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Title: Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia and NAFLD

Short title: Bile acids in meta-inflammatory disorders

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Abbreviations: ASBT (apical-sodium-dependent BA transporter); BA (bile acids); BAAT (bile acid-CoA:amino acid N-acyltransferase); BACS (bile acid:CoA synthase); BAS (bile acid sequestrants); BAT (brown adipose tissue); WAT (white adipose tissue); BSEP (bile salt export pump); BSH (bile salt hydrolase); NAFLD (non-alcoholic fatty liver disease); NASH (non-alcoholic steatohepatitis).

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Abstract

Bile acids are signaling molecules, which coordinately regulate metabolism and inflammation *via* the nuclear farnesoid X receptor (FXR) and the Takeda G protein-coupled receptor 5 (TGR5). These receptors activate transcriptional networks and signaling cascades controlling the expression and activity of genes involved in bile acid, lipid and carbohydrate metabolism, energy expenditure, and inflammation by acting predominantly in entero-hepatic tissues, but also in peripheral organs. In this review, we discuss the most recent findings on the inter-organ signaling and interplay with the gut microbiota of bile acids and their receptors in meta-inflammation, with a focus on their pathophysiological roles in obesity, type 2 diabetes, dyslipidemia and non-alcoholic steatohepatitis and their potential therapeutic applications.

Key words: Bile acids; FXR; TGR5; meta-inflammation.

Enterohepatic Circulation and Metabolism of Bile Acids

Bile acids (BA) are amphipathic steroid molecules synthesized from cholesterol in hepatocytes surrounding the hepatic central vein (perivenous hepatocytes) by the action of ~15 enzymes. BA synthesis occurs *via* two pathways[1]. The classical pathway, initiated by the rate-limiting enzyme cytochrome P450 cholesterol 7α-hydroxylase (CYP7A1), produces the majority of the BA pool. A fraction of the BA pool is synthesized *via* an alternative pathway (between 3-18% of total BA synthesis in healthy humans[2,3]), initiated by cytochrome P450 27α-hydroxylase (CYP27A1). The products of these pathways are the primary BA cholic acid (CA), and chenodeoxycholic acid (CDCA) in humans[1]. In rodents, α- and β-muricholic acid (MCA) are formed from CDCA and ursodesoxycholic acid (UDCA) respectively, by CYP2C70[4]. The hydrophobicity index of the BA pool, reflecting the ratio of highly hydroxylated CA(+MCA) to lower hydroxylated BA (such as CDCA), is an important parameter controlling its physiological functions. This hydrophobicity index differs between rodents (low hydrophobicity) and humans (high hydrophobicity) due to the conversion of CDCA into MCA in rodents. CDCA and MCA synthesis is conditioned by the activity of 12α-hydroxylase CYP8B1, which transforms di-hydroxylated- in tri-hydroxylated-BA. BA synthesis, which follows a circadian rhythm controlled by the clock gene Rev-erbα[5] and the KLF15-Fgf15 axis[6], is regulated by negative feedback mechanisms. High hepatic BA exposure inhibits BA synthesis *via* the Farnesoid X Receptor (FXR), which decreases LRH1-regulation of CYP7A1 *via* induction of Small Heterodimer Protein (SHP/NR0B2) in the liver. BA also induce fibroblast growth factor-19 (FGF19, the human orthologue of murine FGF15) expression and release from the intestine, which activates the FGFR4/βklotho receptor in the liver, thus collectively leading to inhibition of hepatic CYP7A1 and CYP8B1 expression[7,8].

In hepatocytes, primary BA are further conjugated to glycine -mainly in humans- or taurine -mainly in mice- at the C24 position by the enzymes BA-CoA synthase (BACS) and BA-CoA–amino acid N-acetyltransferase (BAAT). Moreover, BA can also be sulfated by the sulfotransferase SULT2A1 (SULT2A9 in mice), or glucuronidated by UDP-glucuronosyltransferases, such as UGT2B4, UGT2B7 and UGT1A3[1]. BA are then secreted into the bile canaliculi *via* the bile salt export pump (BSEP) and the BA transporters MRP2 and MDR1A[1]. The bile containing the secreted BA flows through the biliary tree into the gallbladder, where it is stored and concentrated during the inter-digestive period, until meal ingestion-stimulated cholecystokinin (CCK) secretion by enteroendocrine I-cells induces gallbladder contraction, causing bile release into the duodenum[1].

In the intestine, BA activate pancreatic lipase and form micelles containing dietary fat and lipophilic vitamins (A, D, E and K). The intestinal microbiota transforms primary BA into secondary BA: deoxycholic acid (DCA), lithocholic acid (LCA) and UDCA in humans; and DCA, LCA, ωMCA, hyodeoxycholic (HDCA) and murideoxycholic (MDCA) acid in mice[9]. In the enterocytes from the distal ileum, 95% of intestinal BA are actively re-absorbed through the apical-sodium-dependent BA transporter (ASBT/SLC10A2) and secreted at the basolateral membrane by the heterodimeric organic solute transporters α and β (OST α/β). The unabsorbed 5% of intestinal BA are either deconjugated by the gut microbiota and passively reabsorbed in the colon or lost into feces. The absorbed BA return to the liver through the superior mesenteric and portal veins, where they are cleared by active transporters in the sinusoidal membrane of hepatocytes (NTCP, OAT, OATP, mEH)[10]. Within hepatocytes, free BA are conjugated and secreted into bile canaliculi along with BA newly synthesized from cholesterol, thereby compensating for fecal loss. The small amount of BA

escaping hepatic recapture reaches the peripheral tissues *via* the systemic circulation. These plasma BA circulate bound to plasma proteins -mainly albumin (~80%) and lipoproteins (~20%)[11]- where they may exert signaling functions on peripherally-expressed BA receptors[1].

Reciprocal Interaction between Intestinal Microbiota and Bile Acids: Impact on Host Metabolism

In the intestinal lumen, BA and the microbiota reciprocally control their composition. The gut microbiota transforms BA, present in the intestine at millimolar concentrations, by carrying out numerous reactions, such as hydrolysis of conjugated BA by bile salt hydrolases (BSH); 7α-dehydroxylation of CA and CDCA, forming DCA and LCA, respectively; and oxidation and epimerization of hydroxyl groups at the C3, C7 and C12 positions. The gut microbiota also esterify BA, making them more hydrophobic. Esterified BA (ethyl-esters and long-chain fatty acid esters of LCA and polyesters of DCA) account for approximately 25% of fecal BA[9]. Furthermore, intestinal bacteria reduce the bactericidal effect of BA by transforming DCA and LCA into iso-DCA and iso-LCA (3β-OH epimers) *via* the iso-BA pathway[12]. Indeed, BA act as anti-microbial agents by damaging bacterial membranes and altering intracellular macromolecular structures through detergent actions. Therefore, only microbial populations able to tolerate high BA concentrations can survive in the gut. Whereas free BA are more damaging to bacterial membranes, taurine catabolic end-products promote proliferation of some bacteria strains[13].

BSH is active in *Lactobacillus, Bifidobacterium, Firmicutes, Enterococcus, Clostridium,* and *Bacteroides*, and produces free BA, taurine and glycine. Free BA solubilize intestinal lipids and are reabsorbed less efficiently, resulting in increased

fecal BA loss and an ameliorated metabolic adaptation of the host[14]. Indeed, BSH-over-expressing *E.coli* reduce host weight gain, liver triglycerides and plasma cholesterol in conventionally raised mice by reducing intestinal cholesterol absorption and increasing hepatic uptake of plasma cholesterol for *de novo* BA synthesis to compensate for the fecal BA loss[14].

Gut microbial depletion in germ-free or antibiotic-treated rodents increases the proportion of taurine-conjugated primary BA species –including TβMCA, a rodent-specific FXR antagonist (see below)-, decreases the diversity of the BA pool[15] and concomitantly increases gallbladder and small intestine BA concentrations[16]. In line, treatment of mice with probiotics, such as VSL#3, which enhances deconjugation and fecal excretion of BA, increases hepatic BA synthesis *via* down-regulation of the FXR/FGF15 axis[17]. Intriguingly, colonization of germ-free mice with human microbiota decreases the formation of secondary BA species, especially the FXR antagonist TβMCA, thereby increasing ileal FXR activity and FGF15 expression[18]. As BA receptors have different affinities for distinct BA species (see below), the gut bacteria may hence modulate metabolism and host physiology by altering BA pool composition[9].

Since type 2 diabetes (T2D), obesity and non-alcoholic fatty liver disease (NAFLD) are associated with dysbiosis[19] and changes of the BA pool size and composition[20–22], the interaction of gut microbiota with BA metabolism and its impact on these pathophysiological conditions is of great interest. For instance, NASH patients present dysbiosis associated with increases in BA synthesis, fecal primary BA and the primary:secondary BA ratio[23].

Treatment with antibiotics alters gut bacteria composition. In antibiotic-treated mice[24], BSH-producing *Lactobacillus* decreases, synthesis of the FXR antagonist TβMCA increases, and high-fat diet (HFD)-induced obesity, insulin resistance and NAFLD improve (see below)[25]. However, studies in humans reported no metabolic changes after pro-biotic supplementation[26] or anti-microbial therapy[27]. Hence, although an interplay between BA, gut microbiota and metabolic diseases is evident, the causality and directionality of the interactions remain unclear.

Bile Acid Metabolism in Meta-Inflammatory Disorders

Crosstalk between metabolically active tissues is necessary for proper energy homeostasis. In obesity, T2D and non-alcoholic steatohepatitis (NASH), these organs often present, in combination with metabolic alterations, a chronic low-grade inflammation characterized by the recruitment of immune inflammatory cells, abnormal cytokine and acute-phase reactant production, and inflammasome activation, referred to as "meta-inflammation"[28].

Meta-inflammatory disorders, such as obesity, T2D and NASH, are associated with changes in BA metabolism and pool composition as shown by a large number of observational studies (Table). Chronic inflammation modulates hepatic BA metabolism, as exemplified by the decrease of CYP7A1 transcription in human hepatocytes treated with IL-1β, which acts *via* the JNK/c-Jun signaling pathway[29]. Total BA concentrations increase in obese patients and correlate with body mass index (BMI) irrespective of T2D and NAFLD (Table). In T2D patients, systemic total BA concentrations are increased, in the fasting and post-prandial states. However, reported changes in qualitative BA pool composition differ among studies (Table).

Interestingly, insulin-resistant, but not T2D patients, display an increased 12α-hydroxylated:non12α-hydroxylated BA ratio[20].

In NASH patients, plasma BA and C4[30], as well as hepatic BA concentrations, are increased[23,,31]. Moreover, hepatic BA synthesis may shift to the alternative pathway in livers of NASH patients[21] as suggested by gene expression analysis. The higher BA exposure could lead to cytotoxicity and contribute to the pathogenesis of NAFLD[30].

Fasting peripheral blood BA concentrations consistently increase upon bariatric surgery (Roux-en-Y gastric bypass, RYGB) in humans (Table) and preclinical models[32]. Again, reported qualitative changes of the BA pool after bariatric surgery differ between studies, which may be due to differences in methodological parameters (feeding state, time after surgery). The kinetics of BA pool size and composition alterations after RYGB are unclear: while some studies reported an early 2 to 3-fold increase in BA [33–35], others found BA to be increased only one year post-RYGB[36–38] or after 20% of body weight loss[39]. Both short term[40] and long term[35,37,39–41] augmentations in post-prandial systemic BA concentrations were reported after RYGB, with qualitative changes such as increased conjugated BA species[37,41]. However, the changes in peripheral BA were not correlated to changes in body weight after RYGB[42]. The 12α-hydroxylated:non12α-hydroxylated BA ratio was increased 2 years after RYGB[35], even though insulin resistance improved. Interestingly, patients with RYGB-induced remission of T2D presented higher BA concentrations than patients without remission[43], suggesting a role for the BA in the metabolic improvements.

There is scarce information regarding BA changes after vertical sleeve gastrectomy (VSG), bilio-pancreatic diversion and laparoscopic adjustable gastric banding (LAGB)

(Table). In some studies, fasting plasma BA did not change after LAGB[34,39] or VSG[44]; while others reported a decrease upon 20% weight loss after LAGB[39] and an increase 1 year after VSG[38].

The mechanisms underlying the increase in peripheral BA upon RYGB surgery are still unclear and may include increased hepatic synthesis, increased intestinal recapture potentially associated with microbiota alterations, changes in portal blood flow and/or lower hepatic BA recapture from the portal vein[45–48]. Interestingly, germ-free mice receiving gut microbiota from RYGB-treated mice exhibit weight loss and decreased fat mass[47], suggesting that intestinal microbiota could contribute to the metabolic improvements after RYGB *via* changes in the BA pool.

Alterations in FXR and TGR5 signaling could contribute to the metabolic improvements. Indeed, BA and GLP-1 levels positively correlate after RYGB[38,49] and VSG in humans[38] and in mice[50]. FGF19 has also been reported to increase after RYGB[33,43] and VSG[44]. Direct roles for FXR and TGR5 in the metabolic improvements after VSG have been suggested based on genetic studies in mice[48,50,51].

Since BA have emerged as signaling molecules regulating glucose, lipid and energy homeostasis, and inflammation, it is conceivable that changes in BA pool size and composition in metabolic diseases and upon bariatric surgery alter BA signaling pathways, impacting on metabolic parameters. However, due to methodological heterogeneity and cohort size, it is still unclear how BA pool alterations contribute to and impact on (patho)physiological conditions in humans.

Bile Acids: Signaling Molecules Modulating Meta-Inflammatory Diseases

BA are ligands of the nuclear receptors FXR[52], vitamin D receptor (VDR)[53] and pregnane X receptor (PXR)[54] as well as G-protein coupled receptors (GPCR) such as TGR5[55], sphingosine-1-phosphate receptor (S1PR)2[56], and the muscarinic receptors M2/3[57]. Activation of nuclear receptors by BA requires cellular entry, either by simple diffusion, in the case of hydrophobic free BA, or by active transport for conjugated and hydrophilic BA. In contrast to nuclear receptors, ligands can bind directly to cell surface receptors[1]. Activation of PXR[54], VDR[53,58] and CAR[59] by BA, mainly LCA, induce a xenobiotic detoxification response to stimulate BA excretion under cholestatic conditions[1]. We will here focus on the role of BA in the control of metabolism and inflammation *via* FXR, TGR5 (Figure1) and S1PR2.

FXR (*NR1H4)* is expressed in several organs, including the liver, intestine, kidneys, adrenal glands, white adipose tissue (WAT) and immune cells[1]. Natural FXR agonists are CDCA>DCA>CA>LCA, in order of decreasing potency, while Tα-, Tβ-MCA and possibly UDCA are antagonists[16]. Ligand-bound FXR forms a heterodimer with Retinoic-X-Receptors (RXR α, β or γ) to regulate target gene expression. FXR also indirectly represses gene transcription *via* induction of negative regulators, such as SHP, competition for other nuclear receptors (peroxisome proliferator-activated receptor (PPAR)α[60]) or transcriptional coregulators (CRTC2[61]). In addition to ligands, post-translational modifications also modulate the transcriptional activity of FXR, such as O-GlcNAcylation[62], methylation, acetylation[63] and phosphorylation (*via* AMPK and PKC)[64–66]. Interestingly, acetylation of FXR increases inflammation and deteriorates glucose metabolism by interfering with its sumoylation and transrepressive activity[67].

TGR5 (encoded by the *GPBAR1* gene) is expressed in enteroendocrine L-cells[68,69], brown adipose tissue (BAT), WAT, skeletal muscle, gallbladder, non-parenchymal liver cells and the brain[1]. BA activate TGR5 with different potencies

(LCA>DCA>CDCA>CA). TGR5 activation induces adenylate-cyclase to produce cAMP, which in turn activates protein-kinase A to exert immediate cytosolic effects, or activate the transcription factor cAMP-Responsive Element Binding protein (CREB) to modulate gene expression[1].

Conjugated BA activate S1PR2 in hepatocytes, which activates, *via* the ERK1/2 and Akt signaling pathways, nuclear sphingosine kinase-2 (SphK2). This enzyme, which synthesizes S1P from sphingosine, hence increasing S1P levels in the nucleus. Nuclear S1P inhibits specific histone deacetylases, thus increasing histone acetylation and inducing enzymes involved in lipid and sterol metabolism. By modulating expression of nuclear receptors (including FXR) and proteins involved in lipid and glucose metabolism (LDL-R, SREBP1c and FAS), S1PR2/SphK2/S1P signaling lowers hepatic lipid content. Indeed, S1PR2-deficient mice develop hepatic steatosis upon HFD, whereas S1PR2 over-expression prevents hepatic steatosis[56,70].

Energy metabolism

Distinct AT depots differentially express BA receptors, FXR and TGR5 being expressed in white[71,72] and brown[73,74] adipocytes, respectively. Moreover, both receptors are also expressed in certain immune-inflammatory cells in AT, which may contribute to their anti-inflammatory and insulin-sensitizing effects. The gut microbiota promote diet-induced obesity in a FXR-dependent manner in mice[75]. In AT, FXR regulates adipocyte differentiation and functions by promoting PPAR gamma activity and interfering with the Wnt/β-catenin pathway[72,76]. In BAT, TGR5 stimulates energy expenditure[73] by inducing the expression of iodothyronine-deiodinase type 2 (DIO2), thus converting inactive thyroxine (T4) into 3,5,3'-triiodothyronine (T3), which activates the thyroid hormone receptor to uncouple mitochondrial function and to

increase thermogenesis, and PPARγ coactivator-1α (PGC-1α), a regulator of mitochondrial biogenesis[73].

FXR and TGR5 may be involved in the metabolic improvement induced by VSG. The impact of VSG on body weight and glucose tolerance appears reduced in FXR-deficient mice[48]. TGR5 may mediate the effect of surgery on metabolism by enhancing production of the incretin glucagon-like peptide-1 (GLP-1)[50,51], and on body weight *via* TGR5 activation in BAT[50]. Upon VSG in mice, TGR5 deficiency reduces the 12αhydroxylated:non-12αhydroxylated BA ratio and BA pool hydrophobicity potentially by decreasing CYP8B1[51]. BA also modulate energy expenditure by inducing intestinal expression and secretion of FGF15/19. Administration of FGF19 to HFD-fed mice enhances the metabolic rate and insulin sensitivity, and decreases body weight[77]. The increase of FGF19 associated with T2D remission following RYGB may thus also contribute to the metabolic improvements following surgery in humans[43]. Moreover, pharmacological activation of intestinal FXR with fexaramine, a synthetic FXR agonist with intestine-restricted bioavailability, induces FGF15 in mice, reducing diet-induced weight gain, systemic inflammation and hepatic glucose production[78]. FGF15 induces changes in BA pool composition and increases TGR5 ligand production, leading to both BAT activation, enhanced thermogenesis and WAT browning[78]. Oral administration of CDCA to healthy humans increased BAT activity and energy expenditure likely *via* TGR5 activation in brown adipocytes[74], making TGR5 a target to treat obesity.

Lipid metabolism

FXR regulates lipid and lipoprotein metabolism by acting on hepatic lipogenesis, and lipoprotein secretion, intravascular remodeling and plasma clearance, as well as intestinal cholesterol absorption (Figure2).

FXR reduces TG-rich lipoproteins by several mechanisms. FXR reduces lipogenesis by repressing hepatic SREBP1c expression in SHP-[79] and FGF15/19-dependent manners[80]. FXR also represses microsomal triglyceride transfer protein (MTP) and apolipoprotein (apo) B gene expression[81] thus reducing VLDL secretion[79]. FXR enhances lipoprotein lipase (LPL) activity by increasing expression of apoCII[82] –a LPL activator- while reducing apoCIII[83] –a LPL inhibitor- stimulating intravascular lipolysis of TG-rich lipoproteins. Furthermore, FXR increases VLDL-Receptor (VLDL-R) expression[84]. The inhibition of BA synthesis from cholesterol upon FXR activation results in increased hepatic cholesterol concentrations, hence LDL-R activity decreases and plasma LDL-C increases.

Levels of Lp(a), an atherogenic lipoprotein, decrease due to reduced hepatic apo(a) gene expression through concerned actions of hepatic FXR and FGF19[85]. Interestingly, cholestatic patients present low Lp(a) levels, which increase after removal of the biliary obstruction[85]. FXR also acts on reverse cholesterol transport (RCT) and HDL metabolism by decreasing apoA1[86], increasing Scavenger Receptor–B1 (SR-B1)[87] and CETP[88] expression, thus increasing HDL-C clearance and lowering plasma HDL-C levels *in vivo*. FXR activation thus induces a combination of pro- and anti-atherogenic lipoprotein profile changes. Further attention is thus required to evaluate the impact of FXR activation on cardiovascular risk.

In humans, CDCA and the semi-synthetic FXR agonist obeticholic acid (OCA, INT-747 or 6-Ethyl-CDCA, Intercept Pharmaceuticals) increase LDL-C[89,90], likely *via* FXR-dependent CYP7A1 inhibition, thus decreasing hepatic cholesterol conversion to BA, increasing hepatic cholesterol content, and inhibiting LDR-Receptor (LDL-R) activity (Figure2). In humans, CDCA treatment decreases hepatic mRNA levels of LDL-R

and hydroxymethylglutaryl-Co-enzymeA (HMG-CoA) reductase –the rate-limiting enzyme of cholesterol synthesis[91]. By contrast, in the mouse, OCA inhibits cholesterol absorption and increases RCT, *via* hepatic- but not intestinal-FXR[92].

The hydrophobicity index and conjugation state of the BA pool is an important determinant of intestinal dietary cholesterol and lipid absorption[92], with a more hydrophobic BA pool being most efficient for intestinal cholesterol absorption. In line, CYP7A1$^{-/-}$ mice are protected from HFHC-induced metabolic disorders likely due to upregulation of the alternative BA synthesis pathway and hence a more hydrophilic BA pool[93]. Importantly, studies in mice explaining the increase in RCT[92] and trans-intestinal cholesterol excretion (TICE) upon FXR activation[94] by modifications in the hydrophobicity index of the BA pool, are unlikely to translate to human pathophysiology, as hydrophobic CDCA will predominate in humans *vs* hydrophilic MCA in rodents.

BA sequestrants (BAS) are anionic exchange resins that trap BA in the intestinal lumen increasing fecal BA output, hence decreasing intestinal FXR activity. As a consequence, lower amounts of BA and FGF15/19 reach the liver, deactivating hepatic FXR hence inducing CYP7A1-mediated conversion of cholesterol to BA, increasing LDL-Receptor (LDL-R) expression and thus lowering LDL-C. Concomitantly, the inhibition of lipogenesis by FXR is attenuated. In agreement, BAS decrease LDL-C and increase HDL-C, while also increasing plasma triglycerides and hepatic lipid accumulation. The LRC-CPPT trial showed that the BAS cholestyramine significantly reduced coronary heart disease death in hypercholesterolemic patients[95].

Glucose metabolism

BA regulate glucose homeostasis by acting directly on FXR and TGR5 in the intestine, liver and pancreas, and indirectly by promoting FXR-dependent induction of intestinal FGF15/19 (Figure3). In humans, FGF19 is also produced by the gallbladder[96] and in mice, FGF15 is produced in the hypothalamus where it signals to lower glucagon production[97].

In the intestine, FXR modulates the kinetics of glucose absorption, which is delayed in FXR-deficient mice[98]. FXR reduces post-prandial glucose utilization by inhibiting hepatic glycolysis and lipogenesis[79,99,100], whereas FGF15/19 increases glycogenesis[101]. Thus, in the post-prandial state, FXR lowers splanchnic glucose utilization (Figure3).

In enteroendocrine L-cells, BA regulate the production and secretion of GLP-1 *via* opposite effects on TGR5 and FXR. Activation of TGR5, whose expression parallels L-cell density along the gastrointestinal tract with maximal expression in the colon, induces preproglucagon gene expression and GLP-1 secretion[69,102]. Since TGR5 is rather expressed at the basolateral, than at the apical L-cell membrane, absorption and local release of its agonists appears a pre-requisite for its activation[103]. By contrast, FXR activation represses preproglucagon gene expression and GLP-1 secretion in the ileum by inhibiting glycolysis and ChREBP activity in L-cells[104]. Since TGR5-mediated cytosolic signaling is rapid, whereas transcriptional regulation by FXR is slow, and since FXR expression is more proximal than TGR5 in the intestine, these receptors likely exert opposing effects on GLP-1 production, which are, however, separated in time and space.

FXR and TGR5 are both expressed in pancreatic β-cells, where they positively regulate synthesis and glucose-induced secretion of insulin[105,106]. Moreover, TGR5 activation in pancreatic α-cells induces pro-convertase-1 expression, shifting

glucagon production to GLP-1, hence increasing β-cell mass and function in a paracrine manner[107]. Perturbations in hepatic glucose metabolism alter BA synthesis, impacting on FXR-regulated β-cell glucose-stimulated insulin secretion[108], identifying a liver-pancreas BA signaling connection.

BAS treatment improves also glucose homeostasis, and colesevelam is a FDA-approved oral antidiabetic drug. Chronic treatment with BAS deactivates FXR in intestinal L-cells, enterocytes and hepatocytes, increasing GLP-1 synthesis and secretion, decreasing intestinal glucose absorption, enhancing hepatic glycolysis and lipogenesis thus promoting splanchnic glucose utilization[109] (Figure3). In accordance, pharmacological inactivation or genetic deficiency of FXR in the intestine improve energy and glucose homeostasis[110,111] and NASH due to decreased intestinal ceramide production[25]. Moreover, the BAS sevelamer decreases steatosis, lobular inflammation and endotoxemia in western diet-fed mice[112]. Together, most observations suggest that inactivation of intestinal FXR results in an improved metabolic profile, although treatment with the intestinal-selective FXR agonist fexaramine was also reported to improve metabolism[78].

Inhibition or deficiency of CYP8B1 improves glucose homeostasis by increasing GLP-1 in mice. The decreased CA:MCA ratio upon CYP8B1-deficiency mice impairs micellar absorption of fats and nutrients, increasing luminal free fatty acids in the ileum and GLP-1 secretion[113]. Furthermore, MCA may inhibit intestinal FXR, which enhances GLP-1 production[104]. However, translation of these findings to humans is unlikely (see above).

Initial studies suggested a role of FXR in the regulation of hepatic gluconeogenesis by decreasing the expression of the rate-limiting enzymes phosphoenolpyruvate carboxy-kinase (PEPCK), glucose-6-phosphatase (G6Pase) and fructose-1,6-

biphosphatase-1 (FBP1)[114,115]. However, other studies reported that FXR activation induces PEPCK[116] and that PEPCK and G6Pase are lowered in FXR-deficient mice[99,115]. FXR-deficient mice submitted to a fasting-refeeding schedule presented an accelerated response to high carbohydrate refeeding with induction of glycolytic and lipogenic genes and a pronounced repression of gluconeogenic genes, with concomitant hypoinsulinaemia and hypoglycemia[99]. These studies all employed different conditions in which gluconeogenesis was evaluated (*in vivo*, *in vitro*, fasting, refeeding, HFD...). Thus, the exact role of FXR in fasting-induced gluconeogenesis is still unclear and requires further studies. Similarly, conflicting data exist in humans, since OCA treatment increases insulin-sensitivity measured using hyperinsulinemic-euglycemic clamps in T2D/NASH patients[117], whereas HOMA-IR increased in NASH patients in the FLINT trial[90].

Immune function

FXR and TGR5 are expressed in several immune cell types. TGR5 exerts anti-inflammatory activities, decreasing cytokine production in monocytes, macrophages[118], Kupffer[119] and human dendritic cells[120]. TGR5 activation reduces HFD-induced glucose intolerance, insulin resistance and inflammation by inhibiting NLRP3 inflammasome activation *via* the TGR5-cAMP-PKA axis in mice[121]. Furthermore, TGR5 activation protects against LPS-induced inflammation[122], atherosclerosis[123] and experimental autoimmune encephalitis[118].

Overexpression of CYP7A1 decreases hepatic inflammatory cell infiltration, pro-inflammatory cytokine production and fibrosis in methionine/choline-deficient diet-fed mice by decreasing hepatic free cholesterol, oxidative stress in a FXR-, but not TGR5-dependent manner[124]. Intestinal FXR activation with OCA decreased the pro-

inflammatory genes IL-1β, IL-6 and macrophage chemoattractant protein-1 (MCP-1) in the colon, lowering trinitrobenzensulfonic acid or dextran sodium sulfate-induced inflammation in mouse colonic mucosa[125,126]. Moreover, FXR-deficiency impairs intestinal barrier function, which may enhance hepatic LPS exposure and inflammation[127].

NASH/NAFLD

NAFLD is a progressive liver disease, which initiates with hepatic steatosis and can progress to inflammation and hepatocyte ballooning (NASH), fibrosis, cirrhosis and finally hepatocarcinoma. Besides contributing to liver-related mortality, NAFLD is also strongly associated with a high cardiovascular disease risk. Promoter hypermethylation is inversely correlated to the expression of the CYP27A1, OSTα, BACS and OATP genes in NAFLD livers, which could lead to liver and systemic toxicity[128]. FXR activation may reduce NAFLD, as it reduces steatosis by inhibiting lipogenesis, decreases chemically-induced hepatic inflammation and fibrosis in rats[129] and maintains intestinal barrier integrity, thus protecting the liver from bacteria-derived inflammatory signals[127]. Since FXR expression is low in quiescent and activated stellate cells, its effects on fibrosis may be indirect[130].

A recent large randomized placebo-controlled trial in patients with biopsy-diagnosed NASH showed that OCA treatment improves the histological NAFLD Activity Score and fibrosis of the liver[90]. However, OCA treatment also reduces HDL- and increases LDL-cholesterol and HOMA-IR. In line, OCA reduces the secretion of inflammatory and fibrotic factors, but increases apoB secretion in an *in vitro* reconstituted human liver model[131]. These effects on lipids, also observed in healthy individuals[132], and glucose homeostasis are potentially restricting the clinical use of such semi-synthetic

BA compounds and call for the design of Selective BA Receptor Modulators (SBARMs) devoid of such side-effects.

Therapeutic Modulation of Bile Acid Metabolism

Given the role of BA signaling in the regulation of meta-inflammation, altering the BA pool and BA receptor activities may be valuable therapeutic options to treat meta-inflammatory disorders[133,134].

TGR5

Based on their ability to promote energy expenditure in BAT, GLP-1 secretion in enteroendocrine L-cells and anti-inflammatory properties, TGR5 agonists may be useful in the treatment of obesity and T2D. Semi-synthetic BA-derivatives INT-777[69] and non-steroidal TGR5 agonists[135,136] improve glucose homeostasis in preclinical models. Unfortunately, systemic exposure to TGR5 agonists increases gallbladder volume[135,136] and promotes pruritus[137]. The ideal molecule would thus be a topical intestinal agonist with limited, local absorption to reach the basolateral membrane of L-cells, but without systemic exposure to avoid cholecystomegaly. This would thus preserve GLP-1 secretion, but unfortunately also preclude BAT activation.

FXR

Due to their inhibitory effects on lipogenesis and hepatic fibrosis, FXR agonists, such as OCA, are in development for NASH treatment. Other FXR non-steroidal agonists are GW4064, Px-104 (Phenex), WAY-362450 (Wyeth), EDP-305 and EP-024297 (Enanta Pharmaceuticals), fexaramine, LJN452 (Novartis Pharmaceuticals) and GS-9674 (Phenex-Gilead), some of which are in clinical development. As discussed,

intestinal antagonism of FXR appears to improve metabolic control, whereas hepatic FXR activation may improve hepatic fibrosis. FXR inactivation triggered with BAS has beneficial effects on diabetes and hypercholesterolemia, but increases plasma triglycerides and hepatic steatosis, which precludes their use in patients with hypertriglyceridaemia.

FGF19

Due its metabolic effects[101], FGF19 is a candidate to treat NASH and obesity-related disorders, but FGF19 may increase the risk of cancer. The recombinant FGF19 mimetic peptide NGM282, currently clinically tested in metabolic liver disease, does not induce cell proliferation. Surprisingly, anti-sense FGFR4[138] (ISIS) has also been shown to induce fat burning and energy expenditure in mice. However, potential species differences between FGF19 and FGF15 signaling call for cautions when interpreting FGF19 studies in rodents.

CYP8B1

Based on the beneficial metabolic phenotype of CYP8B1-deficiency[113], inhibition of CYP8B1 by decreasing the 12α-hydroxylated:non12α-hydroxylated BA ratio has potential therapeutic implications. However, CYP8B1 inhibition results in opposite changes in physicochemical properties of the BA pool in humans *vs* rodents. Moreover, it induces the FXR antagonist MCA in rodents, whereas the FXR agonist CDCA is predominant in humans, cautioning the extrapolation of rodent studies to humans.

ASBT-I

The impairment of intestinal BA reuptake by inhibiting ASBT improves features of NAFLD and insulin-sensitivity in HFD-fed mice[139]. Similar as BAS, ASBT inhibitors (ASBT-I) reduce intestinal BA absorption, decreasing hepatic BA supply and thus FXR activation, resulting in de-repression of CYP7A1 and enhanced conversion of cholesterol to BA. Moreover, shifting BA to the distal part of intestine may sustain TGR5-induced GLP-1 secretion as, in contrast to resins, free BA absorption is not impaired upon ASBT-I treatment. Further studies on ASBT-I should be performed to assess the efficacy and safety of this approach in humans or relevant models[139].

NorUDCA

Finally, the UDCA derivative norUDCA improves hepatic steatosis in mice[140,141]. NorUDCA does not activate FXR/TGR5, but may exert FXR antagonistic effects inhibiting intestinal FGF19, enhancing BA synthesis and decreasing plasma cholesterol, but possibly increasing lipogenesis[142].

Conclusion and perspectives

The high BA concentrations in entero-hepatic tissues regulate metabolism in an inter-organ dialogue between the intestine, its microbiota, and the liver. In turn, the liver secretes BA, hence modulating intestinal metabolism. In peripheral organs, BA also contribute to metabolic homeostasis, even though their concentrations are lower in the systemic circulation. Thus, impaired BA metabolism likely contributes to the pathophysiology of metabolic diseases (obesity, T2D and NASH). The bidirectional effects between intestinal microbiota and BA suggest that dysbiosis and associated alterations in BA homeostasis may interactively contribute to the metabolic

dysregulations seen in T2D, obesity and NAFLD, as well as their remission upon bariatric surgery.

An important remaining question is whether FXR should be activated or inhibited in the intestine and/or liver to reverse metabolic abnormalities and NAFLD. Intestinal-specific FXR deactivation, either by natural/chemical antagonism or BA sequestration, prevents obesity, T2D and NAFLD/NASH in rodents (Figure4). Contradictorily, fexaramine, reportedly exerting exclusively intestinal FXR-specific actions, confers similar beneficial effects. A systematic comparison of the activity of FXR agonists and antagonists on the intestinal FXR signaling pathway could provide clues about their mechanism of action, as they clearly differ in their ability to activate subsets of FXR target genes and thus act as SBARMs[143]. In addition, exploring their ability to affect the gut microbiota, and vice-versa, may also identify reasons for their different biological activities.

In the liver, FXR exerts hepatoprotective activities, improving steatosis, inflammation and fibrosis (Figure 4). An important unresolved question is whether, through treating with metabolically-stable, highly-active synthetic BA analogues, interfering permanently with the FXR signaling pathway, whose functions differ in fasting and fed conditions, and BA synthesis, which is submitted to circadian fluctuations, may in the long term induce unwanted effects.

Based on existing preclinical data and clinical use of BAS, it appears that an orally administered inhibitor of intestinal FXR would be preferential to improve glucose and cholesterol metabolism, whereas a liver-targeted FXR agonist would improve liver function and fibrosis.

Caution should also be taken when translating data from preclinical murine models to humans, since BA pool modulation affects the hydrophobicity index differently in mice

vs humans, and mice produce the FXR antagonist TβMCA, absent in humans. The recent identification of the enzyme responsible for MCA synthesis in mice will allow the development of humanized-BA pool murine models. Finally, most beneficial effects of the FGF15/19 pathway have been observed by treating mice with supra-pharmacological concentrations of human recombinant FGF19, which could, due to species-specific differences, result in erroneous extrapolations. Further studies in this exciting field will determine whether pharmacological modulation of the novel BA metabolism targets (TGR5, FXR, ASBT, FGF19, CYP8B1) will convey beneficial clinical effects in humans.

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Figure legends

Figure 1: Role of BA in the control of metabolic and immune homeostasis *via* activation of their receptors FXR and TGR5.

See text and Supplemental references 2 for details.

Abbreviations: BA (bile acids); TGR5 (Takeda G protein-coupled receptor 5); FXR (farnesoid X receptor); FGF15/19 (fibroblast growth factor 15/19); Lp (lipoprotein); HDL (high density lipoprotein); IDL (intermediary density lipoprotein); LDL (low density lipoprotein); Lp(a) (lipoprotein (a)); VLDL (very low density lipoprotein); GLP-1 (glucagon-like peptide-1); ASBT (apical-sodium-dependent BA transporter); TNFα (tumor necrosis factor alpha); IL (interleukin); COX (cyclooxygenase).

Figure 2: BA control lipoprotein metabolism through hepatic and intestinal FXR activation.

FXR and FGF15/19 decrease hepatic lipogenesis. VLDL secretion, apoB and MTP synthesis are inhibited by FXR. Increased ApoCII and diminished ApoCIII enhance LPL-mediated intravascular lipolysis, and thus promote conversion of VLDL to IDL and LDL. FXR inhibits BA synthesis from cholesterol. As a consequence, hepatic cholesterol concentrations increase and LDL-R activity decreases. SR-B1 induction enhances selective CE uptake, and associated with increased CETP and decreased ApoA1 lowers HDL-C. In rodents, FXR enhances TICE by changing the hydrophobicity index. This effect is thus likely to occur in the opposite direction in humans (shown in figure). Inhibition of hepatic synthesis of apo(a) by hepatic FXR and intestinal FGF19 decreases Lp(a).

Abbreviations: Apo (apolipoprotein); BA (bile acids); C (cholesterol); CETP (cholesteryl ester transfer protein); CDCA (chenodesoxycholic acid); CE (cholesteryl esters); FGF15/19 (fibroblast growth factor 15/19); HDL (high density lipoprotein); IDL (intermediary density lipoprotein); LDL (low density lipoprotein); Lp(a) (lipoprotein (a)); MTP (microsomal transfer protein); SR-B1 (scavenger receptor type 1); TICE (trans-intestinal cholesterol excretion); VLDL (very low density lipoprotein).

Figure 3: Modulation of glucose homeostasis by BA.

FXR activation in the enterocyte modulates the absorption of glucose (1) and BA (2), and induces FGF15/19 secretion (3). In the liver, FXR activation decreases glycolysis and lipogenesis *via* inhibition of ChREBP (4) and SREBP1c (5), respectively, hence decreasing VLDL-TG production. Insulin *via* the INSR (6) modulates the proportion of 12α-OH BA by repressing FoxO1, a CYP8B1 activator. Intestinal FGF15/19 activates hepatic FGFR4/βKlotho (7), which decreases GS phosphorylation by inhibiting GSK3β, increasing glycogenesis and decreasing glycemia. In entero-endocrine L-cells, BA increase -*via* TGR5- (8) or decrease -*via* FXR through ChREBP- (9) proglucagon gene expression and GLP-1 secretion. TGR5 activation in pancreatic α-cells induces pro-convertase-1, shifting proglucagon processing from glucagon to GLP-1 (10). FXR and TGR5 activation in pancreatic β-cells promotes insulin secretion, lowering glycemia (11). BA synthesis in the hepatocyte is decreased by FGF15/19-FGFR4/βKlotho activation and FXR activation *via* SHP-LRH-1 (12).

Abbreviations: AcCoA (acetyl co-enzyme A); Apo (apolipoprotein); ASBT (apical-sodium-dependent BA transporter); BA (bile acids); BSEP (bile salt export protein); C (cholesterol); CYP7A1 (cytochrome P450 7α-hydroxylase A1) ; CYP8B1 (cytochrome P450 12α-hydroxylase B1); FGF15/19 (fibroblast growth factor 15/19); FXR

(farnesoid X receptor); GLP-1 (glucagon-like peptide-1); GS (glycogen synthase); GSK3 (glycogen synthase kinase 3); INSR (insulin receptor); SHP (short heterodimer protein); TGR5 (Takeda G protein-coupled receptor 5); L-PK (liver pyruvate kinase); OSTα/β (organic solute transporters α and β); VLDL (very low density lipoprotein).

Figure 4: Differential effects of FXR inhibition *vs* activation in liver and intestine on metabolism, inflammation and fibrosis.

See text and supplemental references 3 for details.

Abbreviations: AMPK (AMP-activated protein kinase); ChREBP (carbohydrate responsive element binding protein); ER (endoplasmic reticulum); FGF15/19 (fibroblast growth factor 15/19); GLP-1 (glucagon-like peptide-1); HSC (hepatic stellate cell); LKB1 (liver kinase B1); L-PK (liver pyruvate kinase); miR (micro RNA); SHP (small heterodimer protein); SREBP1c (sterol responsive element binding protein 1); WAT (white adipose tissue).

Table

Table: Influence of obesity, insulin resistance, type 2 diabetes, NAFLD and bariatric surgery (Roux-en-Y gastric bypass, vertical sleeve gastrectomy, bilio-pancreatic diversion and adjustable gastric banding) on peripheral blood bile acid concentrations.

OBESITY / IR / T2D / NAFLD					
Case (n)	**Controls (n)**	**Feeding state**	**Methodology**	**Outcome**	**Reference**
85 obese	15	fasting	Enzymatic assay	Total BA concentrations positively correlated with BMI	Prinz 2015[1]
15 T2D	15 non-diabetic, BMI-matched controls	fasting OGTT low-fat, medium-fat, high-fat meal test	UPLC-MS/MS	Fasting: ↑ total BA concentrations compared with non-diabetic controls Post-prandial: ↑ total BA concentrations in all the feeding states, positively correlated with increasing meal fat content compared with non-diabetic controls	Sonne 2016[2]
35 T2D	200 non-diabetic patients classified into quartiles based on IR	fasting	LC-MS	T2D patients: ↑total BA concentrations without changes in the 12α-hydroxy:non-12α-hydroxy-BA ratio Non-diabetic IR patients: ↑ 12α-hydroxy:non-12α-hydroxy-BA ratio associated with ↓ insulin sensitivity and ↑ plasma TGs	Haeusler 2013[3]
12 T2D	12 matched non-diabetic controls	fasting meal test	HPLC-MS/MS	Fasting: no changes in total BA in T2D compared with non-diabetic controls Post-prandial: ↑total and Glyco-BA in T2D compared with non-diabetic controls	Vincent 2013[4]
12 T2D	62 non-diabetic volunteers	fasting	HPLC-MS/MS	Fasting: ↑ tauro BA in T2D patients compared with non-diabetic controls	Wewalka 20014[5]
20 T2D 22 obese	14 healthy	fasting	GC-MS	↑ total BA concentrations in obese and T2D patients compared with controls ↑DCA in T2D compared with controls ↑CA in obese patients compared with controls CDCA and CA, and to a lesser extent DCA, positively associated to IR in obese and T2D patients	Cariou 2011[6]

7 NASH	15 healthy	fasting post-prandial		LC-MS/MS	Fasting and post-prandial: ↑ total BA concentrations in NASH compared with controls due to conjugated species	Ferslew 2015[7]

ROUX-EN-Y GASTRIC BYPASS

Case (n)	Controls (n)	Feeding state	Time after surgery	Methodology	Outcome	Reference
9	/	fasting	2y, 4y	HPLC-MS/MS	2y, 4y: ↑ total BA	Patti 2009[8]
19	/	fasting	1m, 3m	HPLC-MS	1m, 3m: ↑ total BA	Nakatani 2009[9]
35	/	fasting	3m	Enzymatic assay	3m: ↑ total BA	Jansen 2011[10]
12	/	fasting	4d, 42d	LC-MS/MS	42d: ↑ total BA	Pournaras 2012[11]
30	/	fasting	1y	Enzymatic assay, HPLC-MS/MS	1y: ↑ total BA; ↓ tauro-BA	Simonen 2012[12]
36 T2D-R 21 T2D-NR	37	fasting	1y	LC-MS/MS	1y: ↑ total BA in T2D-R	Gerhard 2013[13]
8	/	fasting	After 20% of weight loss	LC-MS	↑ total BA	Kohli 2013[14]
21	/	fasting	1m, 6m, 1y, 2y	LC-MS/MS	1m: ↑ total BA due to UDCA, TUDCA, GUDCA 2y: ↑ total BA due to CA, CDCA, DCA, GDCA, HCA 6m, 1y: no changes in total BA	Albaugh 2015[15]
15	/	fasting	1y	LC-MS	No changes in total BA	Sachdev 2016[16]
7	6	fasting	1w, 3m, 1y	GC-MS	1w, 3m: no changes in total BA 1y: ↑ total BA	Steinert 2013[17]

63	/	fasting	15m	HPLC-MS/MS	15m: ↑ total BA	Werling 2013[18]
13	/	fasting	1m, 2y	HPLC-MS/MS	1m: ↓ total BA 2y: ↑ total BA and ↑ 12α-hydroxylated/non12α-hydroxylated BA ratio	Dutia 2015[19]
13 T2D 12 non-diabetic	/	fasting	1w, 3m, 1y	HPLC-MS/MS	1w: ↓ total BA in non-diabetic patients 3m, 1y: ↑ total BA in T2D and non-diabetic patients	Jørgensen 2015[20]
15	/	meal test	1y	LC-MS	1y: ↑ total BA due to conjugated BA	Sachdev 2016[16]
7	6	meal test	1w, 3m, 1y	GC-MS	1w, 3m: no changes in total BA 1y: ↑ total BA	Steinert 2013[17]
5	8	meal test	1w, 4w, 40w	HPLC-MS	4w, 40w: ↑total BA	Ahmad 2013[21]
13 T2D 12 non-diabetic	/	meal test	1w, 3m, 1y	HPLC-MS/MS	1w: ↑ total BA in non-diabetic patients 3m, 1y: ↑ total BA in T2D and non-diabetic patients	Jørgensen 2015[20]
63	/	OGTT	15m	HPLC-MS/MS	15m: ↑ total BAs due to glyco-conjugated BA	Werling 2013[18]
13	/	OGTT	1m, 2y	HPLC-MS/MS	1m: unchanged total BA 2y: ↑ total BA and ↑ 12α-hydroxylated:non12α-hydroxylated BA ratio	Dutia 2015[19]
8	/	post-prandial	After 20% of weight loss	LC-MS	↑ total BA	Kohli 2013[14]
16 GBMIL 14 PBMIL	8	post-prandial	1y	Enzymatic assay	1y: ↑ total BA in GBMIL and PBMIL compared with lean controls No changes between GBMIL and PBMIL	Dirksen 2013[22]

VERTICAL SLEEVE GASTRECTOMY						
Case (n)	**Lean controls (n)**	**Feeding state**	**Time after surgery**	**Methodology**	**Outcome**	**Reference**
7	6	fasting meal test	1w, 3m, 1y	GC-MS	Fasting 1w, 3m: no changes; 1y: ↑total BA Post-prandial: no changes in total BA	Steinert 2013[17]
17	/	fasting	6m, 1y, 2y	GC-MS	6m, 1y, 2y: no changes in total BA	Haluzíková 2013[23]

BILIO-PANCREATIC DIVERSION						
Case (n)	**Controls (n)**	**Feeding state**	**Time after surgery**	**Methodology**	**Outcome**	**Reference**
15	10 BMI-matched non-surgical	fasting	7w, 53w	LC-MS/MS	7w: ↑ total BA mainly due to free BA 53w: ↑ total BA, but lower than 7w	Ferrannini 2015[24]

LAPAROSCOPIC ADJUSTABLE GASTRIC BANDING						
Case (n)	**Controls (n)**	**Feeding state**	**Time after surgery**	**Methodology**	**Outcome**	**Reference**
6	/	fasting	4d, 42d	LC-MS/MS	No changes in total BA	Pournaras 2012[11]
10	/	fasting + post-prandial	After 20% of weight loss	LC-MS	Fasting: ↓ total BA Post-prandial: no changes in total BA	Kohli 2013[14]

References included in Supplemental references 1

Abbreviations: BMI (body mass index); d (days); GBMIL (patients with good body mass index loss after RYGB); GC-MS (gas chromatography–mass spectrometry); IR (insulin resistance); LC-MS (liquid chromatography–mass spectrometry); LC-MS/MS (chromatography–tandem mass spectrometry); m (months); OGTT (oral glucose tolerance test); PBMIL (patients with poor body mass index loss after RYGB); RYGB (Roux-en-y gastric bypass); TG (triglycerides); T2D (type 2 diabetes); T2D-NR (type 2 diabetes without remission); T2D-R (type 2 diabetes with remission); UPLC-MS/MS (ultra-performance liquid chromatography-tandem mass spectrometer); w (weeks); y (years).

Figure 1

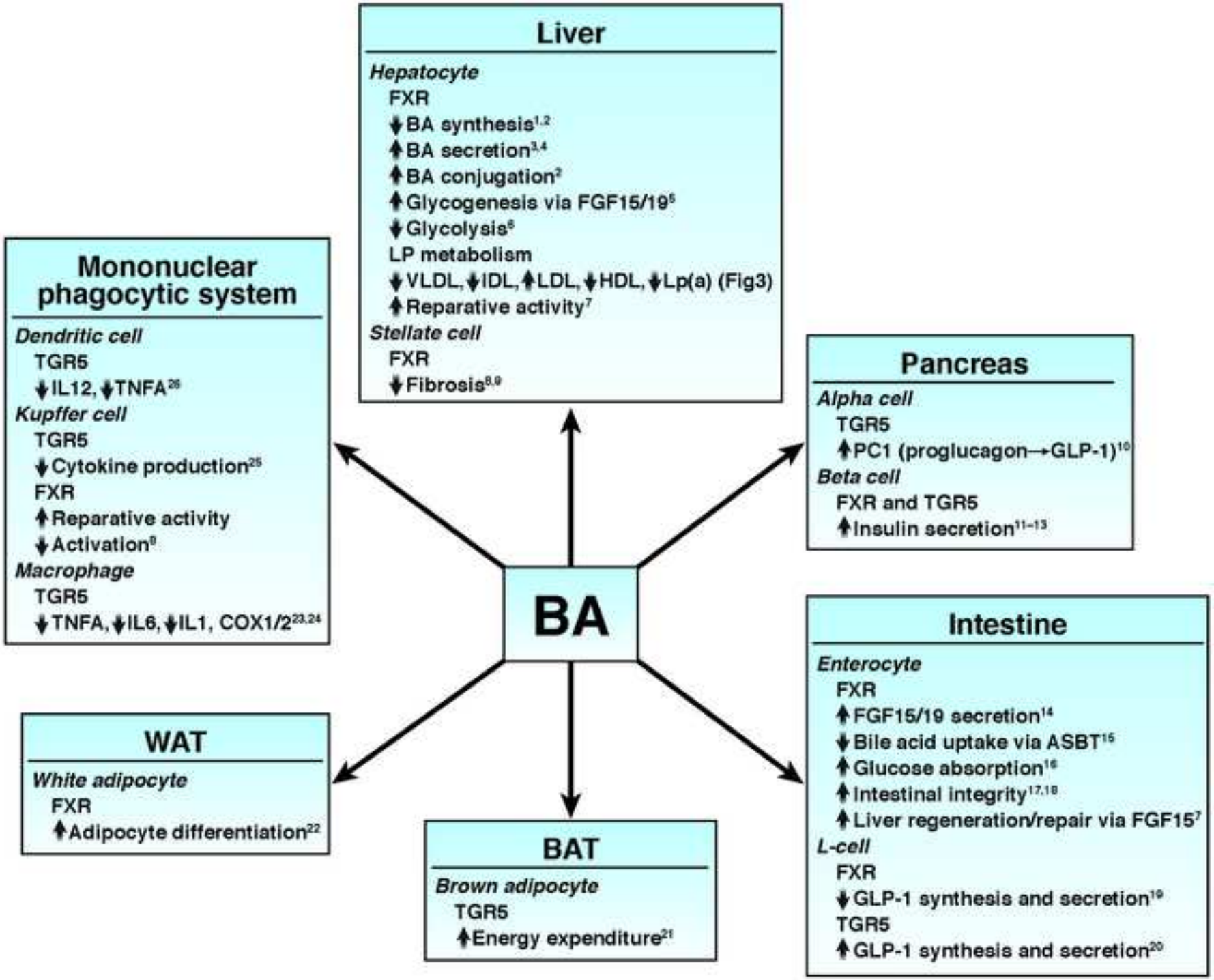

Liver
Hepatocyte
FXR
↓BA synthesis[1,2]
↑BA secretion[3,4]
↑BA conjugation[2]
↑Glycogenesis via FGF15/19[5]
↓Glycolysis[6]
LP metabolism
↓VLDL, ↓IDL, ↑LDL, ↓HDL, ↓Lp(a) (Fig3)
↑Reparative activity[7]
Stellate cell
FXR
↓Fibrosis[8,9]
Mononuclear phagocytic system
Dendritic cell
TGR5
↓IL12, ↓TNFA[26]
Kupffer cell
TGR5
↓Cytokine production[25]
FXR
↑Reparative activity
↓Activation[8]
Macrophage
TGR5
↓TNFA, ↓IL6, ↓IL1, COX1/2[23,24]
Pancreas
Alpha cell
TGR5
↑PC1 (proglucagon→GLP-1)[10]
Beta cell
FXR and TGR5
↑Insulin secretion[11–13]
BA
WAT
White adipocyte
FXR
↑Adipocyte differentiation[22]
BAT
Brown adipocyte
TGR5
↑Energy expenditure[21]
Intestine
Enterocyte
FXR
↑FGF15/19 secretion[14]
↓Bile acid uptake via ASBT[15]
↑Glucose absorption[16]
↑Intestinal integrity[17,18]
↑Liver regeneration/repair via FGF15[7]
L-cell
FXR
↓GLP-1 synthesis and secretion[19]
TGR5
↑GLP-1 synthesis and secretion[20]

Figure 2

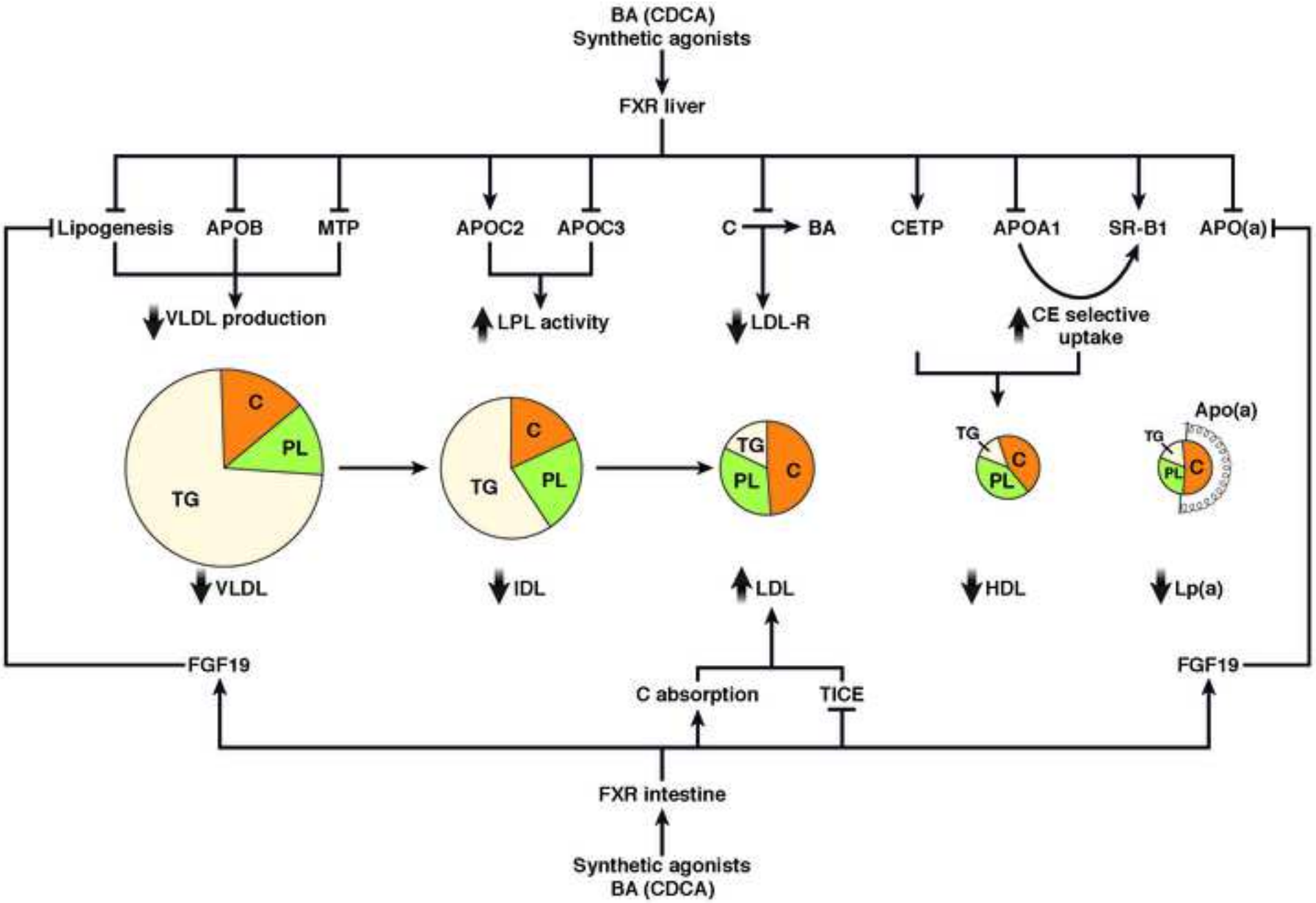

BA (CDCA)
Synthetic agonists
FXR liver
Lipogenesis
APOB
MTP
APOC2
APOC3
C
BA
CETP
APOA1
SR-B1
APO(a)
VLDL production
LPL activity
LDL-R
CE selective uptake
C
PL
TG
VLDL
IDL
LDL
HDL
Lp(a)
Apo(a)
FGF19
C absorption
TICE
FGF19
FXR intestine
Synthetic agonists
BA (CDCA)

Figure 3

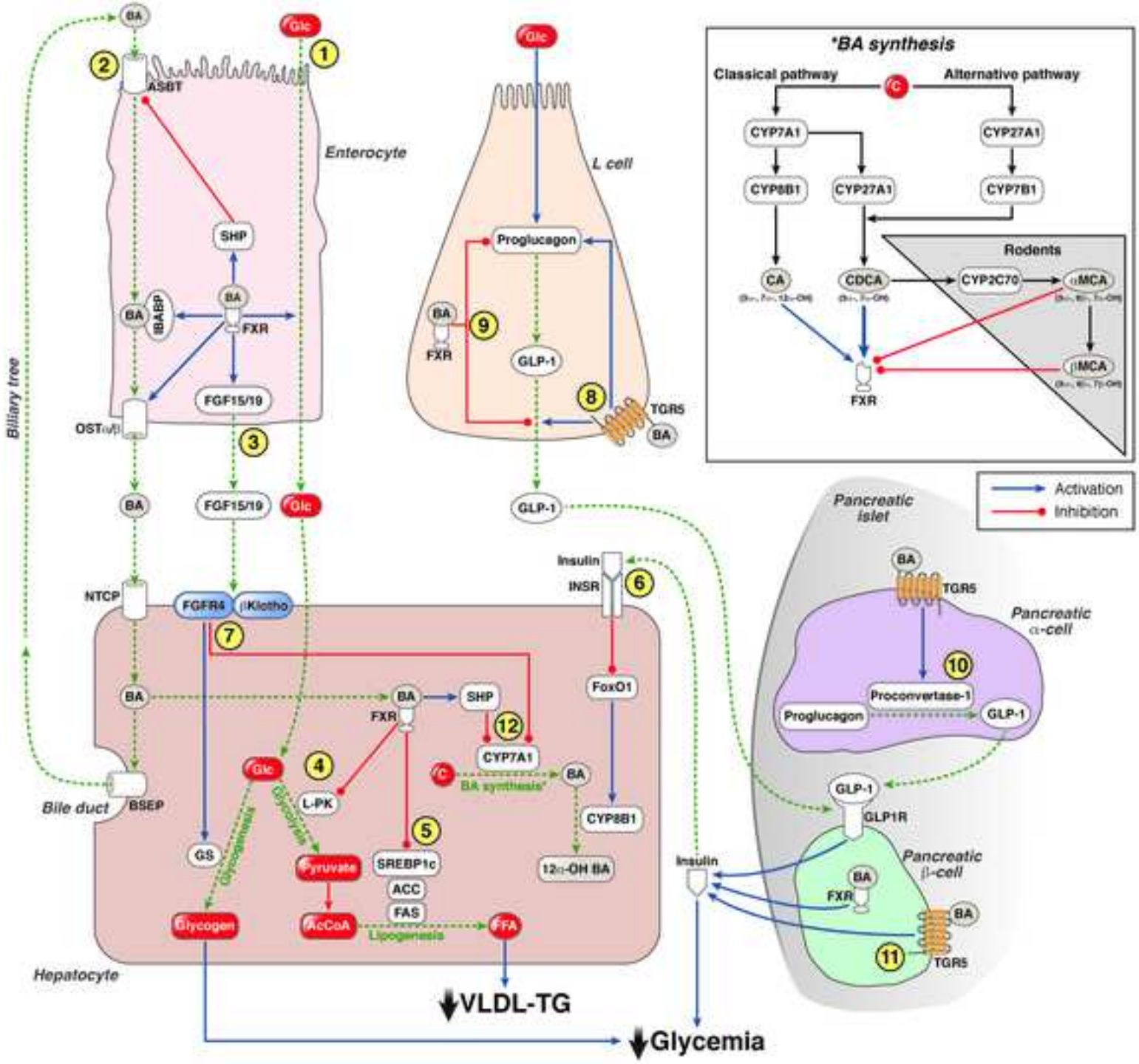
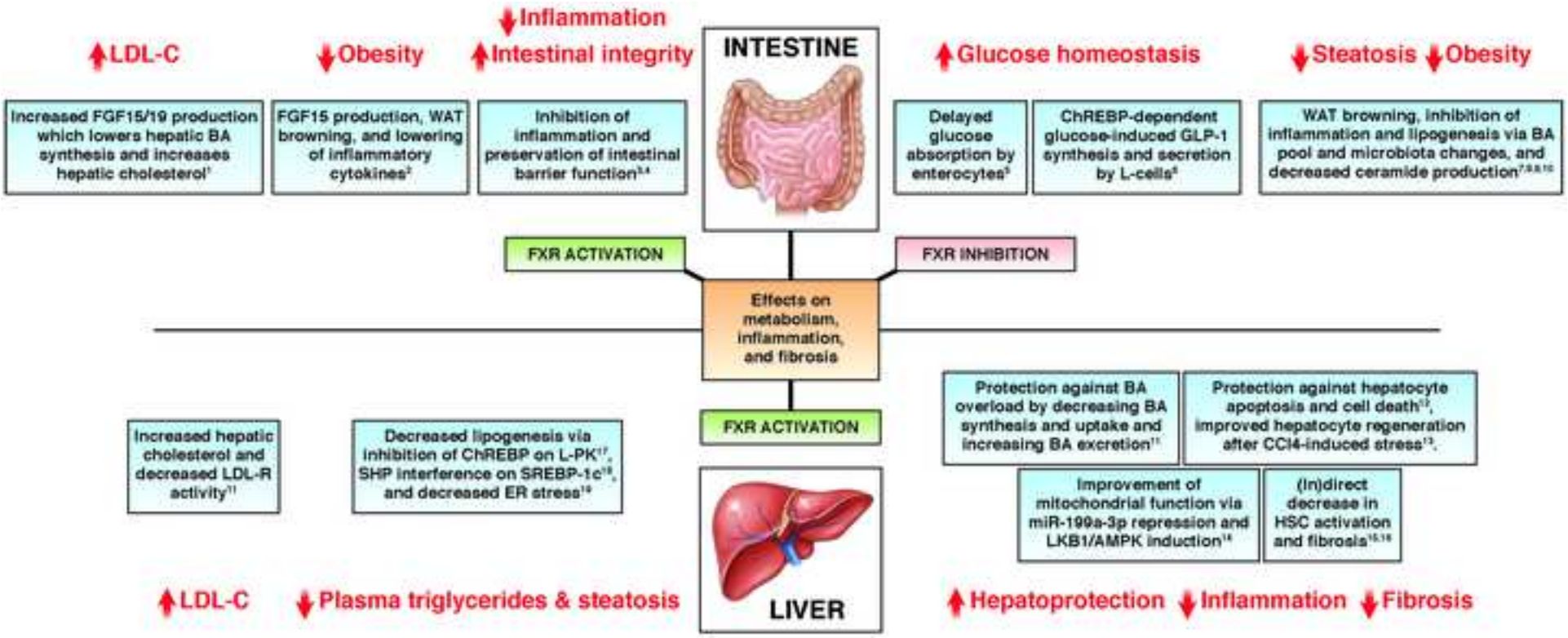

*BA synthesis
Classical pathway
Alternative pathway
CYP7A1
CYP27A1
CYP8B1
CYP27A1
CYP7B1
Rodents
CA
CDCA
CYP2C70
αMCA
βMCA
FXR
Activation
Inhibition
Enterocyte
L cell
Biliary tree
Bile duct
Hepatocyte
Pancreatic islet
Pancreatic α-cell
Pancreatic β-cell
ASBT
SHP
IBABP
FXR
FGF15/19
OSTα/β
Proglucagon
GLP-1
TGR5
NTCP
FGFR4
βKlotho
Insulin
INSR
FoxO1
CYP7A1
BA synthesis*
CYP8B1
12α-OH BA
L-PK
GS
Glycogenolysis
Glycolysis
Pyruvate
SREBP1c
ACC
FAS
Lipogenesis
Glycogen
AcCoA
FFA
BSEP
Proconvertase-1
GLP1R
VLDL-TG
Glycemia

Figure 4


↓Inflammation
↑LDL-C
↓Obesity
↑Intestinal integrity
INTESTINE
↑Glucose homeostasis
↓Steatosis ↓Obesity
Increased FGF15/19 production which lowers hepatic BA synthesis and increases hepatic cholesterol[1]
FGF15 production, WAT browning, and lowering of inflammatory cytokines[2]
Inhibition of inflammation and preservation of intestinal barrier function[3,4]
Delayed glucose absorption by enterocytes[5]
ChREBP-dependent glucose-induced GLP-1 synthesis and secretion by L-cells[6]
WAT browning, inhibition of inflammation and lipogenesis via BA pool and microbiota changes, and decreased ceramide production[7,8,9,10]
FXR ACTIVATION
FXR INHIBITION
Effects on metabolism, inflammation, and fibrosis
FXR ACTIVATION
Increased hepatic cholesterol and decreased LDL-R activity[11]
Decreased lipogenesis via inhibition of ChREBP on L-PK[17], SHP interference on SREBP-1c[18], and decreased ER stress[19]
Protection against BA overload by decreasing BA synthesis and uptake and increasing BA excretion[11]
Protection against hepatocyte apoptosis and cell death[12], improved hepatocyte regeneration after CCl4-induced stress[13].
Improvement of mitochondrial function via miR-199a-3p repression and LKB1/AMPK induction[14]
(In)direct decrease in HSC activation and fibrosis[15,16]
LIVER
↑LDL-C
↓Plasma triglycerides & steatosis
↑Hepatoprotection ↓Inflammation ↓Fibrosis

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