Abstract

Bile acid sequestrants have been used for decades for the treatment of hypercholesterolaemia. Sequestering of bile acids in the intestinal lumen interrupts enterohepatic recirculation of bile acids, which initiate feedback mechanisms on the conversion of cholesterol into bile acids in the liver, thereby lowering cholesterol concentrations in the circulation. In the early 1990s, it was observed that bile acid sequestrants improved glycaemic control in patients with type 2 diabetes. Subsequently, several studies confirmed the finding and recently – despite elusive mechanisms of action – bile acid sequestrants have been approved in the USA for the treatment of type 2 diabetes. Nowadays, bile acids are no longer labelled as simple detergents necessary for lipid digestion and absorption, but are increasingly recognised as metabolic regulators. They are potent hormones, work as signalling molecules on nuclear receptors and G protein-coupled receptors and trigger a myriad of signalling pathways in many target organs. The most described and well-known receptors activated by bile acids are the farnesoid X receptor (nuclear receptor) and the G protein-coupled cell membrane receptor TGR5. Besides controlling bile acid metabolism, these receptors are implicated in lipid, glucose and energy metabolism. Interestingly, activation of TGR5 on enteroendocrine L cells has been suggested to affect secretion of incretin hormones, particularly glucagon-like peptide 1 (GLP1 (GCG)). This review discusses the role of bile acid sequestrants in the treatment of type 2 diabetes, the possible mechanism of action and the role of bile acid-induced secretion of GLP1 via activation of TGR5.

Introduction

In recent years, it has become clear that bile acids are candidate agents in newly identified pathways through which carbohydrate metabolism and lipid metabolism are regulated. Bile acids are ligands of the nuclear farnesoid X receptor (FXR) (1, 2, 3) – a receptor that plays a central role in the regulation of synthesis, excretion and transport of bile acids, as well as lipid, glucose and energy metabolism (4, 5, 6). Bile acids also act as signalling molecules through the cell surface G protein-coupled receptor (GPCR) TGR5 (also known as M-BAR, GPBAR1 and GPR131) (7, 8). In brown adipose tissue (BAT) and skeletal muscles, TGR5 activation results in local activation of thyroid hormone through the stimulation of type 2 iodothyronine deiodinase (D2) (9, 10, 11). Moreover, in enteroendocrine L cells, TGR5 activation leads to the secretion of the incretin hormone glucagon-like peptide 1 (GLP1 (GCG)). In addition to its glucose-dependent insulinotropic effect, GLP1 also has glucagonostatic properties and induces satiety. Other hormonal L cell products with a satiety effect, such as peptide YY (PYY) and oxyntomodulin, may also be released following bile acid-induced TGR5 activation. This suggests that bile acids may regulate glucose homeostasis, appetite and body weight via TGR5 (12, 13, 14, 15). Indeed, bile acids and their
intestinal feedback signal fibroblast growth factor 19 (FGF19) have been suggested to be implicated in the beneficial glucometabolic changes taking place after Roux-en-Y gastric bypass (RYGB). Evidence suggests that manipulation of the bile acid pool with bile acid sequestrants, i.e. bile acid-binding agents, improves glucose control in patients with type 2 diabetes (16, 17). The mechanisms underlying the blood glucose-lowering effect of bile acid sequestrants are incompletely understood, but recent data have suggested that it may be mediated via increased secretion of the insulinotropic gut incretin hormones (13, 18, 19, 20, 21, 22). In the following, the effects of bile acids on glucose metabolism and lipid metabolism are reviewed. Furthermore, a potential role of bile acids in the pathophysiology of type 2 diabetes is described, and the effect of bile acids and bile acid sequestrants on human GLP1 secretion – including potential interplay with other gut hormones – and carbohydrate metabolism is reviewed. Finally, the use of bile acid sequestrants as a possible new therapeutic approach to augment GLP1 secretion is put into perspective.

The physiology of GLP1

Glucose-dependent insulinotropic polypeptide (GIP) and GLP1 constitute the incretin hormones and act in concert to generate the so-called incretin effect. The incretin effect expresses the augmentation of insulin secretion after oral administration of glucose compared with an isoglycaemic i.v. glucose stimulus (23, 24, 25). GIP is secreted from K cells primarily located in the upper small intestine (duodenum and proximal jejunum). GLP1 producing L cells are believed to exist throughout the small and large intestines with the highest cell density in the distal part of the small intestine (ileum) and colon (26, 27, 28). Both GIP and GLP1 are rapidly metabolised by the ubiquitous enzyme dipeptidyl peptidase 4 (DPP4), whereby the biological activity of both hormones is abolished (26). As mentioned above, GLP1 also inhibits glucagon secretion (during high plasma glucose concentrations), an effect that might be as clinically important as the insulinotropic effect of GLP1 (26, 29). GIP, however, has been proposed to elicit glucagonotropic actions (during low plasma glucose concentrations) and, hence, most likely plays a more complex role in glucose metabolism (30). Furthermore, GLP1 reduces food intake (most likely via activation of GLP1 receptors in the CNS) and delays gastric emptying, whereby postprandial glucose excursions are reduced (26).

GLP1 secretion

Meals containing organic nutrients (i.e. carbohydrate, fat and/or protein) are effective stimuli for secretion of GLP1 (31, 32, 33, 34) as well as many other gut hormones (26, 35). The exact mechanisms behind nutrient-induced GLP1 secretion remain elusive. It has been suggested that nutrients interact with luminal microvilli and, in the GLUTag cell model, a correlation among glucose absorption (36), glucose metabolism (37) and GLP1 secretion has been demonstrated. In dogs, blocking the luminal sodium–glucose transporter SGLT1 on intestinal L cells decreases GLP1 secretion, positioning SGLT1-mediated glucose uptake as an important regulator of GLP1 secretion (38, 39). Several GPCRs have been identified on the L cells. These include GPR119 (40), which is activated by N-acylethanolamines (41), GPR120 (42) and GPR40 (43), which are activated by long-chain fatty acids (LCFAs), GPR41 (44), GPR43 (45) and FFAR2 (46), which are activated by short-chain fatty acids (SCFA), and TGR5 (8), which is activated by bile acids. In addition, taste receptors (primarily T1R2/T1R3 and α-gustducin) in the stomach and intestine seem to regulate the secretion of GLP1 (47, 48, 49). Paracrine, neuronal and neurohormonal mechanisms may also be important for the facilitation of postprandial GLP1 secretion (50, 51, 52, 53). As mentioned earlier, bile acids are able to activate TGR5 (7, 8, 9), resulting in GLP1 secretion from intestinal L cells (12, 14). Already in the 1980s, there were reports of bile-induced secretion of GIP (54, 55) and glucagon-like reactive materials in dogs (46, 57, 58, 59) and insulin (60). Since then, various groups have reported similar findings (13, 61, 62, 63, 64).

The concept of GLP1-based treatment revisited

The concept of GLP1-based treatment of type 2 diabetes is rooted in augmentation of peripheral concentrations of GLP1 receptor agonists – either endogenous active GLP1 (via DPP4 inhibitors) or exogenous administration of synthetic DPP4-resistant GLP1 receptor agonists. In general, incretin-based treatment has not yet been able to show GLP1-induced remission of type 2 diabetes in humans – as anticipated from some animal studies (65). Acknowledging the fact that a substantial part of GLP1’s effects is elicited locally, i.e. in close proximity to where GLP1 is secreted (26), a possible explanation for this limitation may be that synthetic GLP1 receptor agonists and DPP4 inhibitors primarily elevate the concentrations of GLP1 receptor agonists in plasma. By contrast, the
elevated plasma concentrations of GLP1 observed after the RYGB (comparable to the GLP1 receptor agonist concentrations in plasma observed during s.c. treatment with synthetic GLP1 receptor agonist) (66, 67, 68) result in diabetes remission rates of up to 80% (69), depending on remission definition (70), in obese patients with type 2 diabetes. This discrepancy may be explained by the fact that local effects of GLP1 secretion in the intestine are not fully exploited during GLP1-based treatment modalities. Such local effects could include stimulation of local afferent sensory nerve terminals (residing in the lamina propria, the portal vein or in the liver), which communicate with the nuclei of the solitary tract and hypothalamus. Hereby, important physiological effects such as reduced gastric emptying, inhibition of appetite and food intake and modulation of pancreatic hormones can be elicited through neural activation. Preclinical studies have provided evidence for an important neural mechanism of GLP1 function (26, 50). By targeting GLP1 secretion, a whole new treatment concept, based on local effects of GLP1, may be unravelled. One approach is potentiation of GLP1 secretion via bile acid-induced TGR5 activation. In fact, stimulation of GLP1 release with agents that are neither deposited (i.e. bile acids or synthetic TGR5 receptor analogues) nor absorbed (i.e. bile acid sequestrants) is captivating and might prove superior in controlling type 2 diabetic hyperglycaemia and obesity compared with current GLP1-based treatment strategies. In the following, some key elements from the current understanding of GLP1 secretion will be outlined in the context of bile acid physiology and bile acid sequestrants.

Bile acids

Bile acids are water-soluble, amphipathic molecules synthesised from cholesterol in the liver. After hepatic conversion of cholesterol, involving ~16 enzymatic reactions (71, 72), bile acids are secreted into the canalicular space between hepatocytes. Bile then flows into the bile ducts and, during fasting, half of the bile – or 450 ml/day – enters the gallbladder and the other half flows into the intestine (4). Owing to isotonic reabsorption of NaCl and NaHCO3 in the leaky epithelium of the gallbladder – mainly mediated by vasoactive intestinal polypeptide (released from neurons innervating the gallbladder) and secretin – bile salts are concentrated up to 20-fold within the gallbladder lumen (73, 74, 75). Upon meal ingestion, the gallbladder contracts and relaxation of the sphincter of Oddi occurs, whereby bile acids from the liver and highly concentrated bile from the gallbladder are released into the intestinal lumen. Herein, they interact with dietary lipids, lipid-soluble vitamins and cholesterol, forming micelles, and thereby facilitate the uptake of these molecules (76). Reabsorption of bile acids occurs primarily in the terminal ileum where bile acids are transported from the lumen into the portal bloodstream and back to the liver (only 5% of bile acids escape intestinal uptake – see below – and are excreted in the faeces) (Fig. 1) (4). Reabsorption occurs via the apical sodium-dependent bile salt transporter (ASBT) and then bile acids are effluxed to the portal vein via the heteromeric organic solute transporter α/β, the multidrug resistance-associated protein 3 and a truncated form of ASBT. This complex transport system constitutes just a small part of the enterohepatic cycling of bile acids, which is reviewed in great detail elsewhere (4).

The human liver produces the primary bile acids cholic acid and chenodeoxycholic acid and their glycine and taurine conjugates. In the intestinal lumen (terminal ileum and colon), primary bile acids may undergo deconjugation and dehydroxylation by bacteria, resulting in secondary bile acids, of which the most important are deoxycholic acid and lithocholic acid (6). The conversion of cholesterol into bile acids by the liver enzymes accounts...
for ~90% of cholesterol breakdown (71, 72). Bile acids also control gut flora by inhibiting the growth of bacteria in the small intestine (77, 78). The mass of circulating bile acids is termed the bile acid pool and can be measured by isotope dilution (79).

**Regulation of the bile acid pool: the role of FXR and FGF19**

Bile acids are powerful detergents and toxic due to their high hydrophobicity. Consequently, the composition of bile acids is strictly regulated, as is their synthesis, secretion, transport and metabolism. Importantly, the biological properties of bile acids depend on their chemical structure, thus indicating that bile acid pool size and composition are regulatory factors for potential bile acid signalling pathways (4, 6, 80, 81). Bile acids feed back to regulate their own synthesis by binding to FXR in the liver (1, 2). FXR is activated by both primary and secondary bile acids, with chenodeoxycholic acid being the most potent natural ligand (1, 2). The activation of FXR in the liver (82) leads to increased conjugation of primary bile acids followed by the excretion of bile acids, thereby promoting bile flow into the lumen of the gastrointestinal tract (4, 80, 83, 84, 85). Furthermore, FXR activation in liver tissue induces transcription of the inhibitory small heterodimer partner (Fig. 1). As a result, transcriptional activity of the nuclear receptors, liver receptor homologue and hepatocyte nuclear factor 4α, is reduced. This leads to impaired activity of the microsomal enzyme cholesterol 7α hydroxylase (CYP7A1), the rate-limiting enzyme of the so-called ‘classic’ or ‘acidic’ pathway of bile acid biosynthesis. Via a small heterodimer partner, FXR activation also inhibits the ASBT (intestine) and the Na⁺-taurocholate co-transporting polypeptide (NTCP) transporter (liver) (4). In addition to FXR activation in the liver, bile acids activate FXR in the distal small intestine, postprandially, and induce expression and secretion of FGF19 (designated as FGF15 in mouse). FGF19 has been established as a postprandial gut hormone (Fig. 2) (1, 2, 3, 6). In recent years, it has become clear that bile acids are signalling molecules with classical endocrine properties (6, 80) and work as metabolic integrators modulating lipid and glucose metabolism (see below) (6, 14). These integrative functions of bile acids are most likely mediated by activation of the TGR5 in the intestine (7, 8) and the FXR and FGF19 signalling pathways in the liver and the intestine (Fig. 2) (1, 2, 3, 6).

**Bile acids activate GPCRs**

As mentioned earlier, bile acids activate TGR5 – one of the three GPCRs activated by bile acids; the others being muscarinic receptors (M1–5) (96, 97) and formyl peptide receptors (98, 99). TGR5 is widely expressed in the gastrointestinal tract and associated glands, including human gallbladder epithelium and cholangiocytes (100, 101, 102), several cell types in the liver (103, 104), spleen (8), colon and ileum (7, 101, 105). In addition, TGR5 is expressed in human monocytes and CD14+ white blood cells (8), and, interestingly, in BAT, skeletal muscle and various areas of the CNS (9, 106, 107). TGR5 is activated by several bile acids, with lithocholic acid being the most potent natural agonist (7, 8). More hydrophilic bile acids, deoxycholic acid, chenodeoxycholic acid and cholic acid, are less potent activators of TGR5 (8). TGR5 has recently been found in human pancreatic islets and shown to release insulin upon stimulation with the TGR5 selective ligands oleanolic acid and INT-777 (a semisynthetic cholic acid derivative, and a potent and selective TGR5 agonist) and also lithocholic acid (108).

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**Bile acids and energy expenditure**

An intriguing effect of bile acids is their ability to affect energy expenditure. FXR activation has been suggested to be involved in bile acid-induced energy expenditure (109), but recent studies in mice have indicated that bile acids increase energy expenditure through activation of the D2 in BAT, resulting in deiodination of the minimally active
thyroid hormone thyroxine (T₄) to the biologically active triiodothyronine (T₃) (9). Interestingly, Thomas et al. showed that administration of INT-777 attenuated weight gain in C57BL6/J mice fed on a high-fat diet compared with control mice not receiving INT-777. These findings were related to enhanced energy expenditure, as indicated by the measurement of O₂ consumption and CO₂ production during indirect calorimetry (14), suggested to be a result of an induction of D2 (DIO2) gene expression (along with an increase in several mitochondrial genes involved in energy expenditure) (9, 14). However, the physiological relevance (bile acid-induced increase in energy expenditure) of these findings is somewhat contentious because INT-777 improves EC₅₀ on TGR5 by 30-fold compared with cholic acid.

Watanabe et al. have recently carried out a study on C57BL/6j mice fed on a normal chow, high-fat diet or high-fat diet supplemented with either the bile acid sequestrant colestimide (2% w/w) or cholic acid (0.5% w/w) for 96 days (10). Notably, both treatments augmented energy expenditure, caused weight reduction and improved insulin sensitivity, and the authors speculated, with support from older studies (9, 10, 110), that these effects may be TGR5 mediated due to changes in the bile acid pool (increased concentrations of cholic acid) and gene expression in liver, BAT, muscle and ileum after both treatments (genes involved in bile acid synthesis, gluconeogenesis and thyroid function (D2)). Presumably, the ability of bile acids to increase energy expenditure is linked to a TGR5-mediated rise in cAMP, which results in augmented activation of D2 and thereby increased conversion of T₄ into T₃ in BAT (rodents) and muscle (humans) (11). However, human studies have yielded conflicting results (111, 112, 113). Patti et al. (21) showed that total bile acids in serum correlate inversely with thyrotrophic hormone (TSH) in patients who have undergone RYGB surgery, and the works by Nakatani et al. (114) and Simonen et al. (115) demonstrated similar results. By contrast, Brufau et al. (111) could not demonstrate any effect of bile acids or bile acid sequestrants on energy metabolism in humans. Thus, further studies are warranted to clarify whether bile acids and bile acid sequestration affect energy expenditure and promote weight reduction in humans.

**Bile acid sequestrants**

Bile acid sequestrants (cholestyramine, coleselam, colestimide and colestipol) are non-absorbable resins that bind negatively charged bile salts in the intestinal lumen. Via this mechanism, bile acids are incorporated into a complex that gets excreted in the faeces – diverting bile acids from the enterohepatic cycle (116, 117). To compensate for the reduction of the bile acid pool, delivery of LDL cholesterol (substrate for bile acid production) to the liver is increased, and bile acid synthesis is increased by a factor four to six. Thus, bile acid sequestrants decrease circulating concentrations of LDL cholesterol. Other contributing factors to the LDL cholesterol-lowering effect are enhanced cholesterol synthesis and up-regulation of LDL receptors (79, 118). The cholesterol-lowering action of bile acid sequestrants has been known since the early 1960s (76), and since then, bile acid sequestrants have been used for the treatment of hypercholesterolaemia. In line with this, studies have shown a decrease in coronary heart disease following treatment with bile acid sequestrants (119, 120).

**Sequestration of bile acids modulate the bile acid pool**

In the study by Brufau et al. (121), 2 weeks of treatment with coleselam altered the synthesis of specific bile acids, which affected their relative contribution to the total pool size. Cholic acid concentration increased by more than twofold (from 30 to 65%) in both control subjects and type 2 diabetic patients, whereas the concentrations of chenodeoxycholic acid and deoxycholic acid decreased in both groups (from ~35 to ~15%). Thus, the ratio of cholic acid to the sum of chenodeoxycholic acid and deoxycholic acid (‘triols’ vs ‘diols’, a surrogate
marker of the hydrophobicity of bile acid pool (6)) resulted in a fivefold increase in both groups, indicating a considerable decrease in the hydrophobicity in the bile acid pool. This pattern has also been observed in older studies after treatment with bile acid sequestrants (122, 123, 124). However, in a recent study by Beyens et al., treatment with colesvelelam has resulted in increased fractional synthesis of both chenodeoxycholic acid and cholic acid from newly synthesised cholesterol, suggesting that individual bile acids respond differently to bile acid sequestrants (22, 125, 126, 127). Interestingly, bile acid sequestrants have been reported to slow colonic transit time (128), which is known to increase deoxycholic acid concentrations in colon and plasma (due to bacterial dehydroxylation (129)), and thus, may possibly also lead to enhanced TGR5 activation in the L cell-rich milieu of the colon (see below).

The physiological significance of changes in bile acid pool composition induced by bile acid sequestration may arise from the altered signalling on the FXR receptor, as well as other receptors (i.e. liver X receptor (LXR) and TGR5) (6). Indeed, concentrations of FGF19 are lowered in type 2 diabetic patients were characterised by increased synthesis in preclinical studies (135, 142, 143) and in recent human studies (22, 111, 121, 140). Of note, both glucose (141) and insulin have been suggested to modulate bile acid synthesis in preclinical studies (135, 142, 143) and in some (131, 138, 144), but not all (137), clinical studies. As noted by Staels & Foncea, the finding that insulin is able to suppress FXR (NR1H4) gene expression (in contrast to glucose, which produces the opposite effect) suggests that diabetes is associated with the dysregulation of FXR expression (141, 145). Indeed, Brufau et al. showed that patients with type 2 diabetes exhibited increased concentrations of deoxycholic acid and decreased concentrations of chenodeoxycholic acid, which was due to the increased synthesis rate of cholic acid and deoxycholic acid. Other human studies have found similar changes in the bile acid pool in diabetes, but these studies are difficult to interpret in a comparative manner due to different methodologies and study populations (131, 136, 136, 137, 138). Recently, Haeusler et al. have reported that there might be a plausible, mechanistic explanation for diabetes-related changes in the bile acid pool composition involving Forkhead box protein 01 (FOX01 (FOX1)), a transcription factor regulating gluconeogenesis, glycogenolysis and liver sensitivity to insulin (140, 146). They showed that mice lacking Fox01 developed a less hydrophilic bile acid pool (146). Moreover, FOX01 activity was shown to be important for Cyp8b1 transcription (12α-hydroxylase). As CYP8B1 determines bile acid pool composition (increases cholic acid production) (147) and was relatively deficient compared with the other enzymes, 12α-OH bile acid concentrations (mainly cholic acid and deoxycholic acid) were found to be lower compared with concentrations of non-12α-OH bile acids (mainly chenodeoxycholic acid) (146). Interestingly, the activity of FOX01 is inhibited by insulin via Akt-dependent phosphorylation and nuclear exclusion of FOX01. Thus, the authors hypothesised that, in diabetes, the inhibition of FOX01 might fall short leading to increased synthesis of 12α-OH bile acids as well as increased hepatic glucose production.

In an elegant study in healthy subjects and patients with type 2 diabetes, Haeusler et al. provided support to this hypothesis with the finding of a significant association between the ratio of 12α-OH/non-12α-OH bile acids and the degree of insulin resistance. By contrast, however, the diabetic population of the study exhibited a higher hydrophobicity index (due to higher concentrations of deoxycholic acid and its conjugated forms, relative to cholic acid and chenodeoxycholic acid and their conjugates), but no disproportionate increase in 12α-OH bile acids despite the marked insulin resistance (140). It bears emphasising that a larger ratio of 12α-OH/non-12α-OH bile acids may theoretically induce less FXR activation owing to relatively lower concentrations of chenodeoxycholic acid – the most abundant non-12α-OH bile acid and a potent FXR agonist in humans. Thus, these results confirm that alterations in the bile acid pool composition and FXR activity may constitute important factors in the pathophysiology of type 2 diabetes. Metabolomic studies have shown that patients with type 2 diabetes exhibit a lower cholic acid concentration and a higher deoxycholic acid concentration compared with control subjects, indicating that cholic acid in type 2 diabetes might be increasingly converted into deoxycholic acid (148).
Similar studies have also revealed that bile acid concentrations become markedly increased in serum in response to an oral glucose challenge, suggesting that systemic bile acids may orchestrate the fine-tuning of human glucose homoeostasis (149, 150) – possibly through FXR signalling (6).

The mechanisms underlying the abnormal composition of the bile acid pool in patients with type 2 diabetes may also be linked to their gut microbiota composition, which has been suggested to be different from that of healthy control subjects (see below) (151, 152). As mentioned earlier, reduced colonic transit time (i.e. constipation), the commonest gastrointestinal symptom of type 2 diabetes, also increases bacterial dehydroxylation of cholic acid to yield deoxycholic acid, possibly explaining part of the postulated alterations of deoxycholic acid concentrations in these patients (128, 129). Despite the unknown causality of the changed bile acid pool composition in type 2 diabetes, it constitutes an attractive field of research into possible new treatment targets – perhaps based on modulation of specific bile acids in patients with type 2 diabetes. In fact, already, sequestration of bile acids in the lumen of the gut represents a way of treating type 2 diabetes.

Sequestration of bile acids alters glucose metabolism

In 1994, Garg & Grundy (16) established clinical evidence that bile acid pool modulation affects glucose metabolism. In addition to the sound effect of the bile acid sequestrant cholestyramine on total and LDL cholesterol concentrations in patients with hypercholesterolaemia and type 2 diabetes, bile acid sequestration also, surprisingly, resulted in improvements of mean plasma glucose concentrations and urinary glucose excretion. The effect of bile acid sequestration on glucose homoeostasis has subsequently been corroborated in recent clinical trials (17, 153, 154, 155, 156, 157, 158, 159), but the exact mechanisms of how bile acid sequestrants improve glycaemic control are contentious (Fig. 3).

Originally, bile acid sequestrants were suggested to affect glucose absorption (160, 161). However, this has not been confirmed in later in vivo studies (121, 162). Indeed, in a pilot study by Schwartz et al. (162), the first dose of colesevelam with a standard meal had no effect on postprandial concentrations of glucose compared with baseline and placebo. Furthermore, there was no effect on peripheral insulin sensitivity measured by the hyperinsulinaemic–euglycaemic clamp technique (164). Neither acute nor chronic treatment with colesevelam seems to affect post-OGTT glucose concentrations (162, 164, 165). However, post-meal concentrations (total area under the curve) have been found to be slightly reduced after both acute (166) and chronic treatments (weeks) with colesevelam (17, 130, 162, 167). Notably, examining glucose kinetics, Beysen et al. (22) could not demonstrate any effect of colesevelam on endogenous glucose production, and recently, Smushkin et al. (167) showed that colesevelam decreased the appearance of meal-derived glucose, without changes in insulin secretion, insulin action or GLP1 concentrations. Thus, the mechanisms behind the glucose-lowering effect of bile acid sequestrants remain a matter of controversy.

As mentioned previously, bile acid sequestrants were originally developed for the purpose of binding bile acids in the intestinal lumen, diverting bile acids from the enterohepatic cycle (116, 117). In this respect, it is important to reiterate that bile acids themselves are increasingly being recognised as modulators of hepatic glucose metabolism via FXR signalling (168, 169, 170, 171, hepatic insulin sensitivity may have been improved (162). However, in another study, colestipol did not appear to affect hepatic or peripheral insulin sensitivity as measured by the hyperinsulinaemic–euglycaemic clamp technique (164). Neither acute nor chronic treatment with colestipol seems to affect post-OGTT glucose concentrations (162, 164, 165). However, post-meal concentrations (total area under the curve) have been found to be slightly reduced after both acute (166) and chronic treatments (weeks) with colestipol (17, 130, 162, 167). Notably, examining glucose kinetics, Beysen et al. (22) could not demonstrate any effect of colestipol on endogenous glucose production, and recently, Smushkin et al. (167) showed that colestipol decreased the appearance of meal-derived glucose, without changes in insulin secretion, insulin action or GLP1 concentrations. Thus, the mechanisms behind the glucose-lowering effect of bile acid sequestrants remain a matter of controversy.
Animal studies have demonstrated that FXR activation inhibit gluconeogenesis (169, 175), whereas others report an overall activation of gluconeogenesis (141, 170, 173). It has been suggested that the control of FXR activation on whole-body glucose homoeostasis is limited to certain time points of the fasting/feeding cycle (80, 176). Furthermore, Fxr-deficient mice are characterised by decreased hepatic glycogen (168) and exhibit reduced insulin sensitivity and secretion (169, 173, 177). Of note, experiments on human islets and β-cell lines have revealed the findings on FXR-dependent insulin secretion (178, 179). With the recent findings outlined above, some of which may constitute new avenues in the search for the target of antidiabetic and antiobesity treatments, it seems to be of tremendous importance to delineate the precise mechanisms by which bile acids and FXR modulate hepatic glucose metabolism, and how FXR activity changes in response to bile acid sequestration in the gut.

Another hypothesis behind the glucose-lowering effects of bile acids and bile acid sequestrants is rooted in FGF19. As already pointed out, FGF19 is secreted upon postprandial bile acid activation of intestinal FXR (87, 180). In addition, FGF19 is secreted from the human gallbladder into the bile at very high concentrations compared with plasma concentrations, suggesting a yet undefined exocrine function of FGF19 (181). A decade ago, Tomlinson et al. (182) demonstrated, in transgenic mice, that FGF19 modulates energy and glucose homoeostasis. Most striking was the observation that FGF19 shares some metabolic actions of insulin, namely the stimulation of protein synthesis and glycogen synthesis (independent of insulin), and inhibition of gluconeogenesis (183, 184, 185). Besides positioning FGF19 as a selective agonist of insulin signalling (without promoting lipogenesis), Kir et al. (184) demonstrated that Fgf15 (the FGF19 equivalent in mice)-null mice exhibited increased blood glucose concentrations after an oral glucose bolus. These studies suggest that FGF19 acts subsequent to insulin as a postprandial regulator of hepatic carbohydrate homoeostasis, utilising signalling pathways independent of insulin (183). In this regard, it is intriguing that FGF19 concentrations, in some studies, are lower in type 2 diabetic patients compared with control subjects (186, 187). However, not all researchers agree on this postulate (121), but, interestingly, it has been proposed that diabetic patients may also exhibit decreased FGF19 signalling and a subsequent impaired FGF19-dependent reduction in bile acid synthesis (121, 188). Lastly, following RYGB surgery, FGF19 concentrations increase, probably reflecting enhanced delivery of bile to the distal intestine and thus increased activation of FXR (21, 189, 190). Thus, FGF19 may constitute an important, postprandial enteroendocrine factor – with possible incretin-like actions – regulating hepatic protein and glycogen synthesis and gluconeogenesis (180, 184). Although fasting FGF19 concentrations may be lower in type 2 diabetic patients, little is known about the physiological relevance of this finding (180). However, the presence of lower FGF19 concentrations in type 2 diabetic patients fits well with the notion of reduced FXR activity, putatively, owing to a less hydrophilic bile acid pool (as mentioned above). Certainly, further studies are required to elucidate the possible contribution of impaired FGF19 signalling to dysregulation of glucose homoeostasis, and importantly, such studies should also outline the importance of the observed cell proliferative effects of FGF19 (191).

As already mentioned, a novel hypothesis takes into account that the gut microbiota composition is altered in type 2 diabetes (151, 152). Notably, gut bacteria are known to exert a great impact on bile acids (and vice versa) (147), cholesterol and glucose metabolism (192). Thus, as recently suggested (193), and elegantly confirmed (194), changes in the gut microbiome may influence glucose metabolism itself. Hypothetically, bile acid sequestrants may, via bile acid binding, be capable of inducing changes in the gut microbiota composition, adding yet another intriguing aspect of bile acid sequestration. Clearly, further studies on this matter are warranted.

Finally, as already outlined, bile acid sequestration is considered to enhance GLP1 secretion via bile acid-induced activation of the intestinal TGR5 receptor. Recent evidence in animal and human studies has provided rigorous proof of this hypothesis and, therefore, in the following, we will discuss the physiological importance of TGR5 in the gut and propose viewpoints as to how bile acid sequestrants may exert their glucose-lowering effects through TGR5-dependent GLP1 release.

**Effects of bile acid sequestrants on GLP1 secretion**

As for the FXR-dependent effects mentioned above, the mechanisms responsible for the enhancement of GLP1 secretion seen after bile acid sequestration remain enigmatic. Recent studies have suggested that cholestyramine and colesevelam improve insulin resistance in diabetic rats by increasing GLP1 release, independent of FXR signalling (activity reduced, see above) (195, 196, 197, 198). As
already mentioned, these findings were not confirmed in type 2 diabetic patients after colesevelam treatment (130, 162). However, colestimide treatment for 1 week has been shown to reduce postprandial glycaemia in type 2 diabetes – an effect that was associated with increased postprandial GLP1 plasma concentrations (18). In line with these results, a recent multicentre, randomised, parallel, double-blind, placebo-controlled study has shown that treatment with colesevelam (3.75 g/day) for 12 weeks in patients with type 2 diabetes increased concentrations of GLP1 (and GIP) and improved both fasting and postprandial glucose homoeostasis (22).

TGR5-dependent mechanisms

Today, it is fairly well established that sequestering of bile acids increases GLP1 secretion from intestinal L cells through activation of TGR5 (18, 22, 195, 196, 197, 198, 199). A widespread hypothesis explaining this phenomenon takes into account that bile acids, when intraluminally bound to sequestrants, are not reabsorbed into the bloodstream. This facilitates the transport of luminal bile acids into the distal L cell-rich parts of the intestine, which, in turn, enhances activation of TGR5 on ‘distal’ L cells and leads to enhanced GLP1 secretion. As noted above, the most potent natural TGR5 agonists are lithocholic acid and taurine-conjugated lithocholic acid, which activate the receptor in nanomolar concentrations, whereas cholic acid, deoxycholic acid and chenodeoxycholic acid activate it in the micromolar range of concentrations (7, 8). TGR5 activation leads to induction of cAMP and activation of protein kinase A, which in turn leads to further downstream signalling (7, 8). In 2005, bile acids were shown to induce TGR5-mediated GLP1 release from the enteroendocrine GLP1-secreting cell line STC1 (12) and, in 2008, also in primary L cells from mice (200). In 2009, Thomas et al. (14) unravelled, in great detail, the physiological function of TGR5-induced GLP1 secretion. The authors used pharmacological and genetic gain-of-function and loss-of-function models to establish the impact of TGR5 activation on GLP1 secretion. However, albeit intriguing, these results are somewhat difficult to translate into human physiology in terms of bile acid-induced GLP1 secretion via TGR5. Reasons for this are, mainly, the use of very high (supraphysiological) doses of lithocholic acid, which is only present in low concentrations in humans (79), and the use of the aforementioned semisynthetic cholic acid derivative, INT-777, which has a 30-fold improved EC50 on TGR5 compared with cholic acid (201, 202). Recently, however, Parker et al. (203) have demonstrated that bile acids exert robust GLP1 secretion from GLUTag cells (L cell model) and primary murine intestinal cultures, revealing evidence for an additive, potentially synergistic interaction between glucose and TGR5 activation. These results suggest that there might be a role for bile acid-induced augmentation of the L cell secretory response to glucose. In the same study, it was also highlighted that delivery of bile acids to the colon, where L cells are believed to be present at a higher density (204, 205, 206) and express greater levels of TGR5, results in enhanced L cell secretion (105, 197, 198, 203). Indeed, Sato et al. (207) has recently demonstrated, in a study in dogs, that biliary diversion to the ileum increases the secretion of the L cell product PYY. Recently, the results from several human studies have supported a role for bile-induced secretion of GLP1 (13, 61, 62, 63, 64).

The notion of bile acid sequestrants binding bile acids in the intestinal lumen, transporting them to more distal regions of the intestine where they constitute a strong stimulus for TGR5-mediated GLP1 release from the high number of L cells present in the distal gut, is in agreement with a recent paper showing that treatment with colestilan in mice increased GLP1 secretion from colon to an extent greater than that from duodenum and ileum (197). Furthermore, in Tgr5−/− mice, it was demonstrated that colonic TGR5 is important for the colestilan-stimulated GLP1 increase. Moreover, an increased delivery of bile acids to the colon by colestilan increased the expression of the GLP1 precursor, preproglucagon (197). This finding turned out to be TGR5 dependent, suggesting that bile acid sequestration enhances the transcription of the glucagon gene in the enteroendocrine L cells of the distal gut (197). Of note, the authors also reported that acute administration of colestimide (3-h treatment) increased GLP1 secretion, a finding that may position bile acid sequestrants as candidate agents for the control of postprandial glucose metabolism. These findings were confirmed by Potthoff et al., who found that administration of colesvelam to diet-induced obese mice inhibited glycojenolysis, increased GLP1 secretion and improved glycaemic control, and that these effects were blunted in Tgr5−/− mice. Interestingly, the authors established the concept that bile acids bound to colesvelam appear to be able to activate the TGR5 receptor and elicit downstream effects (i.e. cAMP production and GLP1 release) (198).

Despite extensive research over the years, it is still not understood how bile acid sequestration results in increased TGR5 activation. It may be anticipated that bile acids primarily activate TGR5 in their unbound form. Furthermore, it may be speculated that the intestinal
milieu in the colon (or terminal ileum) may facilitate the release of bile acids from the complexes (bile acids bound to sequestrants) generated in the proximal intestine; underlining the importance of the ratio between bound and unbound bile acids in the colon (197). Interestingly, fatty acids are also extensively bound by bile acid sequestrants. This phenomenon may play an important role in the bound:unbound bile acid equilibrium in colon, where fatty acids are produced by the gut microbiota (208). In line with this notion, colesevelam, having intermediate hydrophobicity, binds bile acids tightly, as well as being positively cooperative. The latter means that the binding of fatty acids provides additional binding sites for binding of bile acids, and thus enhances the binding capacity of bile acid sequestrants for bile acids (127). Thus, bile acids and fatty acids (or other negatively charged/hydrophobic molecules) may be able to compete under certain conditions. Hypothetically, arriving at the luminal surface of L cells in the terminal ileum or colon, bound bile acids and fatty acids are faced with altered luminal conditions, which disrupt their mutual competition for binding to colesevelam. Intriguingly, factors such as gut microbiota, pH or even the L cells themselves may play a key role in the facilitation of the release of bile acids and fatty acids. Indeed, this puzzle constitutes an exciting area of research.

TGR5-independent mechanisms

It has been suggested that the effects of bile acid sequestrants on endogenous GLP1 secretion might include factors not involving TGR5 signalling. In insulin-resistant rats (F-DIO rats), 8 weeks of treatment with colesevelam or the ASBT inhibitor SC-435 was investigated (196). Colesevelam, but not SC-435, was shown to improve glycaemic control and both fasting and postprandial plasma concentrations of GLP1 were higher after colesevelam treatment compared with SC-435 treatment. These results could indicate that sequestration of bile acids in the intestinal lumen, thereby suppressing the formation of micelles and absorption of fatty acids, increases the amount of fatty acids that reach the ileum and stimulate L cells to release GLP1. The fact that SC-435 failed to affect GLP1 secretion and glucose control might be because SC-435 blocks bile acid uptake with no effects on the formation of micelles. In line with this, Hofmann has suggested that sequestration of bile acids results in a reduction of the solubilisation of fatty acids in micelles, whereby fatty acids will remain in an emulsified form, which reduces the absorption substantially (209, 210). As a result, fatty acids are thought to pass to the ileum where the density of L cells is high, inducing GLP1 secretion via G protein-coupled fatty acid receptors on the L cells (26, 27, 28, 209). Supporting this notion, Knoebel et al. showed that biliary diversion in rats changes the site of fatty acid absorption from the jejunum to both the jejunum and ileum (211). Of interest, Ross & Shaffer (212) suggested, already in 1981, that hydrolytic products of triacylglycerol, mainly LCFAs, were potent incretin secretagogues. Beglinger et al. (213) could confirm these findings in humans recently. Thus, the GLP1-releasing effect of bile acid sequestrants may be exerted via their effects on assimilation of fatty acids.

Another important regulator of bile acid secretion is the gut hormone CCK secreted from duodenal L cells upon ingestion of lipids. In addition to the gallbladder contracting effect of CCK, the hormone exerts direct, stimulatory action on insulin secretion in many mammals including humans (214). The putative effect of bile acid sequestration on the classical enteroinsular axis may also act via a CCK-dependent mechanism. Indeed, CCK release is inhibited by bile acids (215) and, thus, sequestration of bile acids has been reported to increase CCK concentrations (215, 216) and stimulate insulin secretion, possibly by increasing pancreatic β-cell sensitivity to glucose (217). However, in other human studies, CCK antagonism was unable to affect insulin secretion in response to duodenal perfusion with a mixed meal (218, 219). In general, in humans, CCK does not fulfil the criteria for being a physiological incretin hormone, i.e. the ability to augment postprandial glucose-stimulated insulin secretion (220, 221, 223). However, in a small study by Ahrén et al. (220), CCK8 was infused into six healthy and six type 2 diabetic postmenopausal women during a meal test and it has been demonstrated that CCK8 (in doses that have been shown to increase insulin secretion) did not affect the postprandial secretion of GIP and GLP1. Although the majority of studies do not support a role for CCK-induced, postprandial incretin secretion (220, 223, 224, 225, 226, 227), it should be underlined that in the physiological setting of mixed meal intake, a stimulatory effect of CCK on postprandial GLP1 release may easily be overlooked due to the meal response, which could ‘drown’ the effect of CCK itself. Furthermore, any response must be seen in the light of potential effects of endogenous CCK in control experiments. Indeed, in the study by Beglinger et al. (213), the CCK receptor antagonist dexloxiglumide abolished the increase in GLP1 secretion in response to intraduodenal olate. However, CCK concentrations were markedly enhanced. Of interest, a recent human study has demonstrated that treatment with

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colesvelem for 8 weeks improved i.v. but not oral glucose tolerance (130). Surprisingly, incretin concentrations were unchanged, but, as expected, CCK concentrations were augmented. This finding has led the authors to suggest that the improved postprandial glucose tolerance was attributed to CCK-induced delay in gastric emptying. Although gastric emptying was not measured, the study underlines the fact that manipulation of the bile acid pool may inflict multiple changes in gut hormone secretion, possibly affecting several organ functions. In addition, it may be speculated that such actions may prove efficient at modulating postprandial glucose homoeostasis.

A possible role for gallbladder emptying

It may be anticipated that antagonising CCK with dexloxiglumide results in the impairment of postprandial gallbladder emptying (228, 229). It therefore seems relevant to hypothesise that antagonisation of CCK reduces the flow of bile acids into the intestine, resulting in impaired GLP1 secretion. Indeed, bile acids, gut hormones and gallbladder emptying (via intestinal CCK release) have been linked together in human studies of healthy subjects, where bile acid depletion with cholestyramine was shown to increase gallbladder emptying, plasma motilin concentrations and antroduodenal motility (230, 231). Nevertheless, findings from our recent study of postprandial GLP1 concentrations in cholecystectomised patients revealed that postprandial GLP1 release was similar to that in control subjects (age, gender and BMI matched) (232).

To sum up, the role of the individual bile acids, the interplay of gut hormones, gallbladder emptying, fatty acid absorption and metabolism are all of great importance and, as indicated above, the relative contributions of these factors – and many more possible factors – need to be evaluated. Thus, undoubtedly, the role of the gut in the pathophysiology of type 2 diabetes continuously needs to be redefined.

Summary and conclusions

Current evidence suggests that disruption of the luminal enteral communication exerted by bile acid sequestrants might contain the secret behind the captivating effects of these drugs. The role of bile acids, CCK, gallbladder emptying and fatty acid absorption constitute factors that could contribute to the regulation of incretin function and glycaemic control. A multitude of gastrointestinal cells and hormones act in concert to achieve precise neuroendocrine regulation of digestion and metabolic function, probably in a rather adaptive manner (233). Detecting a way to augment endogenous GLP1 secretion, without increasing overall energy intake and deposition, is an attractive concept in the future treatment of type 2 diabetes – simultaneously improving our understanding of endocrine gut physiology. The role of bile acid-induced GLP1 secretion via TGR5 and the effects on glucose and lipid metabolism via FXR activation are probably two of many ways whereby the human body regulates digestion, metabolism and energy expenditure. In addition, unraveling the interactions between gut hormones might be the key to elucidate the complexity of the largest human endocrine organ, the gut. However, it must be importantly noted that most of our knowledge of the influence of bile acids on the signalling pathways outlined here arises from cell, mouse and rat studies, in which relatively high concentrations (30–100 μM) of bile acids or bile acid sequestrants have been used. Obviously, this raises questions about the physiological relevance of the metabolic, regulatory functions of bile acids in humans. Whether TGR5 activation modulates the in vivo control of human intestinal GLP1 release and glucose homoeostasis remains to be fully elucidated. In the attempt to do so, possible downsides of TGR5 activation, such as gallbladder dysfunction (102, 234), cancer risk (235, 236, 237) and pancreatitis (238), should be considered. Nevertheless, targeting the TGR5 signalling pathway could provide a promising incretin-based strategy for the treatment of type 2 diabetes and the associated metabolic diseases.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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