, Rubino et al. 2004, le Roux et al. 2007), whereas some have shown that GLP-1 levels do not rise accordingly (Salinari et al. 2014), inhibition of GLP-1 signalling has no effect on glycaemia following RYGB (Jimenez et al. 2013, Shah et al. 2014) and GLP-1R deficient mice still exhibit improved glycaemia following RYGB and VSG (Wilson-Perez et al. 2013, Mokadem et al. 2014). As such, other gut peptides have been implicated as potential contributors to improved glycaemia following bariatric surgery.

Plasma PYY levels are increased following RYGB (le Roux et al. 2006, 2007, Rodieux et al. 2008) and DJB (Zhang et al. 2011, Liu et al. 2012, Imoto et al. 2014), and a causal link between PYY signalling and weight loss has been suggested for both humans (le Roux et al. 2007, Morimigo et al. 2008) and rodents (Chandarana et al. 2011), although studies investigating the role of PYY in the antidiabetic effects of these bariatric procedures are lacking. Interestingly, PYY action has been correlated with increased sensitivity to GLP-1 and improved glucose tolerance following bariatric surgery (Chandarana et al. 2013), suggesting that studies investigating the role of PYY in the glucoregulatory effects of bariatric surgery are warranted. Another gut peptide identified as a possible mediator of the beneficial effects of bariatric surgery is ghrelin. Plasma ghrelin levels are substantially reduced following VSG (Chambers et al. 2013); however, VSG is equally effective in improving glucose tolerance and lowering food intake and body weight in ghrelin-deficient and wild-type mice (Chambers et al. 2013), indicating that the beneficial effects of VSG are not dependent on reduced ghrelin signalling. Other factors have been shown to be altered following one or more of these procedures such as CCK, GIP and glucagon (Jacobsen et al. 2012, Rhee et al. 2015). Therefore, improvements in glycaemia following bariatric surgery may not be dependent on changes in the action of a single gut peptide, and it is very possible that an adaptive shift increases postingestive feedback, contributing to the rapid lowering of glucose levels.

Given that the rearrangement of the intestinal tract results in an increased flux of nutrients into the jejunum, it was hypothesized that increased jejunal nutrient sensing could mediate the improvements in glucose regulation. Indeed, intrajejunal nutrients lower hepatic glucose production via a gut–brain–liver neuronal axis, independent of changes in circulating insulin levels, while inhibition of these jejunal nutrient sensing pathways altered the rapid glucose-lowering effect of DJB in streptozotocin (STZ)-induced uncontrolled diabetic rats during refeeding (Breen et al. 2012). While lowering of glucose in STZ-Induced uncontrolled diabetic rats following DJB was associated with increased circulating GLP-1 levels, this was not the case following DJB in BBdp rats (Breen et al. 2012), Zucker diabetic fatty rats (Patel et al. 2014) or Goto-Kakizaki rats (Salinari et al. 2014), further arguing against the importance of GLP-1 in DJB. Interestingly, in a follow-up study, it was shown that direct leptin infusion into the jejunum activates jejunal leptin receptor-phosphoinositide-3-kinase signalling to lower endogenous glucose production through a neuronal network, while blocking jejunal leptin receptor signalling abolished the improvements in glucose homeostasis of DJB-diabetic rodents during refeeding (Rasmussen et al. 2014). However, it is important to note that for these studies, the testing period was only 2 weeks following DJB, and while this demonstrates that the rapid remission of diabetes following GBP may be due in part to nutrient- and hormonal-jejunal sensing mechanisms, the long-term potential of these sensing mechanisms remains unknown. The cell-type mediating the effect of jejunal leptin following DJB has not been characterized, the nodose ganglia contains leptin receptors (Li et al. 2011b) and studies suggest that leptin receptors on vagal afferents, rather than intestinal epithelial cells, play a role in the development of obesity and hyperglycaemia (de Artigue et al. 2014). Thus, it is likely that leptin is acting on vagal afferents innervating the gut to regulate glucose homeostasis. However, whether this pathway can be exploited to treat hyperglycaemia remains to be explored. Interestingly, while leptin treatment in obesity is generally unsuccessful due to leptin resistance, cotreatment of leptin with peptides that promote weight loss and leptin sensitivity, such as amylin and CCK, has been shown to be effective in improving glucose homeostasis in rodents (Sadry & Drucker 2013, Trevaskis et al. 2015). Furthermore, human analogues of amylin and leptin were successful in lowering body weight in clinical trials (Ravussin et al. 2009); however, safety concerns lead to the discontinuation of development. Nonetheless, future studies investigating not just leptin receptor activation, but rather vagal signalling in general, may hold promise for the development of novel antidiabetic therapies.
Bile acids

In addition to distal intestinal nutrient sensing and gut peptide changes, rearranging the intestinal tract during bariatric surgery profoundly alters bile acid levels and composition, which has been suggested as key contributor to its success (Seeley et al. 2015). Bile acids have been implicated in the regulation of glucose homeostasis through their effects on glucose production and glucose-induced insulin secretion (Thomas et al. 2008, 2009). Beyond acting as detergent for luminal fat digestion and absorption, bile acids act as endocrine factors, activating the G protein-coupled receptor TGR5, and a ligand-activated transcription factor farnesoid X receptor (FXR) (Fiorucci et al. 2009). In RYGB, bile acids flow undiluted through the biliopancreatic limb and do not mix with food until reaching the common channel of the distal jejunum. As such, increased presence of bile acids in this region could activate TGR5, which is localized on EECs, and can stimulate the release of gut peptides such as GLP-1 and PYY (Katsuma et al. 2005, Pournaras et al. 2012). However, increased gut peptide levels following RYGB have been shown to be independent of changes in bile acids (Bhutta et al. 2014, Jorgensen et al. 2015), and recent evidence shows that increased GLP-1 levels following VSG do not require TGR5 signalling, although TGR5 was shown to contribute to the glucoregulatory benefits of VSG in this study (McGavigan et al. 2015). Interestingly, the effects of VSG on body weight and glucose levels were abolished in Fxr knockout mice, suggesting a role for FXR in the metabolic effects of this procedure (Ryan et al. 2014). Indeed, FXR is essential for normal glucose homeostasis (Ma et al. 2006), and bile acid activation of FXR induces FGF19 (in humans and its mouse orthologue FGF15) release from the ileal intestinal epithelium (Zhang et al. 2013). Improvements in glucose homeostasis following RYGB are associated with changes in FGF 19/15 (Pournaras et al. 2012, Sachdev et al. 2015), possibly via inhibitory effects on hepatic glucose production and lipogenesis through reductions in bile acid secretion (Gerhard et al. 2013). More recently, it has been shown that central FGF 19 improves glucose tolerance, suggesting a central role for the glucoregulatory action of FGF 19 (Morton et al. 2013, Ryan et al. 2013, Marcellin et al. 2014). These studies, therefore, suggest that synthetic FXR agonists could act as potential diabetic treatments. Indeed, obeticholic acid, a semisynthetic FXR agonist, improves insulin sensitivity in type 2 diabetic patients (Mudaliar et al. 2013), while GW4064 has been shown to prevent insulin resistance in rodents (Ma et al. 2013). Furthermore, treatment with a gut-specific FXR agonist, fexaramine, reduces diet-induced increases in hepatic glucose production in mice, likely due to a robust increase in FGF 15 (Fang et al. 2015). In contrast, some studies suggest that intestinal inhibition of FXR may in fact be beneficial via a reduction in intestinally derived ceramides (Li et al. 2013, Jiang et al. 2015), or via increased GLP-1 signalling (Trabelsi et al. 2015), warranting further research into FXR agonism/antagonism for the treatment of diabetes. Interestingly, it has also been proposed that FXR-mediated metabolic improvements are due to alterations of the gut microbiota (Ryan et al. 2014).

Gut microbiota

The gut microbiota, which contains an estimated 100 trillion cells consisting of over 1000 different species of known bacteria, has a major influence on host energy homeostasis, and diabetes is associated with changes in both the bacterial composition and genetic make-up (see for review on gut microbiota and diabetes (Duca & Lam 2014, Tilg & Moschen 2014)). Meanwhile bariatric surgery drastically alters the composition and diversity of the gut microbiota in humans, rats and mice (Zhang et al. 2009, Furet et al. 2010, Li et al. 2011a, Liou et al. 2013, Osto et al. 2013, Ryan et al. 2014, Tremaroli et al. 2015, Yang et al. 2015). Interestingly, germ-free (GF) mice colonized with the microbiota derived from humans, who had undergone RYGB or vertical banded gastroplasty exhibited reduced fat deposition when compared with GF mice colonized with the microbiota of obese controls (Tremaroli et al. 2015). This is in line with the fact that GF animals inoculated with the gut microbiota of RYGB-treated mice gained less weight and have a trend towards improved insulin sensitivity when compared with GF mice receiving sham microbiota (Liou et al. 2013).

Although the mechanisms for gut microbiota-mediated improvements in glucose homeostasis are still not completely understood (Duca & Lam 2014, Tilg & Moschen 2014), alterations in short-chain fatty acid (SCFA) production via fermentation of nondigestible polysaccharides has been hypothesized (Vrieze et al. 2012, Liou et al. 2013). For example, in the RYGB microbiota transplant study described above, levels of propionate were increased in both the RYGB-treated donor and the recipient mice (Liou et al. 2013). Indeed, supplementation with propionate or fermentable fibre is known to reduce appetite and improve glucose tolerance, possibly from inducing distal intestinal gut peptide secretion.
(Everard et al. 2011, Lin et al. 2012, Chambers et al. 2015, Psichas et al. 2015b). Ileal propionate activates mucosal FFA2 to lower hepatic glucose production through a GLP-1-dependent neuronal pathway (Zadeh-Tahmasebi et al. 2016), while increased concentrations of SCFA in the large intestine have been shown to increase circulating levels of GLP-1 and PYY and reduce postprandial insulin and glucose levels (Tolhurst et al. 2012, Psichas et al. 2015b). As such, it is possible that improvements in glucose homeostasis following RYGB are due, at least in part, to changes in the production of SCFA in the ileum and large intestine. Although treatment with SCFAs is not feasible, as they are very unpalatable and do not reach the distal small and large intestine where they are endogenously produced, prebiotic treatment (nondigestible fibre) effectively increases distal intestinal SCFA production and improves glucose homeostasis in rodents (Cani et al. 2006b, Everard et al. 2011), which is thought to be mediated through an increase in gut peptide release (Cani et al. 2004, 2005, 2006b, Everard et al. 2011, Neyrinck et al. 2012). While some evidence exists for the beneficial effects of fermentable dietary fibre in humans (Archer et al. 2004, Cani et al. 2006a, 2009, Parnell & Reimer 2009), intake of dietary fibre is generally low (Howarth et al. 2003), and prebiotic treatment may not readily increase propionate production (Chambers et al. 2015). As such, a novel carrier molecule was developed to selectively increase colonic propionate levels. Treatment with this insulin-propionate ester for 24 weeks reduced weight gain and adipose distribution, and prevented the decrease in glucose tolerance and insulin sensitivity exhibited by the control group (Chambers et al. 2015). In addition, faecal microbiota transfer (FMT) represents another potential mechanism to increase SCFA levels and improve glucose homeostasis, as duodenal transfer of microbiota from lean humans into those with metabolic syndrome results in an increase in butyrate-producing bacteria and insulin sensitivity 6 weeks after the transfer (Vrieze et al. 2012). However, FMT is still experimental, and a more selective change in distal bacterial composition through pre- or probiotics may prove a safer and more efficacious option.

One of the more recently studied bacteria, Akkermansia muciniphila, might hold promise as a probiotic treatment for diabetes. A. muciniphila is a mucin-degrading, Gram-negative bacterium that resides in the mucus layer, and represents 3–5% of the microbial community (Derrien et al. 2004). Levels of A. muciniphila are inversely correlated with body weight, glucose tolerance and type 1 diabetes, while RYGB increases A. muciniphila abundance (Zhang et al. 2009, Hansen et al. 2012a, Everard et al. 2013, Liou et al. 2013, Shin et al. 2014, Schneeberger et al. 2015, Dao et al. 2016). Furthermore, reduced A. muciniphila levels in diet-induced obesity are normalized following prebiotic feeding, which is associated with improvements in metabolic dysregulation (Everard et al. 2013). In humans, A. muciniphila abundance is inversely related to fasting glucose and patients with increased A. muciniphila abundance and gene richness exhibited improved fasting plasma glucose levels and greater improvements in insulin sensitivity following caloric restriction (Dao et al. 2016). Direct chronic treatment in diet-induced obese mice with alive, but not heat-killed A. muciniphila reversed weight and fat mass gain, as well as insulin resistance (Everard et al. 2013). Further, chronic treatment with dietary phenols from grapes, or cranberry extract, improved glucose tolerance and insulin sensitivity which was hypothesized to be due to A. muciniphila abundance (Anhe et al. 2015, Roopchand et al. 2015). Although A. muciniphila remains to be tested in humans, this is one example in an ever-expanding list of potential probiotics that could help lower glucose levels in type 2 diabetes (Le Barz et al. 2015, Stenman et al. 2015a). It is interesting to note that metformin treatment has been shown to alter the gut microbiota, with chronic treatment resulting in A. muciniphila abundance, as well as changes in bile acid levels, suggesting a gut microbiota-mediated role for metformin treatment (Lien et al. 2014, Napolitano et al. 2014, Shin et al. 2014).

Metformin

As mentioned above, metformin is the first-line medication for the treatment of type 2 diabetes, as it potently reduces hyperglycaemia via a reduction in hepatic glucose production (Foretz et al. 2014). Although the main action of metformin was originally hypothesized to be due to activation of hepatic AMP-activated kinase (AMPK) (Shaw et al. 2005), an intracellular energy sensor, via increased AMP levels resultant from inhibition of the mitochondrial respiratory chain complex 1 (Owen et al. 2000), recent work has readily challenged that. Recent studies have demonstrated that metformin can lower HGP by: suppressing glycolytic enzymes in an AMPK-independent fashion (Foretz et al. 2010), antagonizing hepatic glucagon action (Miller et al. 2013), and via an alteration in mitochondrial and cytosolic redox states (Madiraju et al. 2014). Furthermore, the action of metformin in the gut has recently been identified to play a role in its glucose-lowering ability
Indeed, oral/intestinal treatment of metformin results in a greater drop of blood glucose than IV or even portal treatment (Stepensky et al. 2002). Interestingly, delayed-release metformin, which is formulated to avoid absorption and target the lower bowel, was more effective at lowering fasting plasma glucose than currently available metformin (Buse et al. 2016). One possible mechanism may be in the ability of metformin to activate a gut–brain–liver axis to lower hepatic glucose production. Specifically, preabsorptive metformin activates small intestinal mucosal AMPK, triggering a GLP-1R-protein kinase A-dependent pathway to lower glucose production (Duca et al. 2015). This is in line with the fact that metformin can increase both acute and chronic levels of GLP-1 (Foretz et al. 2014). Interestingly, when small intestinal AMPK was virally knocked-down in diabetic rodents, the glucose-lowering ability of acute metformin treatment was diminished by about 50%, indicating a potent and sustained contribution of intestinal AMPK activation to metformin’s effect (Duca et al. 2015). Additionally, this study highlights the potential for more direct targeting of intestinal energy sensors to treat diabetes. For example, in addition to AMPK, the NAD+-dependent deacetylase sirtuin 1 (SIRT1) is expressed in the small intestinal mucosa. Activation of small intestinal SIRT1, via intraintestinal resveratrol infusion, triggers a vagal gut–brain neuronal axis to improve hypothalamic insulin sensitivity to lower hepatic glucose production in high-fat-fed and diabetic rodents (Cote et al. 2015). Interestingly, this effect was also dependent on AMPK (Cote et al. 2015), indicating a possible interactive dependency between these two molecules, although it remains to be tested whether SIRT1 is required for intraintestinal metformin in the gut. Nonetheless, it may be efficacious to develop a ‘gut-targeted’ metformin-like molecule that could activate intestinal mucosal AMPK and/or other energy sensors to potently lower glucose levels in diabetic individuals, given that intestinal AMPK is reduced in diabetes (Harmel et al. 2014).

**Conclusion**

The metabolic potential of the GI tract is becoming increasingly recognized. Drugs aimed at mimicking gut-derived molecules, like GLP-1 receptor agonists, are readily being tested and used for diabetic treatment. As of now, most GLP-1R agonists are administered as a complimentary therapeutic option to metformin, but perhaps in the future, more specialized ‘cocktail’ treatments will be developed to provide a more complete action on complex signalling pathways that often compensate and limit long-term drug potential. Perhaps an even better approach will be to alter endogenous levels of gut-derived hormones, as is done with manipulation of bile acids and gut microbiota following bariatric surgery. Instead of surgery, drugs are also being developed to directly influence the gut milieu, like bile acid sequestrants and pre/probiotics. Although manipulation of a stable gut microbiota has proven difficult, with more research into how to effectively and favourably alter the microbiota in the long-term, pre/probiotics could be a valuable tool in
treating diabetes, possibly as a combination with frontline treatments (Stenman et al. 2015b). Even more intriguing is the possibility that future treatments may involve genetically modified bacteria that contain therapeutic factors to help treat metabolic disease (Chen et al. 2014).

Overall, the GI tract represents a promising avenue for the development of successful targeted therapeutic options for the treatment of diabetes.

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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