

Review article: the emerging interplay among the gastrointestinal tract, bile acids and incretins in the pathogenesis of diabetes and non-alcoholic fatty liver disease

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SUMMARY

Background

Recent research has led to an interest in the role of the gut and liver in type 2 diabetes mellitus (T2DM).

Aim

To review the role of the gastrointestinal system in glucose homeostasis, with particular focus on the effects of incretin hormones, hepatic steatosis and bile acids.

Methods

PubMed and Google Scholar were searched using terms such as incretin, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), dipeptidyl peptidase-4 (DPP-4), hepatic steatosis, bile acid and gastric bypass. Additional relevant references were identified by reviewing the reference lists of articles.

Results

Perturbations of incretin hormones and bile acid secretion contribute to the pathogenesis of T2DM, leading to their potential as therapeutic targets. The incretin hormones (GIP and GLP-1) are deactivated by DPP-4. GLP-1 agonists and DPP-4 inhibitors improve glycaemic control in patients with T2DM. Hepatic steatosis, along with insulin resistance, may precede the development of T2DM, and may benefit from anti-diabetes medications. Bile acids play an important role in glucose homeostasis, with effects mediated via the farnesoid X receptor (FXR) and the cell surface receptor TGR5. The bile acid sequestrant colesevelam has been shown to be effective in improving glycaemic control in patients with T2DM. Altered gastrointestinal anatomy after gastric bypass surgery may also affect enterohepatic recirculation of bile acids and contribute to improved glycaemic control.

Conclusions

Research in recent years has led to new pathways and processes with a role in glucose homeostasis, and new therapeutic targets and options for type 2 diabetes mellitus.

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INTRODUCTION

Diabetes mellitus (DM) refers to a group of endocrine diseases that are all characterised by hyperglycaemia. It is one of the most rapidly rising diseases in the United States, affecting 25.8 million people (about 8.3% of the population), and is now the seventh leading cause of death in the United States.¹ Because of the glycaemic dysregulation related to these diseases, the secondary pathophysiological effects of DM on multiple organ systems impose an additional burden on the healthcare system. DM is the leading cause of end-stage renal disease, lower extremity amputations and blindness in adults, as well as a known aggravator of cardiovascular disease. Type 2 DM (T2DM), the most common type of DM, is classically described as a heterogeneous group of disorders that is characterised by a decline in insulin-producing pancreatic β cells, an increase in peripheral insulin resistance, an increase in hepatic glucose production, or a combination of these factors.

For decades, therapies for T2DM have focused on this 'triad' of characteristics, acting on the liver, pancreas, muscle and adipose tissue to reduce hepatic glucose production (e.g. biguanides), increase insulin secretion (e.g. sulphonylureas), and improve insulin sensitivity (e.g. thiazolidinediones). However, recent research suggests that other organs and processes play a vital role in glucose homeostasis and the pathogenesis of T2DM, and that these new pathways have potent therapeutic potential. Of particular interest has been the role of the gut in glucose homeostasis, through the direct action of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).

Processes occurring in the liver may also play a role in the pathogenesis of T2DM, although a causal link has not yet been definitively demonstrated. Hepatic steatosis (an excess of triglyceride accumulation in hepatocytes) is often observed in the presence of insulin resistance, including in T2DM. Emerging data suggest that hepatic insulin resistance and hepatic steatosis precede the development of T2DM. Diet and pharmacological therapies used in the management of T2DM have been shown to decrease hepatic triglyceride content, in addition to improving glycaemic control.

Emerging research also shows the importance of bile acids in glucose homeostasis, which may lead to an understanding of how bariatric surgery mediates its immediate effect on T2DM. Both the liver and intestines are involved in bile acid metabolism.

Various components of the gastrointestinal system contribute to the pathogenesis of T2DM and may be potential therapeutic targets. In the 2011 American

Association of Clinical Endocrinologists clinical practice guidelines for DM, incretin therapy has been recommended in controlling fasting glucose in patients with DM.² It is likely that incretin therapies will play a much wider role in the treatment of T2DM in the future. In this article, the role of the gastrointestinal system in glucose homeostasis will be reviewed, with particular focus on the effects of incretin hormones, hepatic steatosis and bile acids.

PubMed and Google Scholar were searched using terms such as incretin, GIP, GLP-1, dipeptidyl peptidase-4 (DPP-4), hepatic steatosis, bile acid and gastric bypass. Additional relevant references were identified by reviewing the reference lists of articles.

INCRETIN HORMONES

Incretins, which are hormones released by the gastrointestinal tract in response to nutrients, augment glucose-mediated insulin secretion.³ Their existence was suspected over a hundred years ago,⁴ but was not confirmed until researchers found that the oral administration of glucose resulted in a greater increase in insulin and a more sustained response when compared with glucose administered intravenously.^{5, 6} An estimated 50–70% of insulin secretion after glucose ingestion is attributable to this observation, which is now known as the 'incretin effect'.⁷

To date, only two hormones fulfil the definition of an incretin hormone.⁷ The first incretin hormone to be identified was gastric inhibitory polypeptide, or GIP. Initially, GIP was shown to inhibit gastric acid secretion when tested on dogs.⁸ However, further work on more purified samples in humans revealed that GIP augmented insulin secretion.⁹ As a result, the hormone was renamed to glucose-dependent insulinotropic polypeptide to maintain the original acronym, but to more accurately describe its function.⁷ GLP-1 was discovered later, during the sequencing of mammalian genes. When mapping the proglucagon gene, it was noted that, in addition to glucagon, two more peptides were encoded.^{10, 11} These two peptides were largely homologous to glucagon and hence were named GLP-1 and glucagon-like peptide-2 (GLP-2). However, only GLP-1 was found to stimulate insulin release.¹² Both GIP and GLP-1 potentiate the augmentation of glucose-mediated insulin response in an additive manner and together explain the incretin effect observed in humans.^{13, 14}

Secretion of incretin hormones

Interestingly, the two incretin hormones are synthesised independently by distinct cell types that are mainly organised in two different regions of the human gut. GIP is

synthesised and released from K cells located in the duodenum and proximal jejunum,¹⁵ whereas GLP-1 is produced and released from L cells that are primarily located in the distal jejunum and ileum, although fewer numbers are found scattered throughout the small intestine.¹⁶ They are both released in response to ingestion of nutrients, especially to glucose, carbohydrates and fats.^{17–19} During fasting, the circulating levels of GIP and GLP-1 are low, but both increase rapidly with the ingestion of nutrients.^{17, 19} Furthermore, their release is dependent on the size of the meal, i.e. the ingestion of large meals leads to secretion of higher amounts of both GIP and GLP-1 when compared with smaller meals.²⁰ Neither one is affected by intravenous administration of glucose.

In humans, both fat and protein markedly stimulate GIP secretion.²¹ The release of GIP is directly related to the rate of nutrient absorption rather than the presence of ingested material in the gut lumen.⁷ Hence, in patients with malabsorption, serum GIP levels are low.²² No other stimulator of GIP, besides nutrient absorption, has yet been found.

Serum levels of GLP-1 rise rapidly after ingestion of a meal, and its release occurs in a biphasic pattern.¹⁹ Peak levels occur within approximately 5–15 min after a meal, even though the ingested material has not yet reached the L cells in the distal jejunum and ileum at that point. This phase is followed by a longer 30- to 60-min subsequent phase. The early GLP-1 response to ingested material suggests that an indirect stimulation occurs via endocrine or neural mediators. This effect has been described as a proximal-distal neuroendocrine loop that relays stimulation to ingested foods from the proximal duodenum to distally located L cells that release GLP-1.^{23, 24} Several studies have shown that the autonomic nervous system, through the neurotransmitters gastrin-releasing peptide (GRP) and acetylcholine, contributes to the rapid release of GLP-1.^{25–27} Furthermore, in rats where the vagus nerve was severed, there was no initial peak in release of GLP-1 after a diet rich in fat.²³ Atropine, a muscarinic antagonist, diminished GLP-1 secretion in humans, further bolstering the theory that GLP-1 secretion is stimulated by neural mediators.²⁸ In several rat studies, GIP plays a role in GLP-1 secretion through vagal afferent-efferent pathways and the release of GRP.^{23, 25} However, GIP does not stimulate the secretion of GLP-1 in humans.²⁹ The second phase of GLP-1 release is due to the direct response of L cells to ingested food in the lumen.³⁰

Biological actions of incretin hormones

The incretin hormones mediate their insulinotropic effects mainly in the pancreas. However, GIP and GLP-1

have receptors in many extrapancreatic tissues that contribute to glucose homeostasis. The GLP-1 receptor has also been found in the liver, kidney, stomach, heart, lung, intestines, skeletal muscle, adipose tissue, nodose ganglion neurons of the vagus nerve, and the brain, including the brainstem and hypothalamus.^{31–35} GIP receptors are also expressed in a range of tissues in addition to the pancreas. They have been found in the stomach, small intestine, heart, lung, adipose tissue, adrenal cortex and the brain, including the cerebral cortex, hippocampus and olfactory bulb.³⁶ In addition, GIP has indirect effects on the liver, although no GIP receptors have been found in the liver and the mechanism for an indirect route of action has not been elucidated.^{7, 36} Table 1 summarises the role of the incretin hormones in various tissues.

In the pancreas, both GLP-1 and GIP stimulate glucose-dependent insulin secretion^{43, 44} and β -cell proliferation,^{45, 46} inhibit β -cell apoptosis,^{47, 48} and increase insulin production.^{49, 50} In addition, GLP-1 inhibits

Table 1 | Effects of GLP-1 and GIP on peripheral tissues^{7, 35, 37–42}

	GLP-1	GIP
Brain		
Neuroprotection	Increased	No effect
Appetite	Decreased	No effect
Progenitor cell proliferation	No effect	Increased
Heart		
Cardioprotection	Increased	No effect
Cardiac function	Increased	No effect
Stomach		
Gastric emptying	Decreased	No effect
Liver		
Glucose production	Decreased	Decreased
Pancreas		
Insulin secretion	Increased	Increased
Glucagon secretion	Decreased	No effect
Insulin biosynthesis	Increased	Increased
β -cell proliferation	Increased	Increased
β -cell apoptosis	Increased	Decreased
Bone		
Bone formation	No effect	Increased
Bone resorption	No effect	Decreased
Muscle		
Glucose uptake	Increased	Increased
Glucagon uptake and storage	Increased	No effect
Adipose tissue		
Lipogenesis	No effect	Increased
Glucose uptake	Increased	Increased

GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1.

glucagon production,⁵¹ whereas GIP stimulates glucagon secretion.⁵² However, glucagon secretion by GIP only occurs under basal glucose concentrations, and GIP may play a role in feedback control of glucose homeostasis, as the most profound augmentation of insulin secretion from GIP is seen under hyperglycaemic conditions.⁵²

The role of the incretins in the extrapancreatic tissues is diverse. Both GLP-1 (in the liver and/or kidney) and GIP (in the liver, presumably via an indirect mechanism) inhibit glucagon-stimulated glucose production.^{41, 42} In the gut, GLP-1 impedes gastric emptying and hence delays the rise in glucose after eating.^{53–55} In the central nervous system, GLP-1 has been shown to decrease appetite and food intake,⁵¹ which is mediated through GLP-1 receptors found on the nodose ganglion of the afferent vagus nerve.⁵⁶ In the muscle and adipose tissue, GLP-1 and GIP stimulate glucose uptake.^{35, 38–40}

Although there is some understanding of the release of incretins and which organs they affect, the way in which they control glucose homeostasis is poorly understood. In this case, GLP-1 has been better studied than GIP. There are two proposed mechanisms through which GLP-1 is hypothesised to mediate its effects: (i) the endocrine pathway; and (ii) the neural pathway.⁵⁷ In the endocrine pathway, GLP-1 is released directly into the systemic circulation after the L cells are stimulated by gut nutrients. It then binds to receptors in target organs such as the pancreas, where it increases intracellular cAMP and stimulates glucose-dependent insulin secretion.^{58, 59} GLP-1 also increases β -cell insulin stores through promoting insulin gene expression and stabilising transcription as well as stimulating β -cell proliferation and neogenesis.^{45, 46}

Neurons in the central nervous system contain GLP-1 and GLP-1 receptors – the first hint that GLP-1 has a neural pathway through which it mediates some of its actions. The predominant region of the brain that contains GLP-1 receptors is the nodose ganglion of abdominal vagal afferent nerve that terminates in the nucleus of the solitary tract.³³ GLP-1 promotes satiety and decreases food intake,^{32, 34, 51, 60} and GLP-1 agonists have led to weight loss in human studies.⁶¹

Given these two pathways, it may be that the GLP-1 insulin potentiation may occur as a combination of the two pathways. More than half of the GLP-1 secreted is inactivated before it reaches the systemic circulation.⁶² Furthermore, GLP-1 is metabolised in the liver, leaving only a small amount that actually reaches the pancreas.^{62, 63} It is now presumed that GLP-1 must use local neurons as intermediaries to signal the pancreas.⁶⁴

Incretin hormones in patients with T2DM

The incretin effect is severely reduced in patients with T2DM.⁶⁵ Although the cause of this is likely multifactorial, studies evaluating the secretion levels of incretins and physiological response to their exogenous administrations have shown two salient findings. There is a likely impaired secretion of GLP-1 and decreased activity of GIP.³⁷ In patients with T2DM, GIP secretion is normal or even increased in basal and postprandial conditions.³⁷ However, its insulinotropic activity has been shown to be greatly diminished in patients with T2DM, with response being 54% lower than that of normal controls.⁶⁶ In contrast to GIP, the response to GLP-1 in patients with T2DM was similar to that of controls. However, plasma levels of GLP-1 at meal time appear to be at least modestly diminished.^{20, 67–70} It is noteworthy to point out, however, that a few studies have demonstrated increased or unaltered levels of GLP-1.^{71–74} Finally, patients with secondary DM, such as those with DM secondary to chronic pancreatitis, have similar inhibition of their incretin activity, suggesting that this is a consequence of T2DM rather than a cause of it.⁷⁵

Synthetic GLP-1 agonists [exenatide (Amylin Pharmaceuticals, Inc., San Diego, CA, USA) and liraglutide (Novo Nordisk A/S, Bagsvaerd, Denmark)] are available for the treatment of T2DM when used as monotherapy or in combination therapy requiring subcutaneous administration.^{76, 77} These agents improve glycaemic control and promote weight loss, and are associated with low rates of severe hypoglycaemia.^{78–80} However, there have been reports of an association between GLP-1 agonist use and acute pancreatitis in patients with T2DM.^{81, 82}

The major pharmaceutical target influencing incretins has been DPP-4.⁸³ DPP-4, which cleaves GLP-1 and GIP, is found in many tissues including the gastrointestinal tract, biliary tract, liver, spleen, lungs, pancreas, kidneys and activated T lymphocytes.^{84–86} The protease resides on the surface of endothelial cells of blood vessels from the intestines; hence, it is in a perfect position to rapidly deactivate more than half of secreted incretins.⁶² DPP-4 knockout mice are associated with increased GIP and GLP-1, as well as enhanced insulin secretion after oral glucose administration.⁸⁷ Interestingly, such mice are also resistant to the development of obesity induced by a high-fat diet.⁸⁸ Four DPP-4 inhibitors are currently on the market: linagliptin (Boehringer Ingelheim International GmbH, Ingelheim, Germany), saxagliptin (Bristol-Myers Squibb, Princeton, NJ, USA), sitagliptin (Merck & Co., Inc., Whitehouse Station, NJ, USA), and

vildagliptin (Novartis, Basel, Switzerland; approved in various countries in Europe, Asia Pacific, Africa and Latin America). The DPP-4 inhibitors are generally well tolerated, weight neutral and not associated with hypoglycaemia,^{89–92} and are associated with a rise in plasma incretins after meals.^{93, 94} Glucose-mediated insulin secretion was enhanced, which was consistent with improved pancreatic β -cell function.^{93, 94} They significantly lower blood glucose and haemoglobin A1C levels, and are used either as monotherapy or in combination therapy.^{95–98}

NON-ALCOHOLIC FATTY LIVER DISEASE AND INSULIN RESISTANCE

Alcohol consumption and metabolic syndrome are the two main causes of hepatic steatosis. However, there are many potential causes of hepatic steatosis, including abetalipoproteinemia, acute fatty liver of pregnancy, malnutrition and refeeding syndrome, medications (e.g. amiodarone, methotrexate, tamoxifen), and environmental hepatotoxins (e.g. wild mushroom poisoning).⁹⁹ Hepatic steatosis that is associated with metabolic syndrome is commonly called non-alcoholic fatty liver disease (NAFLD). Insulin resistance and obesity are associated with an increased risk of NAFLD.¹⁰⁰ In patients with NAFLD, the prevalence of obesity is 30%–100% and the prevalence of T2DM is 10–75%.⁹⁹ NAFLD is the most common cause of abnormal liver enzymes in the clinical population of the US and affects approximately 20% of the population.¹⁰¹ In patients with NAFLD, the accumulation of triglycerides in hepatocytes can eventually develop into inflammation (non-alcoholic steatohepatitis [NASH]), fibrosis, cirrhosis and even hepatocellular carcinoma. The molecular and physiological changes that lead to NAFLD have been extensively studied and reviewed.^{102, 103} The current hypothesis for its development is that obesity and insulin resistance increase the release of free fatty acids (FFAs) from adipocytes.¹⁰⁴ Once these FFAs reach the liver, they are either oxidated to generate adenosine triphosphate (ATP) or esterified to produce triglycerides. Triglycerides either become part of very-low-density lipoprotein particles that are exported to the serum, or they are stored within the hepatocyte itself. Defects in any of these processes can result in excessive triglyceride accumulation in the hepatocyte and eventual cell damage and inflammation.

Emerging data suggest that hepatic insulin resistance and hepatic steatosis precede the development of T2DM.^{105, 106} Elevated serum alanine aminotransferase and fatty liver on ultrasound predict the occurrence of

diabetes.^{106, 107} A low-calorie diet is the primary therapy for insulin resistance and hepatic steatosis. Moderate diet-induced weight loss (5–10% of body weight) can decrease hepatic triglyceride content, improve glycaemic control and improve hepatic and muscle insulin sensitivity.^{108, 109} A meta-analysis of randomised trials of treatments for NAFLD found that weight loss was safe and improved histological disease activity in NASH in a dose-dependent fashion.¹¹⁰ However, more than 50% of patients failed to achieve target weight loss, and it remains unclear whether patients were able to maintain the weight loss.

Pioglitazone (Takeda Pharmaceutical Company Limited, Osaka, Japan), a thiazolidinedione, has also shown beneficial effects in patients with non-alcoholic steatohepatitis and impaired glucose tolerance or T2DM; in addition to metabolic improvements, treatment with pioglitazone was associated with reductions in hepatic fat content and corresponding improvements in histological findings.¹¹¹ Although an early meta-analysis of randomised trials for the treatment of NAFLD showed that thiazolidinediones improved histological steatosis and inflammation, but not fibrosis,¹¹⁰ a later meta-analysis that only included randomised, placebo-controlled trials (and hence excluded two open-label trials) showed that there was also an improvement in fibrosis.¹¹² However, patients treated with thiazolidinediones had significant weight gain.

There are also multiple randomised control trials suggesting a possible benefit of metformin in non-alcoholic steatohepatitis, in addition to its glycaemic effects in T2DM.^{113–116} Three trials showed improvement in liver histology after treatment with metformin.^{113, 115, 117} Thiazolidinediones and metformin have both been shown to phosphorylate liver kinase B1 (LKB1), which promotes its nuclear export. LKB1, in turn, activates adenosine monophosphate-activated protein kinase (AMPK) in the liver,^{118, 119} leading to inhibition of anabolic cellular processes such as hepatic lipogenesis and gluconeogenesis, in addition to stimulating catabolic processes such as glycolysis, fatty acid oxidation, and mitochondrial biogenesis.^{119, 120} It should be noted that more recent studies on metformin did not find a benefit in hepatic steatosis, serum markers or insulin resistance when compared with lifestyle modification.^{121–123} However, these results remain controversial, as the studies were conducted in small populations, and there were differences in duration and dose of treatment, and variable time periods between pre- and posttreatment biopsies.¹¹⁶

In addition, enzymes that increase oxidation of FFAs, which can prevent accumulation of hepatic triglycerides, can play a role in the treatment of hepatic steatosis, obesity, and other associated metabolic disorders. AMPK tightly regulates mitochondrial long-chain fatty acid oxidation through the inhibition of acetyl-CoA carboxylase 2 (ACC2).¹²⁴ A product of ACC2 is malonyl-CoA, which is a potent inhibitor of carnitine palmitoyltransferase 1 (CPT1), a mitochondrial membrane enzyme that controls beta-oxidation. Cardiac endothelial cells also oxidise fatty acids in a carnitine-dependent manner, suggesting that this enzyme plays a role in preventing inflammation and coagulation associated with metabolic syndrome and heart disease.¹²⁵ As a result, both CPT1 and ACC2 have become potential therapeutic targets.¹²⁶

THE ROLE OF BILE ACIDS IN GLUCOSE HOMOEOSTASIS

Recent studies show that bile acids play a much larger role in glucose homoeostasis than previously thought. After being released by the gallbladder into the intestines, nearly all of the bile acids (95%) get reabsorbed in the terminal ileum, decreasing the need for *de novo* bile acid synthesis.^{127, 128} Hence, there is frequent cycling of the bile acids (i.e. bile acid pool) between the intestines and the liver in the enterohepatic circulation. Bile acids are endogenous ligands to several receptors, including farnesoid X receptor (FXR) and pregnane X receptor (PXR), constitutive androstane receptor (CAR), vitamin D receptor (VDR) and the G-protein-coupled receptor TGR5. Through various signalling pathways, bile acids regulate cholesterol, fasting and mealtime glucose, and metabolism/energy homoeostasis as well as their own synthesis and blood levels in the enterohepatic circulation.^{127, 128} The composition of the bile acid pool has been shown to be altered in patients with T2DM.¹²⁹ In this section, we will discuss two bile acid signalling pathways, the FXR- and TGR5-mediated changes in homoeostasis, in detail.

Characterisation of several nuclear receptors has led to a better understanding of how hepatic metabolism can be altered by nutrition from the gut. Nuclear receptors have a ligand-binding domain and a DNA-binding domain. Once activated by a ligand, they can induce a transcriptional change in target genes. They provide important means through which cells can maintain homoeostasis in response to changes in their environmental stimuli, such as diet. Studies of several nuclear receptors that were previously thought to be orphaned now reveal that they respond to metabolites such as fatty acids and

oxysterols, in addition to digestive enzymes, such as bile acids.^{130, 131} FXR was a previously orphaned nuclear receptor until bile acids were discovered to be their ligands.¹³²

FXR is primarily found in the liver, kidney and intestines, and overall inhibits hepatic *de novo* bile acid production.^{133–135} Bile acids are produced when cholesterol is oxidised in the liver. The 'classical' pathway of bile acid production is via 7- α hydroxylation of cholesterol by a rate-limiting enzyme, cholesterol 7- α hydroxylase (CYP7A1). An 'alternate' pathway, with 27-hydroxylase in the mitochondria of extrahepatic tissues (e.g. endothelial cells), can also produce bile acids. FXR inhibits *de novo* bile acid formation with the induction of small heterodimer partner (SHP). SHP plays an essential role in feedback regulation of bile acid biosynthesis through repression of CYP7A1 by inhibiting two nuclear receptors: liver receptor homolog-1 (LRH-1) and hepatocyte nuclear factor-4 α (HNF-4 α ; see Figure 1).¹³⁷ *CYP7A1* is a critical regulatory gene in bile acid synthesis. By inhibiting *CYP7A1*, FXR inhibits the classic pathway of *de novo* bile acid formation and moves this process to extrahepatic tissues.

Earlier studies have shown that FXR plays an important role in lipoprotein metabolism,¹³⁸ and FXR knockout mice exhibited elevated plasma triglyceride and cholesterol levels as well as steatohepatitis.¹³⁹ More recent studies have shown that FXR is also important to glucose homoeostasis. FXR knockout mice showed impaired glucose tolerance and decreased insulin sensitivity.¹⁴⁰ The role of FXR in glucose homoeostasis was further bolstered when FXR synthetic agonists or induced overexpression of FXR repressed hepatic gluconeogenesis and enhanced glycogen synthesis and storage, inducing an overall lower blood glucose level.¹³⁵ FXR activation induces expression and secretion of fibroblast growth factor (FGF)19 and FGF21 in the intestine (Figure 1).¹⁴¹ Administration of FGF19 and FGF21 to diet-induced obesity mice increased their energy expenditure and caused weight loss and, as a result, improved insulin sensitivity.^{142, 143} Alterations in bile salts passing through the intestines have strong effects on metabolism by modulating FXR-mediated FGF19 and FGF21 secretion. Because FXR agonists can play a role in both treating steatohepatitis and improving glucose homoeostasis, they have become an area of intense research as potential pharmacotherapy for T2DM and non-alcoholic fatty liver disease.

Another mechanism through which bile acids affect glucose metabolism is a novel cell surface G-protein-coupled

receptor, TGR5 (Figure 1).^{144, 145} TGR5 is found in brown adipose tissue, the liver and intestines.¹⁴⁶ Bile acid stimulation of TGR5 increases intracellular cAMP. The effect of this rise in cAMP differs depending on the cell expressing TGR5. For example, in brown adipose tissue, bile acid induction of TGR5 results in a cascade of reactions that eventually converts inactive thyroid hormone (T4) to its active form (T3), hence modulating energy expenditure.¹⁴⁷ The role of bile acids on glucose homeostasis was reinforced when studies showed that murine enteroendocrine cell lines, when activated by bile acids, secreted GLP-1 via activation of TGR5.¹⁴⁸

Whether TGR5 actually plays a role in glucose homeostasis was not elucidated until a few years later. Thomas *et al.* showed that the TGR5 agonist INT-777 (Intercept Pharmaceuticals, Inc., New York, NY, USA; a bile acid mimetic) induced GLP-1 secretion in human L-cell lines by increasing intracellular levels of cAMP and altering the ATP/adenosine diphosphate (ADP) ratio and causing an influx of calcium, which, in turn, induced GLP-1 secretion.¹⁴⁹ TGR5 overexpression potentiated GLP-1 secretion, whereas TGR5 RNA interference blunted it. Activation of TGR5 by INT-777 in obese mice also prevented weight gain, preserved pancreatic function, and improved insulin sensitivity.

Given these findings, a more complete picture of the normal physiology of meal ingestion and bile acid glucose homeostasis can be formed. After ingestion of a meal, the gallbladder contracts and the amount of bile acids in the intestine increases. This, in turn, causes nutrient-induced secretion of GLP-1 from L cells via activation of TGR5. T2DM dampens gallbladder motility, leading to reduced flow of bile acids to the intestine. With reduced bile acids, there is decreased activation of TGR5 in L cells of the intestine, leading to lower secretion of GLP-1 and poor glucose homeostasis with decreased insulin secretion.¹⁵⁰

Despite growing literature on the direct role that bile acids can play in increasing GLP-1 by activating TGR5 in L cells, there is a paradox in that the main therapeutic target thus far has been to diminish the amount that can act in the terminal ileum. Bile acid sequestrants, such as colestevam hydrochloride (Daiichi Sankyo, Inc., Parsippany, NJ, USA), form non-absorbable complexes with bile acids in the gastrointestinal tract preventing reabsorption and promoting their faecal excretion.¹⁴⁶ Although bile acid sequestrants have been used for control of hyperlipidaemia for decades, they have shown to be effective in improving glycaemic control in patients with T2DM. Studies show that colestevam resulted in a

reduction of haemoglobin A1C of 0.5% when compared with placebo.^{151–153} Significantly greater reductions in fasting glucose levels were seen following 16–26 weeks of treatment with colestevam compared with placebo, in addition to insulin-, metformin-, and sulphonylurea-based therapy, further suggesting that bile acids play a role in fasting plasma glucose control.

An explanation for this paradox was proposed by Hofmann in a letter to the journal *Hepatology*.¹⁵⁴ Passage of fatty acids into the ileum (because micellar solubilisation is not yet completed in the jejunum) may lead to increased GLP-1 release from the ileal L cells. This is supported by the finding that administration of colestevam to rats with diet-induced obesity and insulin resistance led to improvements in glucose tolerance and insulin resistance that were accompanied by increased plasma GLP-1 levels; changes in these parameters were not seen in rats administered SC-435 (which decreases bile absorption in the ileum, leading to inactivation of FXR).¹⁵⁵ Similarly, in patients with T2DM, the addition of colestevam to a regimen comprising a sulphonylurea and/or metformin was associated with improvements in glycaemic control together with increased plasma levels of GLP-1 and GIP, compared with placebo.¹⁵⁶ This could be the mechanism through which glycaemic control improves in patients with T2DM with the bile acid sequestrant colestevam. In addition, this mechanism could explain improvements in glycaemic control following intestinal transposition or other bariatric surgery, as discussed in the next section.

THE EFFECTS OF GASTRIC BYPASS SURGERY ON GLUCOSE HOMOEOSTASIS

A majority of patients who undergo Roux-en-Y gastric bypass (RYGB) surgery are either completely cured of T2DM or have a large improvement despite initial negligible weight loss. A meta-analysis of 136 bariatric surgery studies reported that, of individuals who underwent gastric bypass, T2DM was resolved in 83.7%, and resolved or improved in 93.2%.¹⁵⁷ These patients achieve normal fasting plasma glucose and haemoglobin A1C levels.^{158–161} These changes occur before the patient has lost significant amounts of weight,^{160, 161} suggesting that the improvements in glycaemic control following RYGB may be caused by effects other than weight loss. As the primary objective of these procedures is to manipulate and reorient the gut, it is presumed that this improvement is mediated or potentiated by the gut.

A potential explanation for the improvements in glycaemic control seen following RYGB is altered secretion

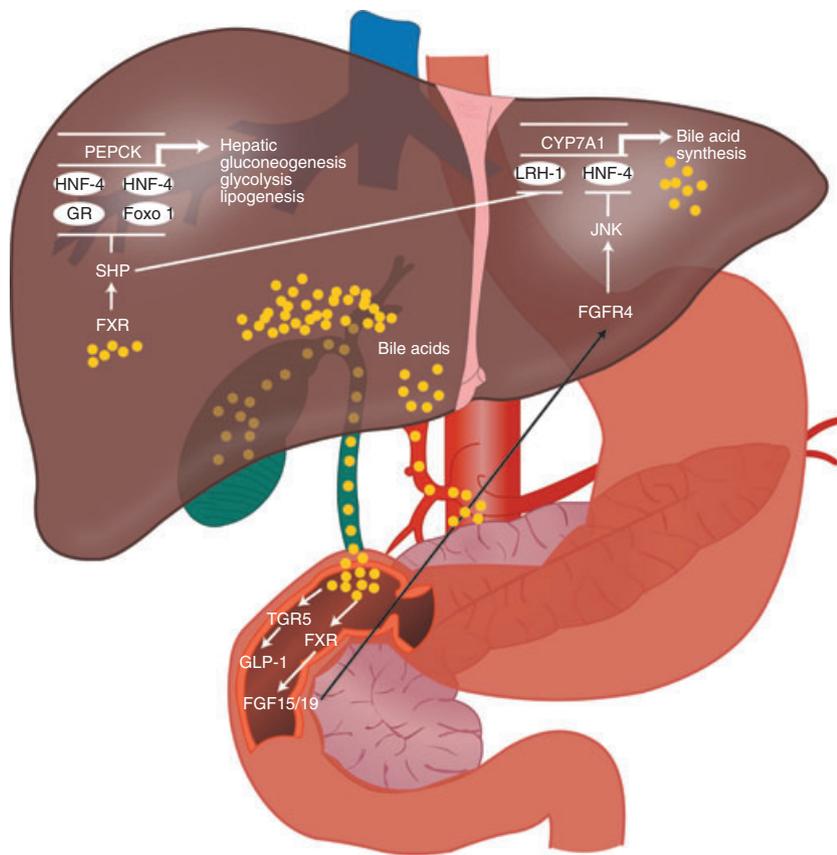


Figure 1 | Potential mechanism(s) of action for the glycaemic effects of a bile acid sequestrant. FGF, fibroblast growth factor; FGFR, FGF receptor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; GR, glucocorticoid receptor; HNF-4, hepatocyte nuclear factor-4; JNK, c-Jun N-terminal kinase; LRH-1, liver receptor homolog-1; SHP, small heterodimer partner. Reprinted with permission from Wright WL.¹³⁶

of GLP-1 secondary to gastrointestinal manipulation and reorientation. Patients who have undergone RYGB have significantly increased postprandial GLP-1 and insulin secretion, compared with obese and lean controls, and patients who had lost an equivalent amount of weight by gastric banding.¹⁶² This could be because more ingested material, especially fats and carbohydrates, are reaching the ileum and causing an increase in GLP-1 release from L cells.

Interestingly, altered gastrointestinal anatomy after RYGB may also affect enterohepatic recirculation of bile acids and contribute to improved glycaemic control. A study of patients who had undergone RYGB revealed that total fasting serum bile acids increased two-fold when compared with overweight or morbidly obese control participants.¹⁶³ A closer analysis showed that multiple bile acid subfractions, including both primary and secondary bile acids, reached statistical significance. Total serum bile acids were inversely correlated with 2-h postprandial glucose levels and positively correlated with GLP-1 levels. Similarly, in another study in Japanese adults who had undergone laparoscopic bariatric surgery, a positive correlation was observed between the changes in serum concentration of primary bile acids and plasma levels of GIP 1 month after surgery.¹⁶⁴ The mechanism

by which bile acids in the enterohepatic circulation increase is not fully understood; Patti *et al.* proposed that their observation that both primary and secondary bile acid levels are increased in the systemic circulation suggests that this occurs via increased uptake of bile acids in the intestines.¹⁶³ This increase could mediate its effects on GLP-1 increases through TGR5 receptors.

CONCLUSIONS

The understanding of the pathogenesis of T2DM is continuing to evolve. Increasingly, there is renewed interest in the gut's neuroendocrine response to nutrition. Although weight loss and diet remain the mainstay of treatment for T2DM, and are the safest, a large majority of patients are either unable to lose a sufficient amount of weight or maintain their lifestyle modification to have a sustained recovery. There is strong indication that bariatric surgery for the morbidly obese who have T2DM helps patients to attain a cure of T2DM by affecting the gut's neuroendocrine response to food. Research of the gut's response to nutrition in recent years has led to new pathways and processes with a role in glucose homeostasis (such as the incretin hormones and the bile acid pathway) and, as a result, new therapeutic targets and treatment options. Further understanding of the

gastrointestinal response to nutrition will help lead a new wave of novel pharmaceutical therapies to treat T2DM and NAFLD.

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