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Intestinal bile acid receptors are key regulators of glucose homeostasis

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In addition to their well-known function as dietary lipid detergents, bile acids have emerged as important signalling molecules that regulate energy homeostasis. Recent studies have highlighted that disrupted bile acid metabolism is associated with metabolism disorders such as dyslipidaemia, intestinal chronic inflammatory diseases and obesity. In particular, type 2 diabetes (T2D) is associated with quantitative and qualitative modifications in bile acid metabolism. Bile acids bind and modulate the activity of transmembrane and nuclear receptors (NR). Among these receptors, the G-protein-coupled bile acid receptor 1 (TGR5) and the NR farnesoid X receptor (FXR) are implicated in the regulation of bile acid, lipid, glucose and energy homeostasis. The role of these receptors in the intestine in energy metabolism regulation has been recently highlighted. More precisely, recent studies have shown that FXR is important for glucose homeostasis in particular in metabolic disorders such as T2D and obesity. This review highlights the growing importance of the bile acid receptors TGR5 and FXR in the intestine as key regulators of glucose metabolism and their potential as therapeutic targets.

Intestine: Glucagon-like peptide 1: Bile acids: Bile acid sequestrants: Type 2 diabetes

Importance of bile acids and regulation of their metabolism

Bile acids, synthesised from cholesterol by periveinous hepatocytes, contain a twenty-four-carbon steroid core and a side carboxyl chain. Due to hydroxyl groups on the steroid core, bile acids are amphipathic molecules. The position and the number of hydroxyl groups on the steroid group allow the classification of the different bile acids. Bile acid synthesis is driven by multiple step reactions divided into two pathways. The classical (or neutral) pathway depends on cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1), which catalyse hydroxylation in position α on C_7 and C_{12}, respectively, of the steroid core thus generating cholic acid (CA), chenodeoxycholic acid (CDCA, predominant in human) and muricholic acids (MCA, predominant in rodents). Schematically, CYP7A1 activity determines the bile acid pool size, whereas CYP8B1 determines the CA:CDCA or CA:MCA ratios thus defining the bile acid pool composition[1,2]. CYP7A1 knockout (KO) mice display only a 66 % reduction in bile acid pool size, pointing to an alternative (or acidic) pathway for bile acid synthesis[3]. This pathway depends on the activity of sterol 27-hydroxylase (CYP27A1) and oxysterol-7α-hydroxylase (CYP7B1). Bile acids, synthesised in the liver by both the classical and the alternative pathways, are the primary bile acids. They are then conjugated to glycine (predominant

Abbreviations: BAS, bile acid sequestrants; CA, cholic acid; CDCA, chenodeoxycholic acid; ChREBP, carbohydrate response element-binding protein; GF, germ-free; GLP, glucagon-like peptide; TGR5, G-protein-coupled bile acid receptor 1; DCA, deoxycholic acid; FGF15/19, fibroblast growth factor 15/19; FXR, farnesoid X receptor; GLP, glucagon-like peptide; IP, insulinotropic polypeptide; KO, knockout; MCA, muricholic acids; NR, nuclear receptors; T2D, type 2 diabetes; TCA, taurocholate; WT, wild type.

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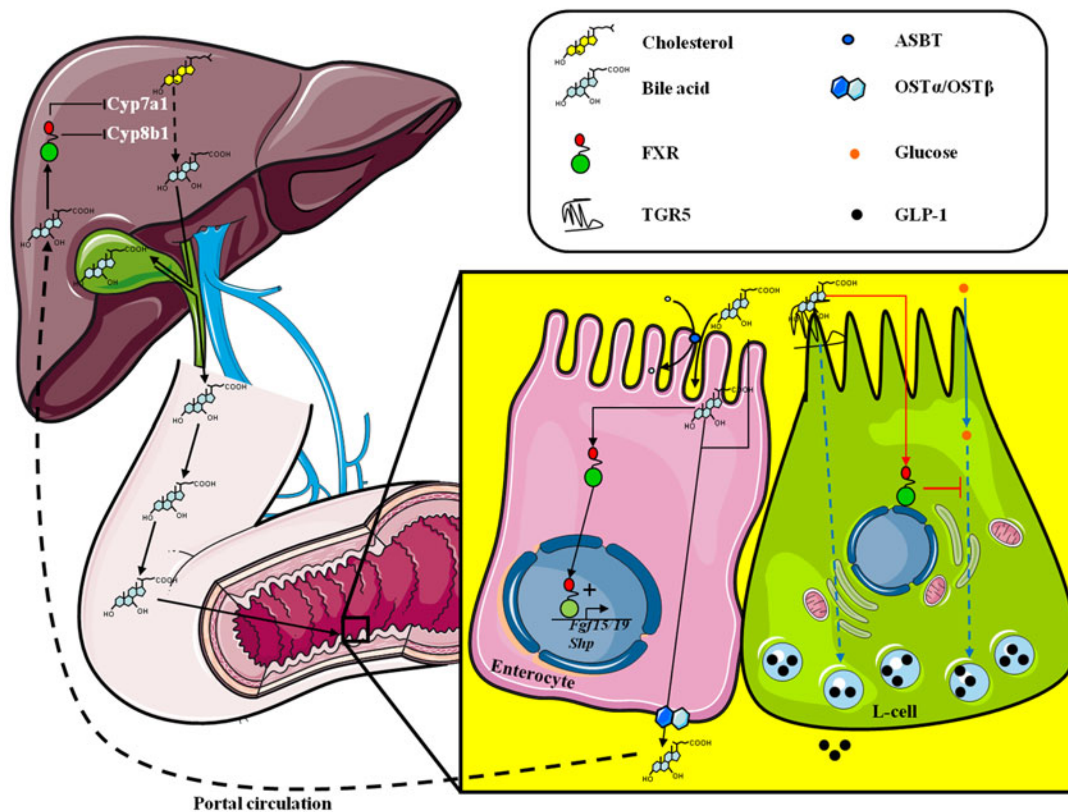

Fig. 1. (Colour online) Enterohepatic cycle of bile acids. Bile acids are produced from cholesterol in the liver. In the fasted state, bile acids are stored in the gallbladder. After meal ingestion, bile acids are expulsed in the intestinal lumen where they emulsify dietary fat. In the ileum, 95 % of bile acids are reabsorbed by the apical sodium-dependent bile salt transporter (ASBT) and basolateral heterodimer organic solute transporter α/β (OSTα/OSTβ). Through the portal circulation, bile acids return to the liver where, by their binding to FXR, they decrease gene expression of the rate-limiting enzymes in bile acid synthesis, i.e. *Cyp7a1* and *Cyp8b1*. In enterocytes, the activated farnesoid X receptor (FXR) increases *Fgf15/19* and *Shp* gene expression thus participating to bile acid metabolism regulation. In L-cells, bile acids bind and activate G-protein-coupled bile acid receptor 1 (TGR5) leading to the secretion of the incretin glucagon-like peptide 1 (GLP-1). By contrast, the activated FXR in L-cells decreases glucose-induced GLP-1 secretion.

conjugation in human subjects) and taurine (predominant conjugation in mice) at C_{24} position by the bile acid coenzyme A:aminoacid *N*-acyl transferase, thus increasing their hydrophilicity and inhibiting their hepatic reflux.

Enterohepatic cycle of bile acids and their importance in intestinal postprandial lipid absorption

Once synthesised, bile acids are secreted into the canalicular space between hepatocytes and reach gallbladder (Fig. 1). Then bile acids are mixed with potassium and sodium ions thereby forming bile salts. The arrival of a meal in the proximal duodenum induces the secretion of cholecystokinin by enteroendocrine I-cells, which subsequently binds to cholecystokinin A receptors on cholangiocytes, induces gallbladder contraction and bile release into the duodenal lumen. There, bile acids facilitate dietary lipid and fat-soluble vitamin absorption and transport by forming, together with phospholipids, TAG, lipid-soluble vitamins and cholesterol, the postprandial mixed micelles. Indeed, it has been shown using the human differentiated Caco-2 cell line, a model frequently used to study apical-to-basolateral lipid transport, that there is no transport of postprandial micelles without taurocholate (TCA)[4]. Bile acids are not absorbed and continue until the distal intestine (i.e. ileum) where they are re-absorbed involving the apical sodium-dependent bile salt transporter, and the basolateral heterodimer organic solute transporter α/β (OSTα/OSTβ) and reach portal blood (Fig. 1). From the portal circulation, bile acids are taken up by the liver via different transporters belonging to the organic anion transporting polypeptide family[5] or via the sodium-TCA cotransporting polypeptide (NTCP/SLC10A1) thus closing their enterohepatic cycle. In human subjects, and depending on when the measure was made but also on the regimen, this cycle occurs six to twelve times daily thus limiting bile acid faecal loss[6,7]. It is reported that 95 % of bile acids are re-absorbed in the distal ileum and 5 % of bile acids arrive in the colon[8]. In the ileum and mainly in the colon, primary bile acids are deconjugated and de-hydroxylated by bacteria belonging to the gut microbiota thus generating secondary bile

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acids. Indeed, some bacteria mainly belonging to the *Clostridiales* and *Bacteroidales* orders display bile salt hydrolase activity transforming (tauro- and/or glyco-) CA and (tauro- and/or glyco-) CDCA acids into deoxycholic acid (DCA) and lithocholic acids (LCA), respectively[9]. These biochemical reactions increase colonic bile acid re-absorption.

Regulation of bile acid metabolism: the role of bile acid receptors

At high concentrations bile acids are cytotoxic molecules. The organism has developed numerous regulatory mechanisms to avoid their overproduction and their accumulation in organs[10–12]. These mechanisms, driven by a negative feedback by bile acids themselves, involve both transmembrane and nuclear receptors (NR). Among them, the membrane G-protein-coupled bile acid receptor 1 (TGR5) and the NR pregnane X receptor and farnesoid X receptor (FXR) participate to alleviate hepatic and intestinal bile acid overload (Fig. 1). The most studied receptors in term of bile acid and glucose metabolism are TGR5 and FXR, we will only focus this review on these two receptors.

TGR5 is a membrane receptor which is activated by oleanolic acid, a triterpenoid molecule, and bile acids (affinity for TGR5: tauro-LCA = LCA > DCA > CDCA = CA)[13]. First described as a regulator of cytokine production in a human monocyte cell line[14], TGR5 is expressed in Küpffer cells, cholangiocytes, adipocytes, myocytes and enteroendocrine cells[15–17]. It has been reported that whole body TGR5 KO mice have a decreased bile acid pool size[15,18,19]. These animals also display more TCA and less tauro-beta MCA and have decreased expression of *Cyp7b1* and *Cyp27a1* gene expression than wild-type (WT) animals[20]. Incubation of murine hepatocytes with culture media from TGR5-activated macrophages decreases *Cyp7a1* gene expression[21], thus highlighting a possible paracrine function of TGR5 in Küpffer cells in hepatic bile acid synthesis regulation. Moreover, the gallbladder is the organ with the highest TGR5 expression levels[17]. TGR5 agonists stimulate gallbladder filling by a mechanism involving cAMP and muscle relaxation[17,19]. Finally, TGR5 is also expressed in the colon in enterochromaffin cells and myenteric neurons where its activation decreases colonic contractility and motility through a 5-hydroxytryptamin/calcitonin-gene-related peptide pathway, a well-known regulatory mechanism of intestinal peristaltism[22–25]. By increasing the delay before defecation, this mechanism can participate to better intestinal bile acid reabsorption but further studies are needed to fully decipher the role of TGR5 in bile acid metabolism.

Bile acids also regulate their own synthesis through binding and activation of the nuclear bile acid receptor FXR, firstly identified in 1995 in rodents[26,27] as a receptor for farnesol[28]. In eukaryotes, the NR superfamily is the largest transcription factor family. Forty-nine NR have been identified so far[29]. FXR is encoded by the *NR1H4* gene. Almost all NR share a common structure with five functional domains. As the other NR, the *N*-terminal domain of FXR is constituted by a ligand-independent activation site called activated function-1 and a DNA-binding domain. These regions are separated from the ligand-binding domain and the ligand-dependent activation site (activated function-2) by a hinge region. Due to alternative splicing and the utilisation of different promoters, four FXR isoforms (FXRα1–4) have been reported (for review[7]). The most transcriptionally active FXR isoforms are FXRα2 and FXRα4 that differ from FXRα1 and FXRα3, respectively, by an introduction of four extra amino acids in the hinge region[30]. FXR is highly expressed in the intestine, liver and kidney and at lower levels in adipose tissue and pancreas. Primary bile acids are the most potent activators of FXRα (called thereafter FXR; affinity for FXR: CDCA > TCA > DCA = tauro-LCA; for review[13]). Moreover, tauro-alpha MCA, tauro-beta MCA and ursodeoxycholic acid have been identified recently as FXR antagonists[31–33]. The development early in the 21st century of whole-body FXR KO mice highlighted the crucial role of FXR in energy homeostasis and more specifically in bile acid metabolism[34]. Indeed, FXR KO mice display an increase in plasma bile acid, as well as TAG and cholesterol levels compared with WT littermates. Once activated by postprandial bile acids, hepatic FXR increases *Nr0b2* gene expression (small heterodimer partner), an orphan NR with co-repressor activities, which decreases *Cyp7a1* and *Cyp8b1* gene expression by direct interaction with liver receptor homologue-1) and the recruitment of corepressors thus decreasing bile acid synthesis[35,36] (Fig. 1). Hepatic activated FXR also decreases *Cyp7b1* and *Ntcp* and increases *Bsep, Osta, Ostβ* and *Mdr3* (multi drug resistance 3) gene expression thus enhancing hepatic bile acid drain (for review[37]). In the intestine, FXR activation up-regulates *Ibabp* and *Ostα/Ostβ* and decreases apical sodium-dependent bile salt transporter gene expression thus enhancing the enteroportal circulation of bile acids[38–41]. In enterocytes, FXR upregulates the expression and the secretion in the portal blood of fibroblast growth factor (FGF; 15 in mice, 19 in human subjects)[42]. Through a pathway not yet fully identified involving β-Klotho, FGF15/19 activates hepatic FGFR4 and decreases *Cyp7a1* gene expression in the liver[43]. Thus, FXR in both hepatocytes and enterocytes controls bile acid metabolism and decreases their cellular toxicity (Fig. 1).

Enteroendocrine cells, glucose and bile acid receptors

Enteroendocrine cells

Even if enteroendocrine cells represent only 1 % of the total intestinal epithelial cells, the length of the intestine makes it the largest endocrine organ. Based on the peptide they secrete and on their expression profile all along the intestine, at least thirteen different enteroendocrine cell types have been identified. Among them, enteroendocrine K- and L-cells secrete the incretins glucose-insulinotropic polypeptide (IP) and glucagon-like peptide (GLP)-1. The incretin effect is based on the observation that oral glucose administration induces a more pronounced insulin secretion

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than an isoglycaemic intraveinous injection. The enteroendocrine L-cells are present all along the upper and the lower intestine following a cephalocaudal gradient with a maximum abundance in the colon. The proglucagon gene, the same gene that produces pancreatic glucagon, encodes GLP-1. After transcription and translation into proglucagon, the action of prohormone convertase 1/3 in L-cells leads to GLP-1, GLP-2, oxyntomodulin and IP2, whereas the action of prohormone convertase 2 in pancreatic α-cells leads to glucagon, glicentin-related polypeptide, IP1 and major proglucagon fragment (for review[44]). In L-cells, the main bioactivity on glucose metabolism is linked to GLP-1. Indeed, in the pancreas, GLP-1 potentiates glucose-induced insulin secretion thus increasing insulin sensitivity of key metabolic organs such as skeletal muscle, adipose tissue and the liver. GLP-1 also inhibits gastric emptying, increases satiety and cardiac function. In blood, GLP-1 half-life is about 1·5–5 min due to a rapid degradation by dipeptidyl peptidase 4. Therapeutic strategies leading to more stable GLP-1 or to a lower GLP-1 degradation have been developed (for review[44]). These drugs are the non-hydrolysable GLP-1 mimetics and dipeptidyl peptidase 4 inhibitors that are successfully used to treat type 2 diabetic patients. Another strategy to increase GLP-1 activity would be to increase its endogenous production and secretion by L-cells.

Glucose is a regulator of glucagon-like peptide 1 production and secretion

Many diet-derived metabolites such as oleoylethanolamine, *n*-3 PUFA, the SCFA butyrate and propionate, glutamine and L-ornithine drive GLP-1 secretion mainly through binding and activation of diverse G-protein-coupled receptors (for review[45]). Glucose also enhances GLP-1 biosynthesis and secretion. Two distinct mechanisms both leading to an increase of intracellular calcium concentrations and membrane fusion of GLP-1 containing vesicles are involved in glucose-induced GLP-1 secretion. The first mechanism involves the sodium-glucose cotransporter 1 where the entry of two sodium ions, concomitantly with one molecule of glucose, induces a difference of potential leading to the opening of a voltage-dependent calcium channel[46,47]. Although this mechanism seems to be the driving force for glucose-induced GLP-1 secretion[48], a second mechanism identified only recently and involving GLUT2-mediated intracellular glucose catabolism through glycolysis pathway has been described[49]. At high extracellular glucose concentrations, the increase in intracellular glucose levels to millimolar range induces glucose catabolism into pyruvate through the glycolysis pathway. Then pyruvate is decarboxylated and conjugated to CoA to form acyl-CoA. By entering in the mitochondrial citrate cycle, acyl-CoA increases the ATP:ADP ratio, which leads to the closure of potassium ATP-dependent channels. The subsequent accumulation of potassium in the intracellular space leads to membrane depolarisation thus opening voltage-dependent calcium channels and the intracellular accumulation of calcium leads to GLP-1 vesicle release[45,49]. A few years ago glucose was identified as a proglucagon gene expression enhancer[50]. Recently, a role for the carbohydrate responsive element-binding protein (ChREBP) in the glucose-mediated proglucagon gene increase has been proposed[51]. ChREBP is a transcription factor activated by glucose metabolites and is highly expressed in enteroendocrine L-cells[52,53]. We have shown that glucose increases proglucagon gene expression in small interference (si)Ctrl, but not in siChREBP enteroendocrine murine L-cells[51]. Moreover, incubation with lactate or 2-deoxyglucose, a non-metabolisable glucose analogue, does not increase proglucagon gene expression. Thus, both ChREBP and glucose catabolism are mandatory for the observed glucose-mediated proglucagon gene expression[51]. However, further studies are needed to fully address the mechanisms behind this ChREBP-dependent glucose-mediated proglucagon gene increase (Fig. 2).

Bile acids are regulators of glucagon-like peptide 1 production and secretion via the bile acid receptors G-protein-coupled bile acid receptor 1 and farnesoid X receptor

As shown for glucose, bile acids also modulate both GLP-1 secretion and proglucagon gene expression. TGR5 is expressed all along the intestine with the maximum levels in the colon, where the enteroendocrine L-cells are predominant[18]. In experiments on L-cells isolated using the transgenic GLU-Venus mouse model, it has been shown that TGR5 expression is mainly restricted to enteroendocrine L-cells[47]. Binding of bile acids to TGR5 in L-cells dissociates the Gαs subunit of the heterotrimeric protein from the Gβ/γ subunits. Thus, activated Gαs activates adenylate cyclase that converts ATP into cAMP. After binding of cAMP to the two regulatory subunits of protein kinase A, the catalytic subunits of protein kinase A are dissociated and shuttle to the nucleus. There, protein kinase A phosphorylates and activates cAMP responsive elements binding protein, which in turn binds to cAMP responsive elements in the promoter of target genes, including proglucagon, thus regulating their expression[18,54,55] (Fig. 2). cAMP produced upon TGR5 activation also triggers GLP-1 secretion through the EPAC2/phospholipase Cε/IP3 and EPAC2/diacylglycerol/protein kinase Cζ pathways[18,55–57] (EPAC, exchange protein directly activated by cAMP) (Fig. 2). Very recently, it has been shown that bile acid-induced GLP-1 secretion is mediated mostly through TGR5 located at the basolateral side of L-cells[58].

Using L-cells sorted by fluorescence-activated cell sorting from GLU-Venus mice, it has been shown that FXR is also expressed in L-cells[51]. Moreover, FXR mRNA levels are higher in L-cells than in non-L-cells. In fresh human jejunal biopsies, GLP-1 immunoreactive cells are also immunoreactive for FXR showing that FXR is expressed in human L-cells. In both human subjects and mice, FXR activation by either bile acids or the specific FXR agonist GW4064 decreases GLP-1 production. More precisely, L-cell-activated FXR is in the same protein complexes containing ChREBP and inhibits glucose-induced ChREBP-mediated proglucagon gene expression as shown by using siChREBP transfected cells

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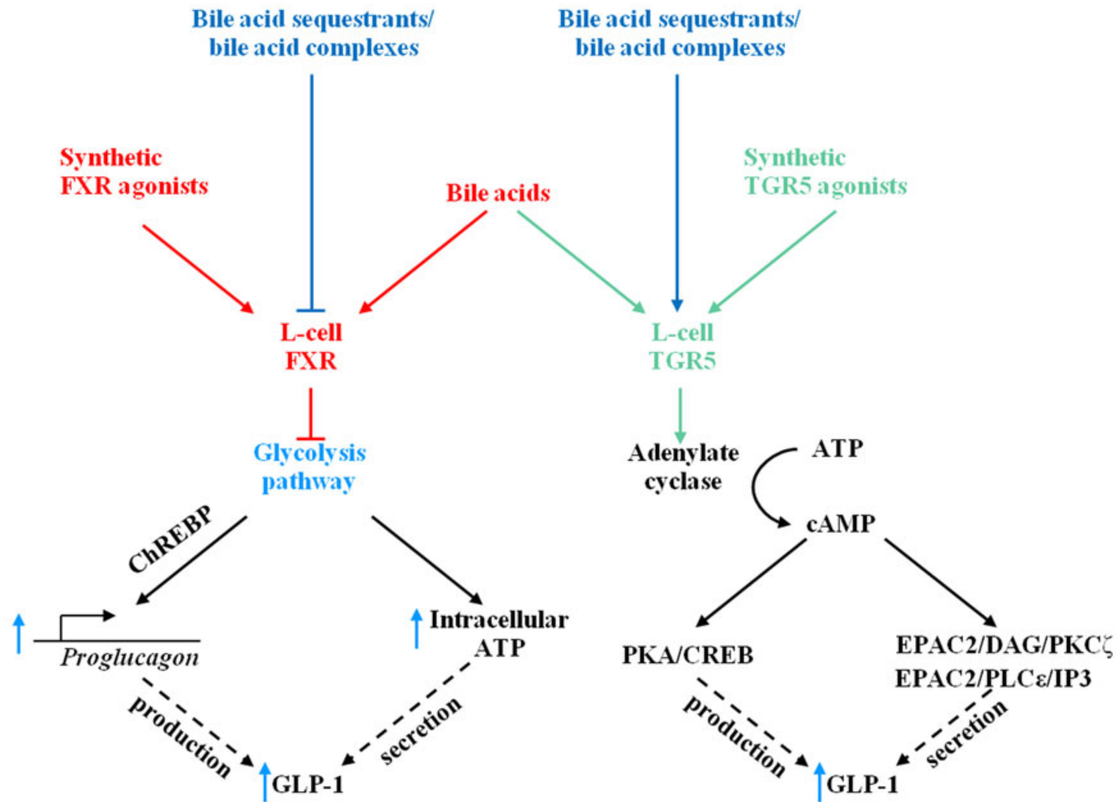

Fig. 2. (Colour online) Activation of bile acid receptors in L-cells modulates glucagon-like peptide-1 (GLP-1) production and secretion. Activation of L-cell G-protein-coupled bile acid receptor 1 (TGR5) increases intracellular cAMP levels, thus leading to an increase in both GLP-1 production and secretion through the protein kinase (PK) A/ cAMP responsive elements binding protein pathway and exchange protein directly activated by cAMP (EPAC2)/ diacylglycerol (DAG)/PKCζ and EPAC2/phospholipase C (PLCε)/insulinotropic polypeptide (IP)3 pathways, respectively. Activation of L-cell farnesoid X receptor (FXR) in the presence of glucose decreases the glycolysis pathway, thus leading to lower intracellular ATP levels. This decrease is associated with lower levels of GLP-1. Moreover, FXR is in the same complex as carbohydrate responsive element-binding protein (ChREBP) and decreases glucose-induced proglucagon gene expression. The bile acid sequestrant (BAS) colesevelam, by inhibiting FXR activation, prevents these decreases. Furthermore, bile acid in complexes with BAS are still able to activate TGR5, thus further increasing L-cell GLP-1 secretion.

and a non-metabolisable glucose analogue. Moreover, FXR activation also decreases glucose-induced GLP-1 secretion. Specifically, in glucose-containing media, FXR activation overall decreases the glycolysis pathway at the gene expression level, lowers intracellular ATP levels and finally lowers glucose-induced GLP-1 release. KCl-induced GLP-1 secretion is not altered by FXR activation, showing the glucose dependency of FXR action on GLP-1 secretion. This glucose dependency is more specifically due to the glycolysis pathway since the glucose-induced GLP-1 secretion is not decreased after GW4064 treatment of murine biopsies challenged with a GLUT-2 inhibitor. These results show that FXR activation decreases both proglucagon gene expression and GLP-1 secretion by interfering with pathways activated by glucose[51] (Fig. 2). The importance of this FXR–GLP-1 pathway as a potential therapeutic target will be discussed later.

In a pathophysiological context of obesity and type 2 diabetes, intestinal bile acid receptors are regulators of energy and glucose homeostasis

Obese and type 2 diabetic patients have altered bile acid metabolism

Obesity and type 2 diabetes (T2D) have been shown to have reached pandemic levels in industrial countries. The WHO estimated 600 million obese and 422 million diabetic individuals in 2014[59,60]. T2D is characterised by fasting hyperglycaemia and insulin resistance which participate together with hypertriglyceridaemia, hypercholesterolaemia, abdominal obesity and hypertension, in the so-called metabolic syndrome[61]. Early and recent studies highlight a disrupted bile acid pool size and/or composition in T2D[62–66]. Patients with uncontrolled T2D have an increase in bile acid pool size, which disappears after insulin treatment[62]. Other studies show no

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differences in bile acid pool size between uncontrolled and insulin treated T2D patients but a change in bile acid pool composition with an increase in the proportion of the secondary bile acid DCA[63,64]. Another study shows that an increase of plasma DCA occurs together with a decrease in plasma CA[65]. Very recently, it has been reported in human subjects that the amplitude in plasmatic postprandial bile acid levels is positively correlated with meal fat content. Moreover, and when compared to age-, sex- and BMI-matched normoglycemic subjects, T2D patients display an increase in total plasma bile acids, mainly due to increases in glycine conjugated bile acids, in DCA and in ursodeoxycholic acid during both oral glucose- and meal tolerance tests[66]. Finally, 12α-hydroxylated bile acids are negatively associated with insulin sensitivity[67]. Even though some discrepancies exist, these studies clearly support the notion of change in bile acid metabolism in T2D. Moreover, in rats fed a high-fat diet, bile diversion to distal intestine improved glucose tolerance[68]. Such an improvement is also observed in mice after bile diversion[69,70] or in mice after ileal interposition, which bypasses bile acid cycling[71]. An increase in GLP-1 secretion is one of the mechanisms evocated to explain these beneficial effects. Furthermore, in healthy subjects, TCA administration stimulates the secretion of GLP-1 by enteroendocrine L-cells and increases fullness sensation[72]. The same year, another team has shown that intrarectal TCA administration increases GLP-1 secretion and decreases blood glucose without hypoglycaemia in obese T2D patients[73]. A better understanding of how bile acids act as signalling molecules through TGR5 and FXR can thus be of interest to develop specific molecules to treat T2D.

It has been difficult to appreciate the exact contribution of each bile acid receptor to energy homeostasis since bile acids are ligands for both TGR5 and FXR. The development of specific synthetic (such as GW4064) or semi-synthetic (such as obeticholic acid (INT-747) and fexaramine) FXR agonists, as well as specific TGR5 agonists (such as INT-777), allow the study of the impact of each bile acid receptor activation to energy homeostasis. Moreover, the development of whole body as well as organ-specific FXR and TGR5 KO animals allows further discrimination of the relative contribution of each bile acid receptor in a specific tissue on glucose metabolism in the pathophysiological context of obesity and T2D (for review[4,5,37]).

G-protein-coupled bile acid receptor 1

TGR5 activation is important in energy metabolism regulation via its capacity to increase energy expenditure and to promote GLP-1 production. Indeed, TGR5 is expressed in brown adipose tissue and skeletal muscle where, through the cAMP/deiodinase 2 pathway, it catalyses the conversion of inactive prohormone thyroxine to active 3,5,3′-tri-iodothyronine[16,74]. This hormone thus enhances brown adipose tissue lipolysis and increases thermogenesis[16]. TGR5 is also involved in lipid metabolism regulation. Whereas both male and female TGR5 KO mice display similar body weight compared to their WT littermates when fed a high-fat diet[15,75], TGR5 KO female animals have less cholesterol in VLDL, LDL and HDL lipoprotein fractions[75]. These mice also display less TAG in VLDL fraction than TGR5 WT mice[75]. Very recently, it has been shown by Donepudi *et al.*[20] that TGR5 KO mice are protected against fasting-induced steatosis. Thomas *et al.*[55] have shown that high-fat-fed mice containing a constitutively active form of TGR5 (TGR5-Tg) display a better glucose tolerance, whereas high-fat-fed TGR5 KO mice have a worsened glycaemic profile. These improvements in glucose metabolism are due to the GLP-1-mediated incretin effect. Indeed, high-fat diet fed TGR5-Tg mice display more GLP-1 and insulin after an oral glucose tolerance test than WT mice thus highlighting the importance of the TGR5/GLP-1 pathway in the improvement of glycaemia by bile acids.

Farnesoid X receptor. Different studies have revealed that, depending on the organ, FXR activation can be beneficial or deleterious for glucose control in obesity. In 2006, Zhang *et al.*[76] demonstrated that overexpressing a constitutively active form of FXR in the liver of *db/db* mice improves glucose tolerance. Moreover, *ob/ob* mice also display an improved glucose tolerance after intra-peritoneal injection of GW4064 (IP, 30 mk/kg mouse, once daily for 10 d) and have a decrease in insulin secretion[77]. A recent study shows that GW4064 treated mice (50 mk/kg mouse, by IP, twice weekly for 6 weeks) gain less body weight when fed a high-fat diet than vehicle-treated mice. GW4064 treated mice also display a better glucose tolerance than vehicle-treated mice[78]. These studies demonstrate that hepatic FXR activation decreases gluconeogenic genes expression. Moreover FXR activation also leads to the induction by glucose of glycolysis gene expression by interfering with the ChREBP pathway[79]. Altogether, these results show that activating hepatic FXR seems beneficial for glucose control. Conversely, mice fed with a high-fat diet mixed with GW4064 display a lower bile acid pool size with a decrease in TCA proportion. They are also more obese and hyperglycaemic than mice fed with the control diet or fed with a high-fat diet enriched with CA 0·1 % showing a deleterious impact of oral GW4064 administration[80]. The involvement of the GLP-1 pathway in this phenotype is unclear since no GLP-1 measurements were performed. However, T2D obese patients treated with TCA have increased GLP-1 and lower blood glucose[73]. Moreover, we have shown in mice that oral administration of GW4064 (by gavage, 30 mk/kg mouse, once daily for 5 d) decreases proglucagon gene expression and intestinal biopsies from mice treated following this protocol failed to secrete GLP-1 in response to glucose[51]. It should be noted that GW4064, as well as fexaramine[81], are not well absorbed by the intestine. Thus, the decreased GLP-1 pathway after intestinal FXR activation can be involved in the deleterious effect of oral GW4064 on glucose metabolism. As indicated earlier, four different isoforms of FXR (FXRα1–4) have been identified (for review[82]). Whereas the expression levels of FXRα1–2 and FXRα3–4 are similar in the liver, the

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intestine expresses higher levels of FXRα3–4 than FXRα1–2[30]. Differences in FXR isoform expression profiles may be involved in the differences between the beneficial effect of FXR activation in the liver and its harmful action in the intestine. Further studies are needed to fully address the importance of each FXR isoform, and especially in the intestine, in energy homeostasis.

The importance of FXR on glucose homeostasis reached a milestone thanks to the development of both whole-body and tissue-specific FXR KO animals. Indeed, many studies demonstrate that whole-body FXR KO mice are protected against diet-induced or genetically induced obesity. These mice also have an improved glucose tolerance[83–87]. Van Dijk *et al.*[83] have demonstrated that whole-body FXR KO mice present a delay in intestinal glucose absorption due to enterocytic accumulation of glucose-6-phosphate. In the hyperphagic *ob/ob* mice FXR gene expression deficiency improves all metabolic parameters and in particular glucose metabolism through an enhancement of peripheral glucose disposal and an increased adipose tissue insulin sensitivity[84]. These improvements are not observed in *ob/ob* mice where FXR gene is specifically invalidated in the hepatocyte thus demonstrating that FXR in extrahepatic tissues drives the beneficial effects of FXR gene expression deficiency on glucose homeostasis.

A recent study shows that mRNA levels of FXR and its target genes small heterodimer partner and FGF19, are increased in the ileum of obese *v.* lean human subjects[88]. Moreover, the expression of these genes positively correlates with BMI. Furthermore, bile diversion to the ileum in obese mice inhibits the expression of these genes and improves glucose metabolism[69]. Jiang *et al.*[88] further demonstrate that administration of a bile acid with specific intestinal FXR antagonism improves metabolic parameters, such as triglyceridaemia and glucose clearance, in obese mice. These improvements are due to a decrease in intestinal ceramide production. Moreover, and according to the phenotype observed in whole-body FXR KO mice, intestinal specific FXR KO mice are protected against diet-induced obesity also through a reduction of intestinal ceramide production[88,89]. Finally, whereas FXR KO mice fed a high-fat diet have a reduced glycaemia after an oral glucose tolerance test. This improvement is lost after GLP-1R antagonism showing that the beneficial effect of FXR gene invalidation on glycaemia is in part mediated through a GLP-1/GLP-1R pathway[51]. Altogether, these results highlight a role of intestinal FXR in glucose metabolism in a pathophysiological context of obesity and T2D.

Direct and indirect inhibitions of intestinal farnesoid X receptor by pharmacological agents as possible treatments for type 2 diabetes

Some recent studies suggest that intestinal FXR inhibition in a pathophysiological context of obesity improves glucose homeostasis. Here we will focus only on bile acid sequestrants and microbiota manipulation as two possible treatments for T2D via an inhibition of intestinal FXR.

Inactivation of intestinal farnesoid X receptor transcriptional activity using bile acid sequestrants

Bile acid sequestrants (BAS) are anionic exchange resins first used to decrease hypercholesterolaemia. Indeed, BAS increase HDL-cholesterol levels by 3–5 % and decrease by 15–30 % LDL-cholesterol without changing or slightly increasing TAG levels[64,90]. By trapping bile acids in the intestinal lumen, these molecules increase their faecal output. Thus, bile acids cannot activate intestinal FXR leading to the inhibition of the negative feedback loop driven by FGF15/19. Hepatocytes continue to convert cholesterol into bile acids thus decreasing plasma cholesterol[64,90]. In the USA, BAS are also used as antidiabetic drugs. Indeed, cholestyramine administration for 5 d to diabetic patients decreases glycaemia by 20 mg/dl and glucosuria by 40 g compared with placebo treated patients[91,92]. The mechanisms behind such improvements are multiple and not fully identified. Among them, an increase in splanchnic glucose utilisation and an increase in GLP-1 secretion can participate in the improvements (for review[93]). Splanchnic glucose utilisation is defined by the hepatic absorption of portal glucose, coming from the intestine, and its metabolisation through glycogenesis or glycolysis. As mentioned earlier, FXR KO mice have a delay in intestinal glucose absorption[83]. Moreover, *ob/ob* FXR KO mice have an improved glucose homeostasis due to an improved glucose clearance and increased insulin sensitivity in adipose tissue. Colesevelam improves glucose homeostasis only in *ob/ob* FXR WT but not in FXR KO *ob/ob* mice showing that the beneficial effect of colesevelam is dependent on FXR[84]. Finally, hepatic FXR activation inhibits glucose-induced glycolytic gene expression[79]. BAS, through lifting these repressions, increases hepatic glucose utilisation. These results are in accordance with the fact that BAS administered to T2D patients increase glucose clearance and insulin sensitivity. This study also demonstrated an increase in incretin secretion after colesevelam[94].

BAS-stimulated GLP-1 secretion has been proposed to occur by inhibiting bile acid ileal reabsorption. Thus, BAS drive bile acids to the colon where L-cell density is the highest. More precisely, the bile acid in complexes with BAS are still able to bind and to activate L-cell-TGR5, thus increasing GLP-1 secretion[18]. Potthoff *et al.*[56] further demonstrated that the improvement of glycaemia after BAS is driven through a TGR5/GLP-1-induced reduction in hepatic glycogenolysis. Using colesevelam, Shang *et al.*[95] have shown in Zucker diabetic fatty rats a decrease in glucose clearance together with an increase in GLP-1 secretion. As indicated earlier, BAS de-activate intestinal FXR activity by inhibiting bile acid flux through enterocytes. Together with an enhanced glucose clearance and a decrease in intestinal small heterodimer partner gene expression, Zucker diabetic fatty rats treated with BAS have an increased glucose-induced GLP-1 secretion[96].

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We have shown that colesevelam treatment of *ob/ob* mice improves glucose clearance after an oral glucose tolerance test and increases GLP-1 production through FXR, because such improvements are not observed in *ob/ob* FXR KO mice[51] (Fig. 2).

Inactivation of intestinal farnesoid X receptor via microbiota manipulation

In recent years, a clear role of the gut microbiota in energy homeostasis regulation has emerged. Mice without intestinal microbiota (germ-free (GF) mice) are protected against diet-induced obesity and have an improved glycaemia compared with conventionally raised mice fed the same diet[97]. Moreover, these GF mice have profound changes in bile acid metabolism. Indeed, GF mice have higher levels of tauro-alpha MCA and tauro-beta MCA, two bile acids with FXR antagonist properties[32]. Proglucagon mRNA levels and GLP-1 positive cells are also increased in GF mice compared with conventionally-raised mice[98]. To link these two observations, we measured ileal proglucagon mRNA in both GF and conventionally raised FXR WT and FXR KO mice. FXR KO mice have increased proglucagon mRNA levels only in conventionally raised mice showing that the impact of FXR gene deficiency on proglucagon gene expression needs gut microbiota[51]. Recent observations show that FXR WT and FXR KO mice have different gut microbiota[87]. Moreover, high-fat fed GF mice transplanted with intestinal microbiota from FXR KO obese mice gain less body weight and display a better glucose tolerance than high-fat diet fed GF mice colonised with intestinal microbiota from FXR WT obese mice[87]. Therefore, the beneficial effect of FXR gene deficiency on glucose tolerance involves the GLP-1 pathway and gut microbiota. Finally, treatment of obese mice with the prebiotic tempol enhances glucose metabolism through increased levels in bile acids with FXR antagonist properties. These improvements due to tempol treatment are not observed in intestinal FXR KO mice[89] and are in line with a study showing that feeding mice with the FXR antagonist GβMCA improves glucose tolerance through intestinal FXR[88]. Thus, prebiotics which increase the levels of bile acids with FXR antagonist properties improve glucose metabolism.

Conclusion

Although some discrepancies remain on the overall role of the bile acid receptors TGR5 and FXR in glucose metabolism, recent studies clearly highlight that these receptors in the intestine seems to be crucial to maintain glucose homeostasis. To summarise, the intestinal bile acid receptors FXR and TGR5 appear valuable targets to treat diabetics. Whereas FXR activation in hepatocytes seems to be beneficial for improving glucose metabolism, its inhibition in intestine seems beneficial to improve glucose clearance and insulin sensitivity. TGR5 activation in L-cells increases the release of the incretin GLP-1 whereas in these cells FXR decreases the glucose-induced GLP-1 secretion. Moreover, in a pathophysiological context of obesity, whole-body, but also intestine-specific FXR gene expression deficiency ameliorates glucose homeostasis. These improvements are not observed in mice with an invalidation of FXR gene specifically in the hepatocyte. Finally, both BAS and the prebiotic tempol improve glucose homeostasis by inhibiting intestinal FXR thus highlighting valuable pharmacological tools to study the interplay between bile acids/FXR/GLP-1 in a pathophysiological context of obesity and T2D. Further studies are needed to fully address to which extend intestinal FXR inhibition is a good option to treat T2D patients.

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Conflicts of Interest

None.

Authorship

M. S. T., S. L., B. S. and X. C. drafted the manuscript; S. L., B. S. and X. C. revised the manuscript for important intellectual content; and X. C. obtained funding.

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