RESEARCH ARTICLE

The emerging role of the intestine in metabolic diseases

William D. Bradley, Catherine Zwingelstein and Cristina M. Rondinone

Metabolic and Vascular Diseases Department, Hoffmann La-Roche Inc., Nutley, NJ, USA

Abstract

The intestine is an important metabolic organ that has gained attention in recent years for the newly identified role that it plays in the pathophysiology of various metabolic diseases including obesity, insulin resistance and diabetes. Recent insights regarding the role of enteroendocrine hormones, such as GIP, GLP-1, and PYY in metabolic diseases, as well as the emerging role of the gut microbial community and gastric bypass bariatric surgeries in modulating metabolic function and dysfunction have sparked a wave of interest in understanding the mechanisms involved, in an effort to identify new therapeutics and novel regulators of metabolism. This review summarizes the current evidence that the gastrointestinal tract has a key role in the development of obesity, inflammation, insulin resistance and diabetes and discusses the possible players that can be targeted for therapeutic intervention.

Keywords: type 2 diabetes, incretins, bariatric surgery, microbiota, gut hormones

Introduction

The intestine is an organ essential for the digestion and extraction of nutrients, such as lipids, carbohydrates and proteins, but its role in metabolic diseases has been poorly investigated over the years. The concept that factors secreted from the gut participate in the regulation of endocrine secretion was raised as early as the beginning of the 20th century (Moore, 1906). Early studies into the gut endocrine system focused on the role of gut hormones in the peripheral regulation of gastrointestinal function, such as secretin on pancreatic secretion, cholecystokinin on gall bladder contraction, and gastrin on gastric acid release. The term “secretin” was first used to define factors regulating pancreas secretion (Bainbridge & Beddard, 1906). Later, the term “incretin” was introduced to describe these potential mediators (Creutzfeldt, 1974). The connection between the gastrointestinal tract and the endocrine pancreas was confirmed in the 1960s, when insulin became measurable in plasma. Clinical studies showed that when an oral and an intravenous load of glucose producing identical increases in plasma glucose levels were compared, the insulin secretory response was greater when glucose was administered orally (Erick et al., 1964). These findings suggested that not only glucose interacted with β-cells in the islets of Langerhans of the pancreas, but also factors released from the gut that could stimulate insulin secretion (Creutzfeldt, 1974; Unger & Eisenraut, 1969).

The incretin effect account for approximately 50 to 70% of the total insulin secreted after glucose ingestion. In humans, two peptide hormones were identified as being responsible for the incretin effect, namely glucose-dependent insulin releasing polypeptide, GIP (formerly called gastric inhibitory polypeptide) and glucagon-like peptide-1, GLP-1. Both peptides are secreted in response to food ingestion and both potentiate the glucose-induced insulin response.

In addition to GIP and GLP-1, a number of other 50 or more peptides and factors are produced and secreted by the gut that affect numerous organs in the body including the brain, liver, fat, pancreas and regulate energy storage, lipolysis, body weight, appetite, satiety, β-cell preservation and glucose metabolism. These peptides include peptide YY, oxyntomodulin, ghrelin and many others (Wren & Bloom, 2007).

Aside from endogenous gut hormones, recent evidence suggests the resident gut microbial community plays a role in the development of insulin resistance and diabetes. Distortions in any one of the microbiota functions or signalling pathways could potentially contribute...
to a wide range of diseases, including cardiovascular diseases (bile-acid-associated regulation of serum cholesterol levels, chronic inflammation), diabetes (carbohydrate uptake and glycemic control), inflammatory diseases (atopic diseases, inflammatory bowel disease, inappropriate immune stimulation) and neoplastic diseases (carcinogen activation, chronic inflammation related hyperproliferation) (Neish, 2009). New sequencing technologies have not only allowed for a thorough analysis of the classifications of bacteria in the human gut, but will also be important in relating these findings to changes in metabolic function triggered by the resident microbiota.

Moreover, bariatric surgery, originally devised to treat obesity, has also been shown to help diabetes, such that it may know be considered a metabolic surgery (Rubino et al., 2010). Indeed, type 2 diabetes is improved or even reversed soon after these operations and well before significant weight loss occurs (Cummings & Flum, 2008; Cummings et al., 2004). Recent studies have shown that improved intestinal gluconeogenesis may also be involved in the amelioration of glucose homeostasis following the surgery. Several mechanisms likely mediate the direct anti-diabetic impact of bariatric surgery, including enhanced nutrient stimulation of L-cell peptides (e.g., GLP-1) from the lower intestine, intriguing but still unidentified phenomena related to exclusion of the upper intestine from contact with ingested nutrients, compromised ghrelin secretion, possible alterations of intestinal nutrient sensing and metabolism that affect insulin sensitivity, and probably additional undiscovered effects (Cummings et al., 2004, 2007). It is increasingly clear that the gut plays a major role in glucose homeostasis, regulating both insulin secretion and sensitivity (Ahren, 2003; Drucker, 2007; Rubino et al., 2006), and bariatric surgery likely influences several GI pathways in complementary ways to improve glucose control.

Further characterization of the role of the gut and identification of other contributing factors promise to reveal novel targets for the pharmacological therapy of diabetes.

### Gut peptides

**Incretins: GIP and GLP-1**

GLP-1 is produced from proglucagon and is mainly expressed in mucosal enteroendocrine L-cells located predominantly in the distal intestine (ileum and colon). The peptide was first identified following the cloning of cDNAs and genes for proglucagon in the early 1980s, and is one of the two principal incretin hormones (Gutzwiller et al., 1999; Mojsov et al., 1987).

GLP-1 acts through a G protein-coupled receptor to exert its functions. This receptor is expressed in many tissues, including pancreatic islets, the central nervous system, lung, kidney, heart, and the gut (Bullock et al., 1996; Thoresn, 1992). GLP-1 is coupled to its receptor through stimulatory Go and adenylyl cyclase to increase intracellular cAMP (Thorens, 1992). GLP-1 can induce other intracellular signals as well, including increases in intracellular calcium (Lu et al., 1993), phosphoinositol 3-kinase (PI3K) activity (Buteau et al., 1999), and mitogen-activated protein kinase activity (Montrose-Rafizadeh et al., 1999). GLP-1 functions to lower postprandial blood glucose via augmentation of glucose-stimulated insulin secretion by β-cells, suppression of appetite, delay of gastric emptying, and suppression of glucagon secretion (Drucker, 2002; Kieffer & Habener, 1999). GLP-1 has extra-metabolic effects as well on the cardiovascular, pulmonary, and hypothalamic-pituitary systems (Kieffer & Habener, 1999).

GLP-1 has a promising potential in the treatment of diabetes mellitus, showing near normalized glucose levels following intravenous infusions in patients with type 2 diabetes (Drucker, 2002; Holst, 2002; Holz & Chepurny, 2003; Nauck et al., 1993b). In addition, trophic effects on the pancreatic islets have been recently discovered in animal models, which make GLP-1 and GLP-1 analogues exciting candidates for diabetes therapy (Thorkildsen et al., 2003; Tourrel et al., 2001, 2002; Xu et al., 1999; Egan et al., 2003). More recently, GLP-1 agonists have been shown to stimulate the growth and differentiation of pancreatic β-cells, as well as exert cytoprotective, anti-apoptotic effects on β-cells (Bulotta et al., 2002; Buteau et al., 2003; de la Tour et al., 2001; Drucker, 2003, Egan et al., 2003). Recent evidence indicates that GLP-1 agonists act on receptors on pancreas-derived stem/progenitor cells to prompt their differentiation into β-cells (Hui et al., 2010). These new findings suggest an approach to create β-cells in vitro by expanding stem/progenitor cells and then to convert them into β-cells by treatment with GLP-1.

Glucose-dependent insulinotropic polypeptide (GIP), also called gastric inhibitory polypeptide is released from the duodenum and jejunum in response to ingestion of a meal containing glucose or fat and potentiates glucose induced insulin secretion (Meier et al., 2002; Pederson et al., 1975; Polak et al., 1973). It has also been shown that under physiological conditions in humans, GIP has a negligible effect on gastric acid secretion (Nauck et al., 1992). GIP modulates ion currents and stimulates proximal and distal steps of the exocytotic cascade (Beguin et al., 1999; Ding & Gromada, 1997) by acting on a seven-transmembrane-spanning G-protein-coupled receptor coupled to stimulation of adenylyl cyclase, activation of phospholipase A₂, and increases in intracellular calcium (Ehses et al., 2001; McIntosh et al., 1996). In addition to enhancing insulin release, GIP further acts as an insulinotropic agent by stimulating pro-insulin gene transcription and translation and up-regulating glucose transporters and hexokinase in the β-cell (Fehmann & Goke, 1995; Wang et al., 1996).

There is controversy regarding the anti-diabetic potential for GIP receptor (GIPR) agonists (McIntosh et al., 2009). The main reasons for this are that many patients with diabetes exhibit reduced responses to GIP and that
elimination of GIP signalling promotes resistance to obesity in rodents (Irwin & Flatt, 2009; Miyawaki et al., 2002; Nauck et al., 1993a). However, pharmacological doses of dipeptidyl peptidase IV (DPP-IV)-resistant GIP analogues are insulinotropic in rodents that are unresponsive to physiological levels of GIP (Hinke et al., 2002; Irwin et al., 2005). Moreover, normalization of glycaemia improves β-cell sensitivity to GIP in diabetic rats and in patients with type 2 diabetes (Hojberg et al., 2009; Meneilly et al., 1993; Piteau et al., 2007) and GIPR signalling promotes survival of cultured β-cells (Kim et al., 2005; Trumper et al., 2002; Widenmaier et al., 2009). In addition, recent studies investigated the effects of chronic treatment of diabetic rats with a long-acting DPP-IV resistant GIP analogue and observed superior β-cell function and increased mass, as well as improved glycaemic control (Porter et al., 2011; Widenmaier et al., 2010).

One of the major drawbacks with the use of either GIP or GLP-1 as potential therapeutic agents is their short duration of action, due to enzymatic degradation in vivo. The enzyme DDP-IV, a serine protease, rapidly metabolizes GIP and GLP-1 (Kieffer & Habener, 1999, Kieffer et al., 1995) so there is a need to identify DDP-IV resistant analogues for the treatment of diabetes.

**Peptide YY**

Peptide YY (PYY) is a 36 amino acid, straight chain polypeptide, which is co-localized with GLP-1 in the L-type endocrine cells of the GI mucosa. PYY shares structural homology with neuropeptide Y (NPY) and pancreatic polypeptide (PP), and together form the “neuropeptide Y family of peptides”, which is also called the “pancreatic polypeptide-fold family of peptides” (Adrian et al., 1985a). PYY release is stimulated by intra-luminal nutrients, including glucose, bile salts, lipids, short-chain fatty acids and amino acids. After release, DPP-IV cleaves the N-terminal tyrosine and proline residues forming PYY(3-36). PYY(1-36) represents about 60% and PYY(3-36) 40% of circulating PYY. PYY inhibits many GI functions, including gastric acid secretion, gastric emptying, small bowel and colonic chloride secretion, mouth to cecum transit time, pancreatic exocrine secretion and pancreatic insulin secretion (Adrian et al., 1985b; Allen et al., 1984). In addition, selective activation of the neuropeptide Y2 receptor by PYY (3-36) suppresses appetite and provides a promising approach to obesity management. PYY(3-36) reduces acute food intake in different animal models, and daily administration of PYY(3-36) causes body weight loss in DIO and ob/ob mice, rats, and rabbits (Batterham et al., 2003; Challis et al., 2003; Moran et al., 2005; Pittner et al., 2004; Sileno et al., 2006), and increases insulin action and glucose disposal (van den Hoek et al., 2004, Vrang et al., 2006). PYY(3-36) does not reduce food intake in NPY2 knockout mice, or when it is co-administered with the NPY2 antagonist BIIE0246 in wild-type rats, suggesting that the anorexigenic effect of PYY(3-36) is mediated specifically through the NPY2 receptor (Abbott et al., 2005; Batterham et al., 2003). This property makes NPY2 receptor modulation an attractive mechanism for the therapeutic management of obesity. Indeed, intravenous and nasal administration of PYY(3-36) to humans reduces caloric intake (Batterham et al., 2003; Degen et al., 2005). However, the effect of PYY(3-36) is short-lived, and it may be as short as 4h in humans (Brandt et al., 2004). Therefore it is desirable to discover a selective, long-acting NPY2 receptor agonist of potential superior therapeutic benefit to PYY(3-36).

**Microbiota**

The human gut is home to more than a trillion microorganisms collectively deemed intestinal microbiota that normally coexist in a mutualistic relationship with their host (Backhed et al., 2005). This collection consists mostly of bacteria, and includes thousands of different species, which contain an estimated 100 times the number of genes found in the human genome (Xu & Gordon, 2003). This additional genetic information generally offers advantageous functions not performed in the human gut, such as vitamin synthesis and breakdown of complex polysaccharides (Backhed et al., 2005). Indeed, microbiota also contribute to a number of developmental and homeostatic processes, including immune system maturation (Mazmanian et al., 2005) and response to injury of the epithelium (Rakoff-Nahoum et al., 2004). However, several studies have directly linked microbiota to diseases, such as inflammatory bowel disease and gastric carcinoma, and indirectly to a number of other disorders (Sekirov et al., 2010). Interestingly, recent work has identified a role for microbiota in the onset of obesity and metabolic disorders, such as type 2 diabetes, with new studies emerging uncovering specific mechanisms linking these gut microbes to cellular pathways with detrimental physiological outcomes.

The link between microbiota and obesity was first uncovered based on the observation that mice reared in a germ free environment (thus devoid of intestinal bacteria) contained 42% less body fat than mice raised conventionally, despite ingesting more food (Backhed et al., 2004). Interestingly, conventionalization of the germ free mice with microbiota from conventionally raised (thus populated with intestinal bacteria) donor mice led to a 57% increase in total body fat content, along with increased fasting glucose and insulin levels, and insulin resistance. Subsequent experiments revealed putative mechanisms by which this adiposity increase occurs, as well as uncovered significant differences in the proportion of specific types of bacteria in the gut that may contribute to the pathogenesis of obesity and metabolic disorders.

Intestinal microbes process complex polysaccharides into simpler sugars, more readily absorbed by the host epithelium. Studies revealed glucose uptake is increased in conventionally raised mice compared to germ free mice, leading to the hypothesis that this increases monosaccharide delivery to the liver, resulting in the documented elevated expression of lipogenic...
enzymes (Backhed et al., 2004). In conjunction with this mechanism, microbiota-induced adiposity also appears to involve the secreted protein Fiaf, or fasting-induced adipocyte factor, which displays increased expression in germ free mice. Fiaf is an endogenous lipoprotein lipase (LPL) inhibitor, and high LPL activity leads to increased triglyceride accumulation in adipose. Upon conventionalization of germ free mice, Fiaf expression is suppressed, leading to increased LPL activity, and subsequent triglyceride storage. Germ free fiaf–/– mice have the same amount of body fat as conventionalized littermates, and only gain minimal amounts of fat when conventionalized, indicating that Fiaf is necessary for the increased adiposity following colonization of the intestine with bacteria (Backhed et al., 2004). Interestingly, germ free mice are also resistant to diet induced obesity (DIO). These mice not only show elevated Fiaf expression, but also display increased AMPK phosphorylation, which leads to an increase in fatty acid oxidation and energy consumption (Backhed et al., 2007). Together, these mechanisms indicate that microbiota have the ability to induce adiposity by processing complex polysaccharides into absorbable mono-saccharides, thus inducing expression of lipogenic signals in the liver. By complementary, but yet uncharacterized mechanisms, microbiota also lead to reductions in AMPK phosphorylation and Fiaf expression, which result in reduced fatty acid oxidation, increased LPL activity leading to generation and accumulation of triglycerides in adipose. More research is required to understand the molecular mechanisms by which microbiota signal to the host cells triggering these signalling mechanisms.

Following the intriguing findings that microbial conventionalization has such profound effects on inducing adiposity, subsequent comparative metagenomic analyses of the microbial community of obese ob/ob mice and their lean littermates uncovered stark differences in the proportion of the two dominant bacterial divisions found in both mice and humans, the gram negative Bacteroidetes and gram positive butyrate-producing Firmicutes (Ley et al., 2005). In these experiments, obese mice displayed a 50% reduction in total Bacteroidetes with a proportional increase in Firmicutes when compared to lean mice (Turnbaugh et al., 2006). Interestingly, DIO mice also display increased Firmicutes compared to littermates fed a normal chow diet, however, the DIO mice also show a more pronounced enrichment in a specific class of Firmicutes, the Mollicutes (Turnbaugh et al., 2008). Importantly, studies displayed that conventionalization of germ free mice with the microbiota from obese ob/ob mice induced a significant increase in adiposity compared to mice conventionalized with microbiota from lean littermates (a 47% increase in fat mass compared to 27%, respectively) (Turnbaugh et al., 2006). Similarly, germ free mice conventionalized with microbiota from DIO donors experienced a significantly greater increase in adiposity when compared to mice conventionalized with microbiota from chow-fed donors (Turnbaugh et al., 2008). Both of these results indicate the composition of the transferred microbial community had a causal effect on the increase in fat mass in the recipient mice. However, it is important to keep in mind that the change in proportions of gut bacterial populations results from hyperphagia (ob/ob mouse) or a high caloric fat- and sugar-rich diet (DIO mouse), indicating the presence of a feed forward loop in which the increased exposure to nutrients likely promotes favourable conditions for Firmicutes, which then further contributes to enhanced adiposity. Indeed, studies show that a DIO-resistant mouse model (the RELMβ–/– mouse) shows similar changes in microbial composition as wild type control, indicating diet alone is sufficient to induce the initial change in microbial population, but the altered microbiota is not sufficient to induce adiposity (Hildebrandt et al., 2009).

Following these observations, it is essential to understand how increased exposure to food leads to a change in the ratio of these two bacterial divisions, and why the increased proportion of Firmicutes leads to obesity. One suggested mechanism for the latter is that Firmicutes has the ability to extract more caloric content from ingested food (Turnbaugh et al., 2006). Interestingly, the Mollicutes class of bacteria show an enrichment for genes involved in the import and metabolism of simple sugars (Turnbaugh et al., 2008), which could result in the release of short chain fatty acids that are readily absorbed by the host. It was experimentally determined that mice conventionalized with microbiota from ob/ob mice do extract more calories from food, and show increased by-products of fermentation, than mice conventionalized with microbiota from lean littermates (Turnbaugh et al., 2006). Moving forward, it is important to understand what specific conditions and/or nutrients promote Firmicutes growth, and if other mechanisms contribute to their ability to induce obesity.

Importantly, some of these findings were confirmed in humans, as obese humans show increased Firmicutes and decreased Bacteroidetes when compared to lean controls (Ley et al., 2006). Of particular interest is the observation that the abundance of Bacteroidetes in the gut is positively correlated with reductions in body weight when obese subjects were placed on a carbohydrate- or fat-restricted low calorie diet, again indicating that diet has a direct effect on the composition of gut microbiota.

**Microbiota and inflammation**

While microbiota conventionalization-induced fat gain was shown to be independent of both innate and adaptive immune systems in mice (using MyD88–/– and Rag1–/– mice) (Backhed et al., 2004; Turnbaugh et al., 2008), obesity and type 2 diabetes are characterized by the incidence of low-grade inflammation that affects metabolic homeostasis (Eckel et al., 2005; Hotamisligil and Erbay, 2008; Pradhan, 2007). Recent studies indicate that there may be more to microbiota than just the ability to extract more calories from diet.
It was recently shown that mice lacking the innate immune system pattern recognition receptor Toll-like receptor 5 (TLR5) are hyperphagic, and develop obesity and metabolic syndrome (Vijay-Kumar et al., 2010). While these mice do not display altered levels of either Bacteroidetes or Firmicutes when compared to wild type littermates, they do experience changes in 116 other phyotypes. Importantly, treatment of TLR5–/– mice with antibiotics prevents obesity, and conventionalization of germ free wild type mice with microbiota from TLR5–/– causes the recipients to become obese. While not entirely consistent with the studies mentioned above, it is still clear that the gut bacterial population plays a causative role in developing obesity. Commensurate with changes in the microbial community of the TLR5–/–, these mice also exhibit increased triglycerides and cholesterol, increased pro-inflammatory cytokines and insulin resistance, leading to a lack of glycaemic control.

Indeed, other studies have linked gut microbes with host innate immune responses. Lipopolysaccharide (LPS) is a component of the cell wall of gram negative bacteria, and interacts with Toll-like receptor 4 (TLR4) to initiate an innate immune response (Medzhitov, 2001) leading to release or pro-inflammatory cytokines and chemokines. Initially, it was uncovered that endogenous free fatty acids, which are elevated in circulation in the obese state, activate TLR4 in macrophages and adipose to release pro-inflammatory cytokines and chemokines. Initially, it was uncovered that endogenous free fatty acids, which are elevated in circulation in the obese state, activate TLR4 in macrophages and adipose to release pro-inflammatory cytokines, leading to insulin resistance (Shi et al., 2006). TLR4–/– mice show reduced cytokine induction and suppression of insulin resistance.

Subsequent studies identified a role of bacterially-derived LPS causing insulin resistance in a CD14 (a member of the TLR4 signalling complex) dependent manner (Cani et al., 2007). These studies displayed significant increases in circulating LPS in mice following 4 weeks on a high fat diet, with commensurate changes in microbiota populations in the gut. Interestingly, these mice, as well as mice infused with LPS directly, displayed whole body weight gain and hepatic insulin resistance, which was abrogated by the lack of a functional TLR4 complex in the CD14–/– mouse. Treatment of DIO or ob/ob mice with antibiotics led to decreased gut microbiota, LPS concentration, and improvements in inflammation and glycaemic control (Cani et al., 2008), indicating a direct relationship between microbiota, LPS concentration, and insulin resistance. However, analysis of the types of bacteria present in the high fat diet fed mice compared to chow fed mice showed a decrease in many classes of bacteria, including the gram negative (and thus LPS-containing) Bacteroidetes and Bacteroidetes-like bacteria, as well as the gram positive Eubacterium rectale, Clostridium cocoides, and Bifidobacterium (Cani et al., 2007). The group hypothesizes that reductions in Bifidobacterium may ultimately contribute to the increase in LPS, as this group of bacteria has been shown to reduce intestinal LPS. Yet, this hypothesis has not yet been tested. While mechanistic details of this system remain unclear, recent evidence indicates the endocannabinoid system may be involved (Muccioli et al., 2010), and further study is required to understand in what context.

**Gut microbiota as a drug target**

It is well established that creating an energy deficit by consuming fewer calories than one expends, or simply eating a healthy low-fat, low-sugar diet can lead to weight loss and improved metabolic parameters (Knowler et al., 2009). Studies have shown that such diets can increase Bacteroidetes, which correlates with weight loss and improved metabolic features (Ley et al., 2006). However, there is no definitive mechanistic study directly showing that a specific change in the proportion of Bacteroidetes and Firmicutes in the gut leads to such improvements. It is essential for future research to not only identify the mechanisms by which pathogenic bacteria cause their deleterious effects, but also to show that modulation of these mechanisms results in metabolic improvements. Interestingly, there are studies in genetically obese mouse models showing that antibiotics can lead to lower body weight, and improved metabolic features, such as fasting glycaemia, glucose tolerance, and inflammation (Cani et al., 2008; Membrez et al., 2008). One potential therapeutic avenue of interest is to capitalize on selective antibiotics or pre- or probiotics that promote the growth of beneficial bacteria, while hindering the growth of pathogenic strains.

Understanding the mechanistic details of how bacterial signal to the host cells of the intestine is essential in developing any sort of rational therapeutic agent. Indeed, recent studies have identified specific gut bacterial molecules that trigger signalling cascades in the host. These include the previously mentioned LPS, as well as polysaccharide A (PSA), a molecule secreted by Bacteroides fragilis that induces the conversion of a specific T cell to secrete the anti-inflammatory IL10 (Round and Mazmanian, 2010). This mechanism is dependent on Toll like receptor 2 (TLR2), and has been shown to not only prevent, but also cure experimentally induced colitis in a mouse model. Identifying and understanding how molecules such as this work mechanistically will allow for greater confidence in how modulation of the gut microbial environment can contribute to improvement in metabolic disorders. Alternatively, understanding how host mechanisms alter the growth environment for microbiota in the gut is also important. Recent studies identified intestinal alkaline phosphatase (IAP) as a key regulator of microbial homeostasis in the gut (Malo et al., 2010). Gaining insight into how molecules like IAP and other intestinal proteins involved in anti- or pro-microbial conditions, such as mucins and defensins, is imperative to develop a clear picture of the complex interactions between microbe and host.
Bariatric surgery

Gastrointestinal bypass operations were initially developed for the treatment of obesity nearly a half century ago (Mason and Ito, 1967), yet in recent decades have received increased attention for their ability to ameliorate metabolic disorders, such as type 2 diabetes, at a staggeringly high rate of success. The initial reports of this success, published in 1995 (Pories et al., 1995) have led to a number of recent studies attempting to understand the molecular and physiological mechanisms behind the surgery’s success. The “gold standard” surgery, Roux-en-Y gastric bypass (RYGB) involves stomach stapling, leaving only a small gastric pouch, and surgical rearrangement of the small intestine resulting in ingested food bypassing the duodenum and proximal portion of the jejunum, and being directly delivered to the distal jejunum (Rubino et al., 2010). The initial report and a subsequent meta-analysis of several clinical studies confirm diabetes remission in 83–84% of patients following this procedure, with some ceasing all other diabetes medications just days after surgery (Buchwald et al., 2004; Pories et al., 1995; Schauer et al., 2003). Other bypass surgeries, including biliopancreatic diversion (BPD), ileal transposition (IT), and duodenal-jejunal bypass (DJB) also exhibit high rates of diabetes remission in human patients and rat model systems (Buchwald et al., 2004; Cohen et al., 2007; de Paula et al., 2006; Patriti et al., 2005; Rubino and Marescaux, 2004; Scopinaro et al. 2007; Strader et al., 2005).

Interestingly, the most striking diabetes remission rates occur following bypass operations, whereas rates are much lower for purely gastric restrictive surgeries, such as laparoscopic adjustable gastric banding (LAGB) and vertical banded gastroplasty (VBG) (Buchwald et al., 2004). While remission following restrictive surgeries is thought to be the result of the dramatic weight loss observed, diabetes remission following bypass surgeries occurs well before weight loss (Schauer et al., 2003). In fact, head to head studies of bypass versus restrictive procedures, or bypass versus non-surgical caloric restriction show greater improvements in glycaemic control in patients receiving gastric bypass, indicating a weight independent mechanism triggering early disease resolution (Korner et al., 2007; Laferriere et al., 2008; le Roux et al., 2006). Several hypotheses have been posited in an attempt to explain these exciting results.

Hindgut hypothesis

One of the most likely mechanisms playing a major role in diabetes resolution is the hindgut hypothesis, which suggests that enhanced nutrient delivery to the distal small intestine due to bypass of the duodenum and proximal jejunum, leads to increased secretion of the gut hormones GLP-1 and PYY from entero-endocrine L-cells. Several groups have published experimental results showing increased plasma concentration of both hormones following RYGB (Korner et al., 2007; Laferriere et al., 2007; le Roux et al., 2006). Additional support for this hypothesis comes from the observation that IT, which places an L-cell rich segment of the ileum early in the digestive tract, also leads to improved insulin sensitivity, glycemic control, and importantly, enhanced plasma levels of both GLP-1 and PYY (de Paula et al., 2006; Patriti et al., 2005; Strader et al., 2005). The underlying notion is that increased GLP-1 and PYY may act via several mechanisms, including as an appetite suppressant via the “ileal-brake” mechanism and signalling to GLP-1 receptors in the brain that modulate satiety (Ashrafian and le Roux, 2009). Indeed, one report shows that plasma levels of GLP-1 and PYY post-surgery correlate with reduced appetite and post-surgery weight loss (le Roux et al., 2007). In addition to its role as appetite suppressants, GLP-1 plays a well-known role in stimulating insulin secretion from β-cells, as well as stimulating β-cell proliferation and reducing apoptosis (Drucker, 2006). A recent study performed in a diabetic rat model shows increased levels of PDX-1, a transcription factor required for β-cell proliferation, following RYGB (Li et al., 2010), suggesting increased GLP-1 concentrations may trigger this up-regulation leading to disease resolution.

Foregut hypothesis

While RYGB enhances nutrient exposure to the distal small intestine, it also eliminates exposure to the duodenum and proximal jejunum. In this regard, the foregut hypothesis posits that nutrient exclusion may alter secretion of a yet unknown hormone that may antagonize either incretins or insulin, or even promote insulin resistance (Rubino and Marescaux, 2004; Rubino et al., 2010). The success of the DJB surgery in rat models, as well as humans, supports this theory (Rubino and Marescaux, 2004). However, recent conflicting results suggest DJB does not improve metabolic features in all rat models (Kindel et al., 2011). In this study, non-diabetic, but insulin resistant diet-induced obese Long Evans and Wistar rats did not show metabolic improvements following DJB. This differs from previous reports using diabetic GK and ZDF rats (Rubino and Marescaux, 2004, Rubino et al., 2005), suggesting the level of β-cell function may play a role in the efficacy of DJB in improving metabolic symptoms. In support of the foregut hypothesis is the endoluminal sleeve (ELS) device that is inserted in the proximal small intestine and prevents exposure of nutrients to the duodenum and proximal jejunum, does not require physical re-routing of the intestinal tract (Rubino et al., 2010). ELS has successfully triggered improvements in glycaemic control in clinical trials (Rodriguez-Grunert et al., 2008).

Ghrelin

In addition to incretin and hypothesized anti-incretin effects, it is also suggested that alterations in ghrelin
levels may contribute to improved metabolic features following bariatric surgery. Ghrelin is orexigenic, stimulates glucagon secretion, suppresses the insulin-sensitizing adipokine adiponectin, and is considered pro-diabetic (Cummings et al., 2002; Cummings et al., 2005; Thaler and Cummings, 2009). Initial reports indicated ghrelin levels are reduced following RYGB (Cummings et al., 2002), which could enhance insulin sensitivity observed following bariatric surgery. However, conflicting reports indicate ghrelin levels may increase following certain bariatric procedures (Holdstock et al., 2003; Sundbom et al., 2007). While more work is necessary to understand this discrepancy, it has been hypothesized that surgical technique involving treatment of the vagus nerve may dictate which ghrelin response is observed (Ashrafian et al., 2010; Thaler and Cummings, 2009). Indeed, it would make sense that reduced ghrelin levels could contribute to diabetes resolution post-surgery by reducing appetite, but also by allowing increased adiponectin and reduced glucagon levels. However, further careful study is required for a better understanding of its putative role in these processes.

### Bile acid and nutrient sensing

One hypothesis that has recently emerged is that altered bile acid metabolism following bariatric surgery may contribute to diabetes resolution. It is known that bile acid sensing plays a role in modulation of glucose homeostasis (Thomas et al., 2009), and a recent report indicates that serum bile acid levels are increased following bariatric surgery (Patti et al., 2009). The anatomical rearrangement of bariatric surgery results in increased un-sequestered bile acids in the bypassed duodenum and proximal jejunum, as well as an increase in free lipid in the jejunum just distal to its reattachment site at the stomach before the two are rejoined further down the digestive tract. It is currently unknown what signalling and hormonal responses these alterations invoke specifically, although it has been hypothesized that increased lipid sensing may be involved in meal termination and inhibition of hepatic glucose production signals being transmitted to the brain (Wang et al., 2008).

### Gut microbiota

In addition to these innate mechanistic responses, recent reports indicate that the gut microbial community also changes following bariatric surgery. Consistent with the results described above, obese patients display a significant decrease in *Firmicutes* and the methanogenic *Archaea* post-RYGB (Zhang et al., 2009). Additional studies show that the *Bacteroidetes* and *Prevotella* bacterial groups are lower in obese patients when compared to lean controls at the time of surgery, but then exhibit a significant increase three months post-RYGB (Furet et al., 2010). While these results are consistent with bacterial community changes observed in obese patients put on fat-restricted or carbohydrate-restricted low calorie diets (Ley et al., 2006), it is unknown if the changes observed post-RYGB are a result of the altered intestinal nutrient flow, changes in gut hormone levels, or simply the restricted-diet patients eat following surgery. Interestingly, in the same study, the proportion of *Lactobacillus* was found to be decreased in the gut three months after bariatric surgery. Whereas pro-biotic treatment of obese patients undergoing RYGB with *Lactobacillus* led to statistically significant greater weight loss at six weeks and three months post-surgery compared to control patients (Woodard et al., 2009). This indicates that the changes in the microbial community post-surgery are not necessarily the ideal composition for optimal weight loss, and that alterations via selective supplementation can have an additive weight loss effect. Further research is required to understand why microbial changes specifically occur following bariatric surgery, and how do specific classes of bacteria contribute to the weight loss effect observed.

While each mechanism detailed above alone does predict positive glycaemic outcome, it is more likely that each of these mechanisms is not mutually exclusive, and collectively contribute to the improved physiological effects observed post-surgery. In fact, the collective physiological effects are now being referred to as the “BRAVE” effect (Ashrafian et al., 2010) for observed changes in bile acid flow, reduction of gastric size, anatomical gut rearrangement, vagal manipulation, and enteric gut hormone modulation. Indeed, it is important to understand how each of these mechanisms contributes to the overall metabolic improvements observed following bariatric surgery, and equally important is the identification of which synergies the combination of mechanisms may provide. Ultimately, this will allow for more efficient and streamlined surgeries making only the necessary anatomical alterations, and may also aid in drug discovery efforts eliminating the need for surgical intervention altogether.

### Conclusions

In recent years, the intestine has emerged as an important regulator of whole body metabolic homeostasis. While it is well established for its role in digestion and nutrient absorption, the fact that gut hormones specifically signal to various other organs and systems makes it an intriguing target for understanding novel mechanisms of metabolic control (Figure 1). The discovery of incretins and identification of their important role in stimulating insulin secretion has led to a wave of interest in developing novel therapeutic mimics. The success of GLP-1 analogues, such as Byetta (exenatide), at improving glycaemic control in patients with diabetes furthered this interest (Gentilella et al., 2009; Madsbad, 2009), with recent efforts focused on identifying pharmacological methods of stimulating endogenous incretin release or preserving its activity (via inhibition of DPP-IV). Similarly, GIP and PYY mimics continue to be attractive therapeutic modalities, as do combination treatments that mirror...
the physiological response observed following bariatric surgery (GLP-1 and PYY increases) that likely triggers diabetes remission.

While bariatric procedures, such as RYGB, are attractive therapies for treating type 2 diabetes, they are still dramatic surgical interventions that require lifelong care and adherence to specific diet. It is important to gain as much mechanistic detail as possible to understand how these surgeries cause such dramatic improvements in glycaemic control and other metabolic parameters, with the hope that molecules and mechanisms that are necessary and sufficient for these responses can be identified. This will allow for streamlined less invasive surgical interventions, or possibly even a combination therapeutic that will alleviate the need for surgery altogether.

Lastly, the emerging field of metagenomics and its relationship with the study of the microbiome has become a hot topic in recent years. Understanding how our diet affects the microbial population of our gut, and how this ultimately affects various metabolic mechanisms will be
an important field of research in the years to come. The hope is that this will identify a number of pathways that can be modulated to promote an environment that can nurture bacteria beneficial to human health. Along the same lines, understanding if anti-, pre-, or pro-biotic treatments can achieve similar results will undoubtedly continue in earnest.

Acknowledgements

The authors want to thank the Metabolic and Vascular Diseases Department at Hoffmann-La Roche Inc. and Dr. Francesco Rubino’s team at Weill-Cornell Medical College for their dedication and valuable discussions.

Declaration of interest

WDB, CZ, and CMR are employees of Hoffmann-La Roche Inc.

References


