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REVIEW

Bile acids and their effects on diabetes

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Abstract **Diabetes is a widespread, rapidly increasing metabolic disease that is driven by hyperglycemia. Early glycemic control is of primary importance to avoid vascular complications including development of retinal disorders leading to blindness, end-stage renal disease, and accelerated atherosclerosis with a higher risk of myocardial infarction, stroke and limb amputations. Even after hyperglycemia has been brought under control, "metabolic memory," a cluster of irreversible metabolic changes that allow diabetes to progress, may persist depending on the duration of hyperglycemia. Manipulation of bile acid (BA) receptors and the BA pool have been shown to be useful in establishing glycemic control in diabetes due to their ability to regulate energy metabolism by binding and activating nuclear transcription factors such as farnesoid X receptor (FXR) in liver and intestine as well as the G-protein coupled receptor, TGR5, in enteroendocrine cells and pancreatic β-cells. The downstream targets of BA activated FXR, FGF15/21, are also important for glucose/insulin homeostasis. In this review we will discuss the effect of BAs on glucose and lipid metabolism and explore recent research on establishing glycemic control in diabetes through the manipulation of BAs and their receptors in the liver, intestine and pancreas, alteration of the enterohepatic circulation, bariatric surgery and alignment of circadian rhythms.**

Keywords bile acids; metabolic memory; diabetes; circadian rhythm; bariatric surgery

Introduction

Diabetes is a widespread and increasing global health problem that is driven by hyperglycemia. Intensive efforts to control hyperglycemia include administration of insulin or drugs that: (1) increase the endogenous release of insulin, (2) decrease release of glucose from the liver, (3) increase the use of glucose in both skeletal muscle and adipose tissue, (4) delay glucose absorption from food and, (5) upregulate the incretin system [1,2]. The importance of early intensive glycemic control to reduce risk of micro- and macrovascular complications has been shown by many large studies [3–5]. As a result of hyperglycemia-induced vascular complications, diabetic patients may develop retinal disorders leading to blindness, end-stage renal disease, and accelerated atherosclerosis that leads to a higher risk of myocardial infarction, stroke and limb amputations [6].

Regulation of hepatic glucose metabolism is of key importance to overall energy metabolism because the liver orchestrates not only the production of glucose via gluconeogenesis and glycogenolysis but also its storage via glycogenesis and lipogenesis. Hyperglycemia can eventually exhaust glycogen storage and glucose will then be diverted into the production of lipids that are then exported as very low density lipoproteins which in turn, are converted into triglycerides in adipose tissue. Therefore increased blood glucose leads to increased lipidemia and obesity [7].

The early glycemic environment is proposed to be "remembered" and this "metabolic memory" is thought to be due to the accumulation of reactive species during hyperglycemia that persist after glycemic control is established. Four key pathways (Fig. 1) have been suggested to be involved in diabetic complications; (1) increased flux of the polyol pathway, (2) increased production of advanced glycosylation products (AGE), (3) increased amounts of dihydroxyacetone phosphate, and (4) increased flux of the hexosamine pathway (Fig. 1) [2,6]. Whereas, treating type I diabetes (T1D) with insulin as early as possible may have merit, the situation is different for type II diabetes (T2D) where insulin is usually not the first line treatment. Therefore a complete

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understanding of metabolic control of nutrients, in particular, glucose and lipid metabolism, is essential in order to be able to intervene aggressively, perhaps by means other than insulin administration, to combat hyperglycemia for T2D patients at its onset [1]. In the following review we will explore recent research on the effect of bile acids (BAs) on glucose and lipid metabolism, the enteroendocrine system, as well as glycemic control via alteration of enterohepatic circulation of BAs, bariatric surgery and alignment of circadian rhythms. The articles evaluated in this review were chosen from recent articles published within the past 5 years in peer-reviewed journals. The keywords listed above were used to generate a search of the PUBMED database and representative articles pertaining to the involvement of BAs in the glycemic control of T2D were then selected.

Bile acid control of hepatic glucose and lipid metabolism via farnesoid X receptor

Bile acids (BAs) are synthesized in hepatocytes via cytochrome P450-mediated oxidation of cholesterol, which can occur through two pathways, the "classical" and "alternative" pathways. The "classical" pathway produces the primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA) through the actions of the enzymes, CYP7A1 and CYP8B1 [11]. CYP7A1 is the rate-limiting enzyme in the classical pathway and converts cholesterol to CDCA and the 12α-hydroxylase, CYP8B1 mediates the production of CA. Cholesterol will be converted to CDCA when CYP8B1 is deficient and thus CYP8B1 controls the CA/CDCA ratio [12]. The alternative pathway which involves CYP27A1 results in the

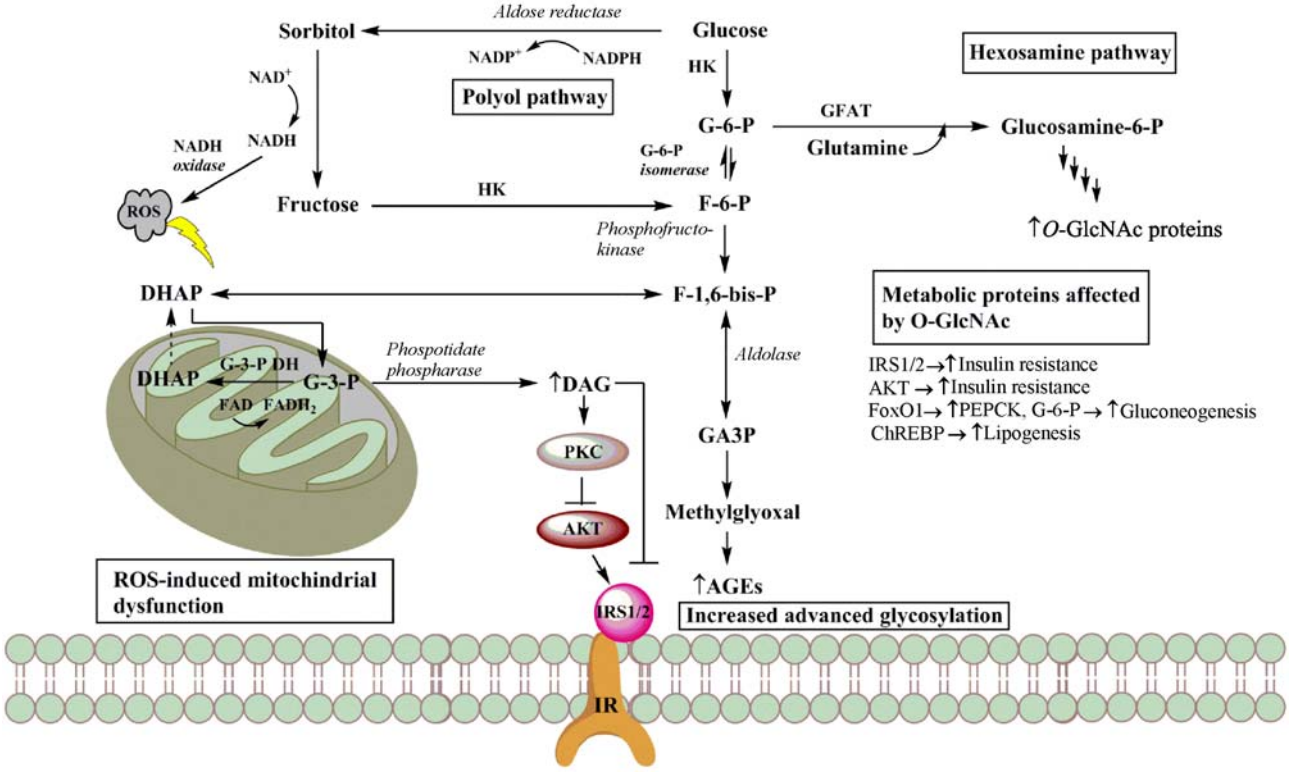

Fig. 1 The Effects of hyperglycemia on the development of metabolic memory. Excess glucose can exceed glycogen storage capacity and use for energy. When this happens, several alternative glucose utilizing pathways become active. The polyol or aldose reductase pathway converts glucose to sorbitol with concurrent production of additional NADH which when reacted with NADH oxidase leads to the production of excess ROS ultimately causing mitochondrial dysfunction. Sorbitol can also be converted to fructose in the polyol pathway and enter the glycolytic pathway to become F-6-P and then onto F-1,6-bis-P which in turn can become converted to GA3P and then methylglyoxal, a precursor for AGEs. Alternatively, F-1,6-bis-P can be biotransformed into DHAP which then enters 2 cycles, one which leads back to F-1,6-bis-P and more AGEs and another which leads to synthesis of G-3-P which can go on to be converted to DAG. DAG blocks AKT→ IRS1/2 leading to decreased insulin sensitivity. In the glycolytic pathway, G-6-P can be diverted to the hexosamine pathway which leads to an increase in *O*-GlcNAc proteins that in turn can cause increased insulin resistance, gluconeogenesis and lipogenesis [2,6,8–10]. Abbreviations: hexokinase (HK), glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), fructose-1,6-bis-phosphate (F-1,6-bis-P), glyceraldehydes-3-phosphate (GA3P), advanced glycosylation end products (AGEs), reactive oxygen species (ROS), dihydroxyacetone phosphate (DHAP), *O*-linked-*N*-acetylglucosamine (*O*-GlcNAc), glucosamine-fructose-6-phosphate amino-transferase (GFAT), glucose-3-phosphate (G3P), glucose-3-phosphate dehydrogenase (G-3-P DH), diacylglycerol (DAG), protein kinase C (PKC), protein kinase B (AKT), insulin receptor substrate 1/2 (IRS1/2), insulin receptor (IR), forkhead box protein O1 (FoxO1), carbohydrate response element binding protein (ChREBP), phosphoenolate pyruvate carboxylase (PEPCK).

production of CDCA. Notably, BA synthesis is under control of the farnesoid X receptor (FXR). Hepatic FXR via its effect on transcription of small heterodimer partner (SHP) suppresses the expression of CYP8B1 and to a lesser extent, CYP7A1, while intestinal FXR activation results in the production of FGF19 (15 in rodents) that upon binding to hepatic FGFR4/β-Klotho (bottom right, Fig. 3) causes ERK1/2 activation and suppresses both hepatic CYP7A1 and CYP8B1 expression [12]. After the primary BAs are synthesized they are then conjugated to either taurine (mainly in rodents) or to glycine (predominant in humans) by BA:CoA synthetase (BACS) and BA-CoA: amino acid N-acyltransferase (BAAT) enzymes to form TCA, TCDCA, GCA, and GCDCA. Next they are secreted from the liver into the bile caniculus via the canicular bile salt export pump (BSEP) where they are further modified via sulphation (sulfotransferase family 2A member 1 enzyme or SULT2A1) or via glucuronidation (UDP-glucuronosyltransferase or UGT enzymes) and secreted from the liver into the bile via the multidrug resistance-associated protein 2 transporter (MRP2) [13,14]. After a meal, the gallbladder contracts and releases bile salts in the form of mixed micelles which contain bile salts, cholesterol, and phospholipids, into the intestinal lumen, thus facilitating the emulsification and absorption of lipids in the small intestine [11]. Once bile salts are in the small intestine, the gut microbiota use bile salt hydrolase enzymes (BSH) to deconjugate the bile salts and a portion of these are further transformed in the large intestine into the secondary BAs, i.e., CA is converted into deoxycholic acid (DCA) and CDCA is converted into lithocholic acid (LCA) which can be further metabolized by gut microbiota to ursodeoxychlic acid (UDCA) [15,16]. Both bile salts and unconjugated BAs are transported from the intestinal lumen into the enterocytes via the apical sodium-dependent transporter (ASBT) and passive diffusion, respectively. Upon entry into the enterocyte, BA species are coupled to the ileal BA carrier protein (IBABP) which transports them across the enterocyte [17] to the basolateral organic solute transporters α and β (OSTα/β) where they are then transported into the portal vein for recycling back to the liver [14,17,18]. From the portal vein, bile salts are taken up by the sodium-dependent taurocholate cotransporting polypeptide (NTCP) transporter and unconjugated BAs are taken up by the organic anion transporters-1,4 (OATP1,4) [18]. This cycling of BA species between the intestine and liver is termed, enterohepatic circulation [19].

BAs are thus the end products of cholesterol catabolism and provide a means for the elimination of excess cholesterol in the body. They also facilitate the absorption of nutrients and drugs. In addition, BAs play an important role in regulating energy metabolism [11]. For example, hepatic gluconeogenesis, when excessive, can be a major cause of fasting hyperglycemia as well as increased postprandial hyperglycemia [20,21]. BAs have been shown to regulate gluconeogenesis by repressing transcription of gluconeogenesis rate-limiting enzymes such as phosphoenolypyruvate carboxylase (PEPCK), glucose-6-phosphatase (G6Pase) and fructose-1,6-bis phosphatase (FBP1). The complete mechanism is detailed in the legend for Fig. 2 [22]. When glucose enters the liver via the glucose transporter 2 (GLUT 2), it can be shunted either to glycolysis or to glycogenesis. The genes for the enzymes that promote these metabolic pathways are in turn, under the control of the transcription factor, liver X receptor (LXR) (see Fig. 2) [23]. The two downstream targets of LXR, carbohydrate response binding protein (ChREBP) and sterol response element binding protein-1c (SREBP1c), as well as being responsible for transcription of genes for key glycolytic/glycogenesis enzymes, also act in a synergistic way to transcribe genes for enzymes that are important for the *de novo* lipogenesis (DNL) pathway [23,24]. BA activated FXR is an antagonist of LXR, causing transcription of peroxisome proliferator-activated receptor-α (PPARα) which, in turn, transcribes genes for substances that increase β-oxidation of fatty acids (FAs) [25]. In this way, BAs are positive regulators of metabolism in the liver, via FXR. BA activated FXR leads to a decrease in the glycolysis → DNL → triglyceride (TG) axis, thus reducing hepatic lipid accumulation and also via increased transcription of PPARα, causing increased usage of lipids for energy via the β-oxidation pathway (Fig. 2).

In humans, an inverse relationship has been observed between metabolic disease and serum ceramides (Fig. 3) [28,29]. Intestinal FXR signaling has been found to be increased in distal ileum biopsies from obese individuals [30]. In mice on a HFD and administered tempol, a gut microbiota modifying agent which reduces microbiota with high BSH activity, suggested that inhibition of FXR signaling was responsible for the resistance of these mice to obesity and insulin resistance as there were increased amounts of T-β-MCA observed (FXR antagonist) and decreased amounts of the unconjugated form, β-MCA (FXR agonist) [31]. Subsequently, a synthetic, intestine-specific small molecule, G-MCA, which cannot be hydrolyzed by gut microbial BSH, has been used to selectively inhibit intestinal FXR in mice and when exposed to HFD, these mice were protected from weight gain, insulin resistance, hyperglycemia and hepatic steatosis [30]. The mechanism by which intestine selective inhibition of FXR signaling reduces diet-induced metabolic disease was shown to involve the modulation of ceramide synthesis as FXR targets genes in the ileum for the ceramide synthesis enzymes, sphingomyelin phosphodiesterase 3 (*Smpd3*) and serine palmitoyltransferase long-chain base subunit 2 (*Sptlc2*) (lower left, Fig. 3) [30]. More direct evidence for the link between ceramide and metabolic disease came from studies of the WAT of

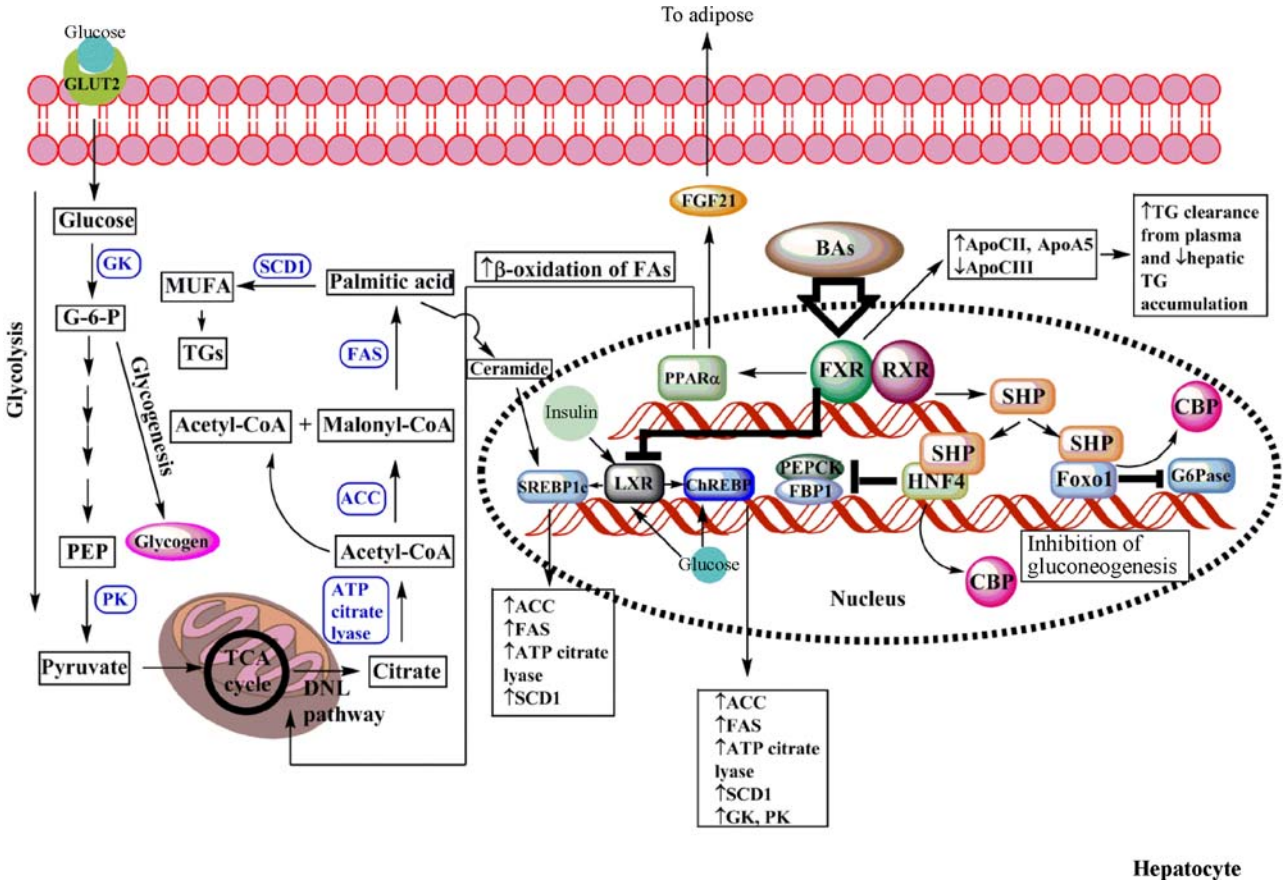

Fig. 2 The effects of BAs on glucose and lipid metabolism in the normal liver. BAs are considered positive regulators of metabolism in the liver. BA activation of FXR causes its translocation to the nucleus where it forms a heterodimer with RXR. Several signaling pathways are initiated as a result. The co-factor, SHP becomes active in binding the transcription factor, HNF4 which in turn, releases its co-factor CBP. This results in inhibition of the transcription of two important enzymes in gluconeogenesis, PEPCK and FBP1. SHP also can bind to the transcription factor FoxO1 which in turn releases its co-factor, CBP with the result that the enzyme G6Pase does not get expressed. In this way BA activated FXR inhibits gluconeogenesis and prevents release of more glucose into the circulation. BA activated FXR also modulates the transcription factor LXR. LXR is a positive regulator of the transcription factors, ChREBP and SREBP1c which act synergistically to control DNL and glycolysis. Both insulin and glucose can also activate LXR directly. FXR is a negative regulator of LXR and thus BAs via FXR have the effect of decreasing TG formation and accumulation in the liver. Decreased glycolysis leading to pyruvate may also lead to increased shunting into the glycogenesis pathway. BA activated FXR also increases expression of the transcription factor PPARα which leads to increased transcription of genes important for β-oxidation of FAs, therefore increasing the use of FAs for energy rather for storage. PPARα activation also has the effect of stimulating the expression of FGF21 which has important anti-diabetic effects in adipose (Fig. 3) tissue. Lastly, FXR controls the transcription of genes important in lipid handling and transport such as increased expression of ApoCII which increases the activity of LPL and therefore more FAs are taken up by adipose, thus lowering plasma TGs. FXR also causes increased production of ApoA5 which acts to reduce hepatic uptake of TGs. FXR last of all, causes decreased expression of ApoCIII which has the effect of increasing FA uptake by the liver and thus lowers serum FA levels [11,24–27]. Abbreviations: bile acid (BA), farnesoid X receptor (FXR), glucose receptor 2 (GLUT2), glucokinase (GK), glucose-6-phosphate (G6P), phosphoenolpyruvate (PEP), pyruvate kinase (PK), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), steroyl-CoA-desaturase-1 (SCD1), liver X receptor (LXR), sterol response element binding protein-1c (SREBP1c), carbohydrate response element binding protein (ChREBP), retinoic acid receptor (RXR), fibroblast growth factor (FGF21), small heterodimer partner (SHP), hepatic nuclear factor-4 (HNF4), cAMP response element binding protein (CBP), forkhead transcription factor FoxO1 (FoxO1), glucose-6-phosphatase (G6Pase), phosphoenolatepyruvate carboxylase (PEPCK), fructose-1,6-bis-phosphatase (FBP1).

obese human subjects which identified a positive correlation between ceramide synthase enzyme 6 (CerS6) with BMI and hyperglycemia and subsequent studies in CerS6 deficient mice that exhibited reduced C16:0 ceramides and were protected from diet-induced obesity and glucose intolerance [29]. The effects of high serum ceramide (upper part of Fig. 3) include increased FA production in the liver via ceramide-induced activation of SREBP-1c in

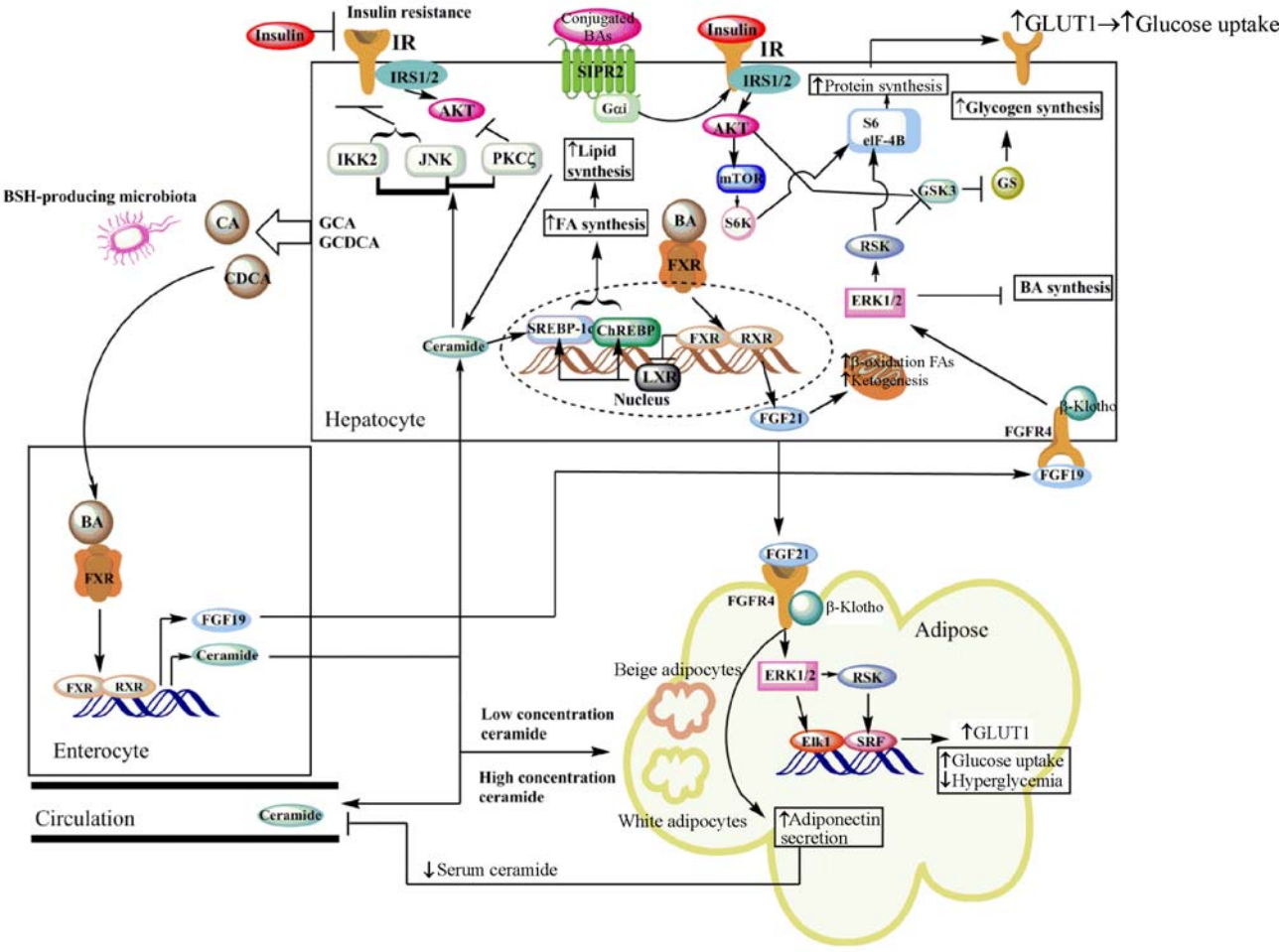

Fig. 3 The effect of BAs on FGF19/21 and ceramide signaling relative to lipid and glucose metabolism. BA activated FXR has tissue specific effects with respect to hormone production. In the intestine, BAs activate FXR to cause production of FGF21 which is then secreted from the liver and binds to its receptor, FGFR4/β-Klotho on WAT causing activation of the ERK1/2→ RSK →Elk1/SRF pathway which in turn, causes increased expression of GLUT1 receptors on the adipocyte surface thus reducing hyperglycemia by increasing glucose uptake into the cell. FGF21 in WAT also causes increased secretion of adiponectin which has the effect of decreasing serum ceramide levels and increasing the number of beige of adipocytes thereby, increasing the energy utilization capabilities of the adipose tissue to help fight obesity. In the liver, FGF21 stimulates β-oxidation of FAs, as well as, increased ketogenesis. BA activation of FXR in the intestine targets two genes for enzymes important for the synthesis of ceramide, *Smpd3* and *Sptlc2,* leading to increased serum levels of ceramide. In addition to the previously discussed effect on WAT, ceramide, when it is taken up by the liver becomes an activator of several signaling pathways which together act to block insulin signaling, hence, insulin resistance results. Ceramide also directly acts on the transcription factor, SREBP-1c, to increase DNL and lipid synthesis. The other consequence of intestinal FXR activation is the production on FGF19 which is secreted and then binds to its receptor, FGFR4/β-Klotho, in the liver where it acts to decrease BA synthesis and activates a hepatic ERK1/2 signaling pathway which results in increased protein synthesis, including GLUT1 receptors to increase glucose uptake and also increased glycogen synthesis to increase storage of glucose as glycogen. Both effects act to decrease hyperglycemia [28,32,36–38]. Abbreviations: bile acid (BA), cholic acid (CA), chenodeoxycholic acid (CDCA), farnesoid X receptor (FXR), retinoid X receptor (RXR), fibroblast growth factor 19/21 (FGF19/21), insulin receptor (IR), insulin receptor substrate 1/2 (IRS1/2), inhibitor of nuclear factor κB kinase subunit β (IKK2), c-Jun N-terminal kinase (JNK), protein kinase C ζ (PKCζ), fatty acid (FA), protein kinase B (AKT), sterol response element binding protein-1c (SREBP1c), carbohydrate response element binding protein (ChREBP), S6 ribosomal protein (S6), eukaryotic translation initiator factor-4B (elF-4B), glycogen synthase kinase 3 (GSK3), glycogen synthase (GS), ribosomal S6 kinase (RSK), extracellular signal-related kinase 1/2 (ERK1/2), fibroblast growth factor receptor-4 (FGFR4), ETS domain containing protein-1 (Elk1), serum response factor (SRF), bile salt hydrolase (BSH), white adipose tissue (WAT), GLUT1 glucose transporter (GLUT1).

the nucleus and subsequent steatosis. Ceramide also activates JNK, IKK2, and PKCζ (Fig. 3, upper left) and increases insulin resistance [28].

In contrast, BA activation of intestinal FXR also leads to the production of the hormone fibroblast growth factor-19 (FGF19) (Fig. 3, upper right) that binds to the fibroblast growth factor receptor-4 (FGFR4) to inhibit BA synthesis, increase production of GLUT1 receptors allowing for increased glucose uptake into the liver (Fig. 3, upper right) and increased synthesis of glycogen, thus increasing

hepatic glucose storage [30,32]. These effects lower blood glucose and increase glycogenesis thus preventing usage of glucose in several other pathways that are responsible for metabolic memory which are described in detail in the legend for Fig. 1 and Table 1 [33]. Briefly, increased *O*-GlcNAc of IRS-1,2 and AKT in the insulin signaling pathway diminishes their phosphorylation status and results in decreased insulin sensitivity and glucose uptake into the cell [34]. *O*-GlcNAc also has an impact on the transcription factors that control gluconeogenesis and DNL. *O*-GlcNAc of FoxO1 causes upregulation of PEPCK and glucose-6-phosphate and thus increases gluconeogenesis [35]. ChREBP can also be *O*-GlcNAc causing increased lipogenesis [10]. Therefore, increased *O*-Glc*N*Ac due to hyperglycemia, causes a continuous, toxic cycle of increased glucose and lipids [8]. The final consequence of an enhanced polyol pathway activation are elevated levels of DHAP that can form methylglyoxal which, in turn, reacts with arginine and/or lysine to produce the advanced glycation end products, 5-hydro-5-methylimidazolone and *N*-carboxyethyl-lysine (AGEs) (Fig. 1) [9]. Higher plasma AGEs have been linked to increased cardiovascular events in T2D patients [9].

The contribution of fasting blood sugar levels (FBS) and post-prandial glucose levels (PPG) to the diurnal blood glucose concentration were studied in 290 T2D patients and the major findings were that the relative contribution of PPG was predominant in patients with glycemic control and a hemoglobin A1C (A1C) $\leqslant$ 8.4% (68 mmol/mol) whereas, for patients with poor glycemic control (A1C $>$ 8.4% or 68 mmol/mol), FBS was the greater contributor to the diurnal hyperglycemia [39]. Subsequently, a study was done that examined changes in postprandial BAs in T2D patients relative to non-diabetics (NGT) when subjected to 4 different meals, oral glucose tolerance test (OGTT), low, medium and high fat [40]. BAs are released following a meal and are therefore in a position to regulate postprandial glucose homeostasis and overall glycemic control for T2D patients with A1C $<$ 8.4% (68 mmol/mol), via BA activation of FXR and Takeda G-protein coupled receptor 5 (TGR5) [11,39]. The results of this study were that postprandial serum concentrations of secondary BAs, cholic acid (CA) and total BAs were elevated in T2D vs. NGT patients that corresponded to lower serum FGF19 levels, a known inhibitor of BA synthesis. Total BAs were increased after the meal in a dose dependent way according to fat content with the highest fat content meal generating the highest level of BAs for both T2D and NGT. FGF19 levels corresponded with the total BA level for NGT but not T2D subjects which maintained a low, basal level of FGF19. Chenodeoxycholic acid (CDCA), the most potent FXR agonist was not significantly different between T2D and NGT but T2D patients had higher DCA (weaker FXR agonist), CA (not considered a strong FXR agonist) and higher UDCA which is considered an FXR antagonist [11,41]. In conclusion, the investigators proposed a T2D-BA-FGF19 phenotype that results in altered FXR/FGF19 signaling in the intestine [40]. Therefore, alteration of the BA pool in T2D may be a contributing factor for decreased glycemic control due to the decreased insulin-like action of FGF19 in the liver (Fig. 3).

The fact that for early T2D which has not progressed to an A1C of $>$ 8.4% (68 mmol/mol) it is the postprandial glucose that contributes more to the overall diurnal blood glucose concentrations and that BAs are peaked at postprandial times opens up a possibility for use of a particular BA or manipulation of the BA pool perhaps via

Table 1 Effects of FGF-19/21 on metabolism [32, 36, 37, 42]

FGF-19	1° Targets	2° Targets	Cellular effects	Metabolic effects
Originates from BA activation of intestinal FXR	Hepatic FGFR4/β-Klotho receptor complex	ERK1/2	↓Hepatic BA synthesis	↓BA concentrations in liver and gut
		RSK	↑Hepatic GLUT1 receptors	↑Hepatic glucose uptake ↓Blood sugar
		GSK3	↑Glycogen synthase	↑Glycogen synthesis/storage from glucose ↓Blood sugar
FGF-21	1° Targets	2° Targets	Cellular effects	Metabolic effects
Originates from BA activation of hepatic FXR	Adipose FGFR4/β-Klotho receptor complex	ERK1/2 + RSK	↑Adipose GLUT1 receptors	↑Adipose glucose uptake ↓Blood sugar
			↑Adiponectin secretion	↓Serum ceramide concentration
			↑Number of beige adipocytes	↑Energy utilization by adipo cytes ↓Lipid storage/obesity
	PPARα/FGF21 synergism with PPARα agonists in WAT	Upregulation of *Ucp1* and *Pgc1α* in WAT but not BAT	↑Production of beige adipo cytes in WAT	↑β-oxidation of FAs ↑Ketogenesis ↓Lipid storage/obesity ↓Blood sugar ↓Blood insulin

adjustment of the gut microbiota as an option to achieve and maintain glycemic control and prevent metabolic memory for early onset T2D patients.

FGF21 results from BA activation of hepatic FXR and after secretion, FGF21 binds to the FGFR4 receptor in adipose causing GLUT1 upregulation [37] and increased secretion of adiponectin [36]. The FGF-19/21 pathway details are shown in Fig. 3.

Hepatic FGF21 has also been found to be crucial for PPARα agonist-induced improvement in energy and glucose metabolism in obese mice [42]. BA activation of FXR causes increased expression of PPARα (Fig. 2). PPARα is highly expressed in liver, brown adipose tissue (BAT), heart and kidney. A recent study compared the effects of fenofibrate, a PPARα agonist on plasma FGF21 levels and improvements in glucose homeostasis in HFD-induced obesity in mice and compared results to lipodystophic A-Zip/F1 mice which lack WAT [43]. The results showed that in DIO mice, plasma glucose and hyperinsulinemia were reduced under fenofibrate treatment relative to untreated controls. HFD feeding led to a reduction of FGF21 plasma levels that was greatly increased with fenofibrate treatment. When A-Zip/F1 mice, which exhibited severe glucose intolerance, hyperglycemia, hyperinsulinemia and insulin resistance, were treated with fenofibrate, there was improvement in hypertriglyceridemia but no improvement in hyperglycemia. Plasma levels of FGF21 were increased in the A-Zip/F1 mice as well as other genes targeted by PPARα but the lack of affect on hyperglycemia suggested that WAT was essential for the fenofibrate-induced reduction of glucose metabolism dysfunction [42].

In the same study a comparison of FGF21 KO mice with WT controls was done to study the effects of fenofibrate treatment. Fenofibrate treatment lowered plasma triglycerides in both phenotypes, suggesting that this was not due to FGF21. The expression levels of PPARα target genes other than FGF21 were similar in both WT and FGF21 KO mice, suggesting that hepatic PPARα activation was not dependent on FGF21. In WAT, FGF21 expression was not induced by fenofibrate, suggesting that adipose tissue does not contribute to plasma FGF21 levels. In WT but not in FGF21 KO mice, fenofibrate induced genes related to brown adipocyte function such as *Ucp1* and *Pgc1α* in WAT but not in BAT, suggesting that treatment-induced increases in FGF21 led to browning in white adipocytes (Fig. 3). HFD feeding of both WT and FGF21 KO mice, produced obesity in both phenotypes but treatment with fenofibrate reduced body weight and improved glucose tolerance/hyperinsulinemia only in the WT mice indicating that FGF21 was responsible for the induced increases in energy expenditure and anti-diabetic effects [42]. Table 1 summarizes the metabolic effects of FGF19/21 hormones.

The effects of FGF15, FGF21 and an FGFR1/β-Klotho-activating antibody on the nervous system to regulate body weight and glycemia in mice were investigated very recently, employing genetic loss of function of the shared co-receptor, β-Klotho, in liver, adipose and the nervous system [19]. These tissue-specific β-Klotho knockout mice were generated in parallel with a floxed control. Initially, mice lacking β-Klotho in hepatocytes were generated (Klb^{Alb} mice) and tested for expression of the rate-limiting BA synthetic enzyme, CYP7A1, which was significantly elevated, thus confirming a previous study [44] that showed that FGF15/β-Klotho-FGFR4 signaling was essential for physiologic regulation of BA homeostasis. Table 2 summarizes the important findings for this research article [19].

No significant correlations between FGF15/21 and ceramide concentrations were observed in this study although a modest decrease in adiponectin was seen in the control mice. Hepatic diacylglycerol (DAG) concentrations were significantly reduced by FGF15 in both control and Klb^{adipo} mice. Gene expression studies showed that FGFs acted directly on adipocytes by increasing expression of dual specificity phosphatase 4 (*Dusp4)* mRNA which is involved in the feedback regulation of ERK1/2, in both BAT and WAT in control mice only. The last analysis was in mice with loss of function for β-Klotho in the nervous system, Klb^{Camk2a} mice. After 2 weeks of FGF15, only control mice showed weight loss and improvement in glycemic control. Sympathetic nerve activity was measured in BAT and was increased in a dose dependent manner with FGF15 administration indicating that FGF15 acts on the nervous system similar to the effects of FGF21 determined previously [45]. Final confirmation for the longer term effects of FGF15/21 came from an experiment using an antibody, bFKB1, a selective activator of the FGFR1 receptor. Only the Klb^{Camk2a} mice were resistant to loss of body weight and improvement in glycemic control which mirrored the result of FGF15/21 in this mouse model. The final conclusion for this study was that longer term changes in weight and glycemic control require β-Klotho containing FGFR complexes in neurons and therefore, therapeutic strategies must consider access of the brain [19].

BA activated Takeda G-protein receptor 5 (TGR5) control of enteroendocrine cell signaling to regulate metabolism

It is well known that dietary fat is first broken down by pancreatic lipases that, in turn, degrade triglycerides into long chain fatty acids (LCFAs) that act as agonists for the Gαq-protein-coupled G-protein couples receptor 40 (GPR40) (shown in Fig. 4A). The remaining product of this lipase activity is 2-monoacylglycerol (2-MAG) which is an agonist for Gαs-coupled GPR119. All of these GPRs are expressed on enteroendocrine cells (EEC) that secrete

Table 2 Effects of tissue-specific β-Klotho loss of function in mouse liver, adipose and nervous system on the nervous system relative to a floxed control [19]

Tissue	FGF15	Conclusion for FGF15	FGF21	Conclusion for FGF21
Liver KO (Klb^{Alb} mice)/ treatments	↓CYP7A1 ↓FGF15/β-Klotho-FGFR4 signaling	FGF15/FGFR4 essential for BA homeostasis	No acute effect on CYP7A1, ERK1/2	No acute, direct effect on hepatocytes
HFD (7 days)	DIO both for KO and control with no changes in glucose production, uptake despite elevated FGF15	Hepatic FGF15 not essential for systemic glucose metabolism		
2-week administration of FGF15/21	↓Body weight, serum glucose, hepatic TG	Improved metabolic parameters do not require FGF15/21 in liver	↓Body weight, serum glucose, hepatic TG	Improved metabolic parameters do not require FGF15/21 in liver
Adipose KO (BAT,WAT) Klb^{adipo} mice/ longterm treatment FGF15/21	↑Whole body insulin sensitivity ↓Body weight, plasma insulin/glucose, hepatic TGs ↓Hepatic DAG for both control and model mice ↑Expression of *Dusp4* in control mice only	The effects of these hormones do not require direct action on the adipocytes in Klb^{adipo} mice but do directly affect ERK1/2 signaling in control mice	↑Whole body insulin sensitivity ↓Body weight, plasma insulin/glucose, hepatic TGs ↓Hepatic DAG for both control and model mice ↑Expression of *Dusp4* in control mice only	The effects of these hormones do not require direct action on the adipocytes in Klb^{adipo} mice but do directly affect ERK1/2 signaling in control mice
NS KO (Klb^{Camk2a} mice) Longterm treatment FGF15	Only control mice showed weight loss and improved glycemic control Sympathetic nerve activity in BAT was ↑ in a dose dependent way with FGF15 treatment	FGF15 acts on the NS in a way similar to FGF21 (previous study)[45]		
Administration of bFKB1 Ab to the FGFR1 receptor	Klb^{Camk2a} mice were resistant to weight loss and improved gylycemic control	This confirmed the result in the FGF15/21 model	β-Klotho/FGFR1 complexes are essential in the NS	

Abbreviations: dual specificity phosphatase 4 (Dusp4), nervous system (NS).

glucagon-like peptide-1(GLP-1) [46]. It is also well-known that ingestion of dietary fat causes BA secretion into the gut and that BAs are agonists for the Gαs-coupled BA responsive G-protein coupled receptor, TGR5, also causing secretion of glucagon-like protein-1 (GLP1) [47]. Fig. 4 A shows the GPCR signaling pathway that leads to secretion of GLP-1 and other incretins such as GIP and PYY from EECs.

A recent study examined the relative importance of Gαs and Gαq signaling in GLP-1 secretion from EECs using primary crypt cultures, which allow cells to remain in a crypt organization rather than be plated out using single cell suspensions [48]. The underlying hypothesis was that both Gαs and Gαq-coupled pathways were important for the stimulation of GPR40 to secrete GLP-1 and that the effect was synergistic and not simply additive. The study was prompted by the fact that GPR119 agonists (Gαs-coupled), although promising in animal models never achieved clinical improvement in glucose tolerance in phase II trials. GPR40 (Gαq-coupled) agonists also showed only modest effects on GLP-1 and insulin secretion. However, a second generation GPR40 agonist, AM-1638, produced a more robust response in terms of GLP-1 secretion. Selective pharmacological blockage of the Gαs signaling pathway using cholera toxin (CTX) and also of the Gαq signaling pathway using UBO-QIC and GPR40 receptor selective agonist, AM-1638, at low concentrations resulted in a decreased response in GLP-1 secretion with CTX (0.9-fold) and an even greater decrease (0.4-fold) with UBO-QIC. Addition of both CTX and UBO-QIC abolished GLP-1 secretion in response to AM-1638 stimulation completely. Therefore, the combined Gs + Gq agonist, Am-1638, produced a more robust response and therefore, GPR40 was found to signal both by Gαs and Gαq pathways (Fig. 4A) explaining the lack of efficacy of the first generation GPR40 agonists, TAK-875 and MK-2305 that affect only the Gαq activation of the receptor.

Next, synergistic effects of GPAR40, GPR119 and TGR5 were probed for by administration of the Gαq-only GPR40 selective agonist, MK-2305 in combination with either the GPR119 selective AR246881 or the TGR5 selective Merck V agonists at low concentrations. When given alone, each had a small GLP-1 secretion response but when MK-2305 (GPR40) and Merck V (TGR5) were administered together, a synergistic effect was observed. A similar trend was observed for GPR119 but did not reach statistical significance. These results were confirmed in mice that were fasted and administered MK-2305, AR246881 and Merck V agonists by oral gavage. Therefore Gαq-coupled GPR40 acts in synergy with the Gαs-coupled TGR5 and GPR119 receptors with respect to GLP-1 secretion. The results support the notion that

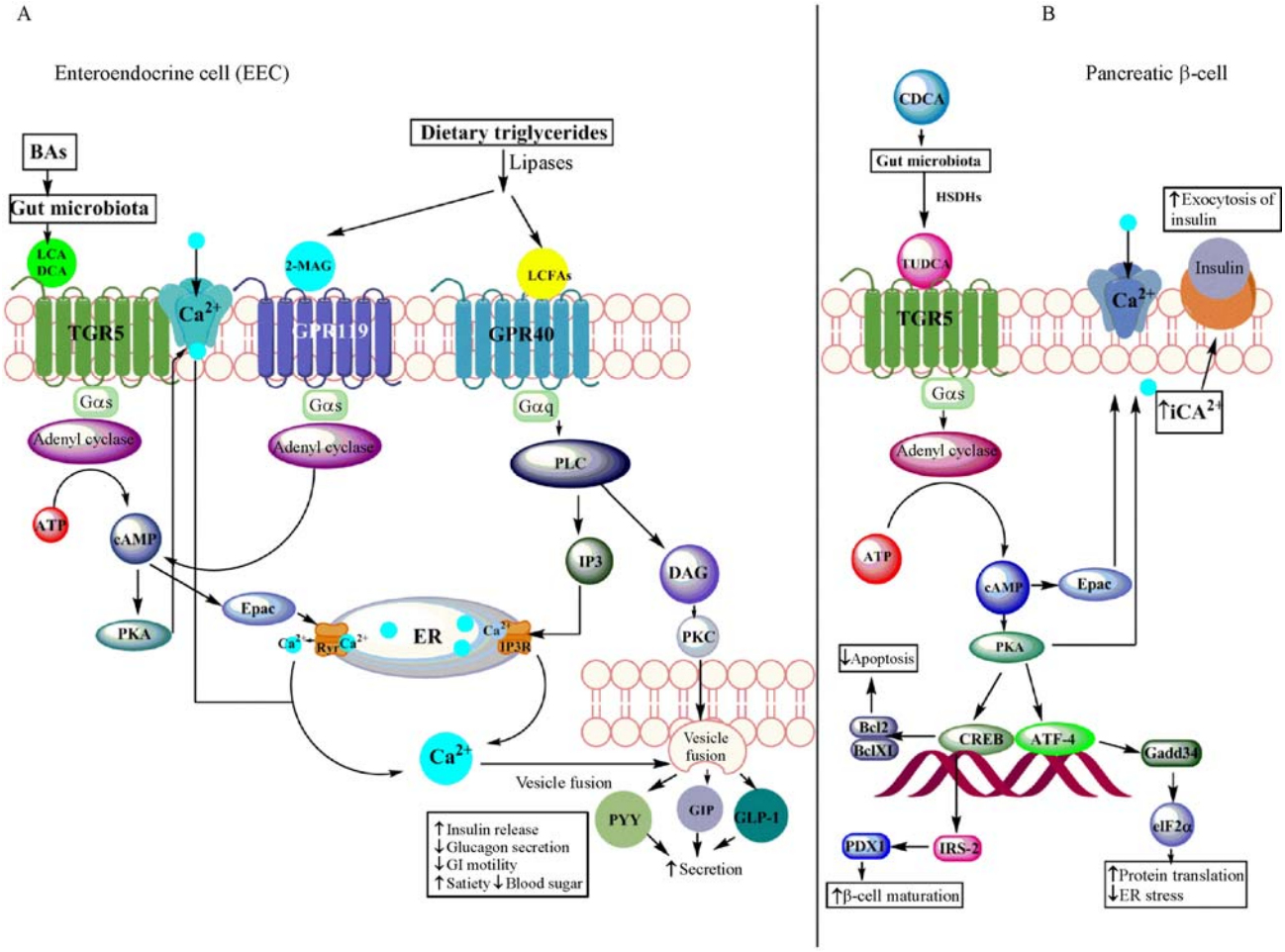

Fig. 4 The effects of BA activated TGR5 signaling in enteroendocrine cells (EECs) and in pancreatic β-cells. (A) EECc produce and secrete important hormones that affect energy metabolism and preserve pancreatic β-cell function. In EECs, TGR5 and GPR119 are coupled to Gαs G-proteins while GPR40 is coupled to a Gαq G-protein. Secondary BAs are agonists for TGR5 while 2-MAG and LCFAs are agonists for GPR119 and GPR40, respectively. All three GPCRs promote secretion of GLP-1, GIP and PYY, incretins that have important effects on glucose homeostasis. Gαs protein coupling to TGR5 and GPR119 results in the recruitment of adenyl cyclase which subsequently activates cAMP to increase intracellular Ca^{2+} via PKA or Epac pathways and ultimately increase secretion of GLP-1 and other incretins. Gαq protein coupling to GPR40 activates an alternate pathway to increase intracellular Ca^{2+} , PLC → IP_3. Furthermore, Gαq signaling via PLC can activate DAG →PKC to cause increased incretin secretion via a Ca^{2+} independent pathway. It was determined that both Gαs and Gαq coupling to GPCRS worked in a synergistic way to increase intracellular Ca^{2+} levels which in turn stimulated vesicle fusion and increased secretion of incretins. (B) In the pancreatic β-cell, the BA TUDCA was used to activate TGR5 and this was found not only to increase the secretion of insulin via Ca^{2+} stimulated exocytosis using the same mechanism as seen in EECs for increased incretin secretion but TGR5 activation also led to activation of the CREB→ Bcl2/BclXL pathway to decrease apoptosis and additionally, activation of the CREB → IRS-2 → PDX pathway which leads to increased maturation of β-cells. The net result of these pathway activations is increased β-cell mass. TUDCA activation of TGR5 also activates ATF-4 → Gadd34 →eIF2α signaling to cause increased protein translation which acts to decrease ER stress [46 – 52]. Abbreviations: lithocholic acid (LCA), deoxycholic acid (DCA), adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), guanine nucleotide exchange factor (Epac), ryanodine receptor (RYR), long chain fatty acid (LCFA), inositol triphosphate (IP_3), inositol triphosphate receptor (IP_3R), endoplasmic reticulum (ER), diacylglycerol (DAG), phospholipase C (PLC), protein kinase C (PKC), peptide YY (PYY), glucose dependent insulintropic polypeptide protein (GIP), glucagon-like peptide-1 (GLP-1), hydroxysterol dehydrogenase (HSDH), chenodeoxycholic acid (CDCA), tauroursodeoxycholic acid (TUDCA), cAMP response element binding protein (CREB), activating transcription factor-4 (ATF-4), B cell lymphoma-2 (Bcl-2), B cell lymphoma extra large (BclXL), pancreatic and duodenal homeobox-1 (PDX1), insulin receptor substrate-2 (IRS2), growth arrest and DNA damage induced protein-34 (Gadd34), eukaryotic initiation factor 2α (elF2α).

LCFAs stimulating GPR40 under physiological conditions act in synergy with 2-MAGs acting on GPR119 and BAs on TGR5 to jointly stimulate GLP-1 secretion. The high efficacy of the second generation GPR40 agonist AM-1638 and its likely mechanism of stimulating GLP-1 secretion not only through the Gαq pathway but also via Gαs, thus increasing cAMP in addition to IP3 and Ca^{2+}, were also demonstrated (Fig. 4A). The enhanced GLP-1

secretion observed in this study via a combination of agonists that bind GPR40 and the BA sensitive TGR5 is therefore a step forward in glycemic control of T2D.

BAs also exert an effect on glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells which is diagrammed in Fig. 4B. In particular, the BA, tauroursodeoxycholic acid (TUDCA), when incubated with mouse pancreatic islets in 11 mmol/L or higher glucose concentrations, enhanced GSIS [49]. No differences were seen in total insulin between test and control cells and thus changes in insulin release were not related to changes in insulin synthesis. No differences were observed in glucose-induced NAD(P)H fluorescence levels between TUDCA treated and untreated cells, indicating that mitochondrial metabolism was unaffected by the BA. Diazoxide, a potent K_{ATP} channel opener, caused a decrease in GSIS at 11 mmol/L glucose, but in the presence of TUDCA, insulin secretion increased even in the presence of diazoxide, indicating that TUDCA action was via another mechanism than the effects of K_{ATP} closure. To further confirm this, the effect of TUDCA on glucose-induced Ca^{2+} signals were measured, and no differences were observed between BA treated and untreated cells at 11 mmol/L glucose stimulation. Therefore, the effect of TUDCA was not due to K_{ATP} channel-dependent mechanism or Ca^{2+} signals.

Next, the BA receptor TGR5 became a point of focus as TUDCA has poor affinity for FXR. TGR5 relies on the Gαs stimulatory subunit which leads to activation of adenyl cyclase → cAMP→ PKA (Fig. 4A). The Gα inhibitor, NF449, did not change GSIS at 11.1 or 22.2 mmol/L of glucose stimulation but did abolish TUDCA effects on GSIS. The GSIS stimulation was mimicked by the TGR5 selective agonist INT-777 at 11 mmol/L glucose concentration whereas it had no effect at lower glucose concentrations. Incubation with the FXR antagonist TβMCA did not alter the effect of TUDCA on GSIS, indicating that FXR was not involved. Therefore, TUDCA was proposed to increase GSIS at higher but not basal glucose concentrations via the TGR5 receptor. Lastly, to determine whether TUDCA modulated the cAMP/PKA pathway, the effects of the PKA inhibitor H89 and (Rp)-cAMP, a competitive inhibitor of PKA activation by cAMP, were examined. In both cases, the effect of TUDCA on GSIS was inhibited. TUDCA was also found to increase phosphorylation of PKA (increase its activation) and CREB phosphorylation (target protein of PKA). These effects lead to less loss of pancreatic β-cells along with increased protein translation and decreased endoplasmic reticulum (ER) stress. Therefore, a TUDCA →TGR5 → adenyl cyclase → cAMP → PKA → CREB → GSIS pathway was proposed as shown in Fig. 4B.

Thus, because TUDCA administration causes increased insulin secretion only under hyperglycemic conditions, a possibility for early therapeutic glycemic control using BA-induced secretion of endogenous insulin may be feasible for early onset T2D with the view of preventing metabolic memory.

Metabolic effect of altered enterohepatic circulation of BAs

BA sequestrants, cholestyramine, colevelam, colestimide and cholestipol, are resins that are not absorbed in the intestine and are able to bind negatively charged bile salts within the intestinal lumen. The sequestration process has the effect of removing those bile salts from the enterohepatic circulation and thus causes increased return of low-density lipoprotein cholesterol (LDL-C) to the liver and increased hepatic BA synthesis [53,54]. BA sequestration has also been shown to increase GLP1 secretion and improve glycemia in HFD-induced obese mice but not in $TGR5^{-/-}$ mice, indicating that BAs activated TGR5 [55]. Notably, a higher number of L-type enteroendocrine cells are found in more distal parts of the gut and BA sequestration allows BA transportation to these regions. Treatment of mice with colestilan was shown to cause increased secretion of GLP1 from the colon relative to duodenum or ileum [56]. A meta-analysis of 15 different human trials, involving patients with T2D, of the effects of BA sequestrants revealed that high-density lipoprotein cholesterol (HDL-C), LDL-C, hemoglobin A1C (HbA1C), TGs and glycemia were all improved with this treatment [57]. Another meta-analysis of 17 trials involving 2950 T2D patients that focused only on glycemic control showed significant improvement of A1C and FBS with the use of BA sequestration [58]. The conclusion derived from both of these meta-analyses was that BA sequestrants were beneficial in glycemic control of T2D patients.

An alternative way to affect the enterohepatic circulation that has been proposed as a means to improve glycemic control in T2D patients is the inhibition of the apical sodium BA transporter (ASBT). A recent study of the ASBT inhibitor 264W94 was done on Zucker diabetic rats. Oral administration of the inhibitor for 2 weeks increased fecal BAs and elevated plasma GLP1 levels. There was significant improvement seen in A1C levels and glycemia [59]. $ASBT^{-/-}$ mice were found to display a similar metabolic phenotype to that described for BA sequestrants and ASBT inhibitors. For example, they have increased expression of hepatic CYP7A1 resulting in increased BA synthesis causing increased flux of BAs into the colon with potential for increased activation of TGR5 and increased GLP1 secretion. There was also suppression of hepatic SREBP1c and an improvement in TG metabolism [60]. In summary, the adjustment of enterohepaic circulation may be another viable means for achieving early glycemic control for T2D subjects and avoidance of metabolic memory.

The metabolic effect of bariatric surgery on BAs and T2D

It has been hypothesized that postprandial variations of bile salts in the gut lumen and circulation could be important for the rapid establishment of glycemic control following bariatric surgery that cannot be accounted for by weight reduction. In a recent study, vertical sleeve gastrectomy (VSG) or sham surgery was performed on HFD $Tgr5^{+/+}$ and $Tgr5^{-/-}$ mice [61]. VSG was found to decrease food intake and body weight, increase energy expenditure and circulating bile concentrations, improve fasting glucose, oral glucose tolerance (GTT), GSIS, increased GLP1 secretion and favorable shifts in gut microbiota for both mouse genotypes relative to sham WT controls. However, body weight independent improvements in fasting glucose, GTT, hepatic insulin signaling and islet morphology after VSG were attenuated in $Tgr5^{-/-}$ relative to $Tgr5^{+/+}$ mice. The favorable BA alterations after VSG were also blunted in $Tgr5^{-/-}$mice. TGR5 signaling was found to contribute to long-term maintenance of body weight independent improvements in glucose tolerance and did not contribute to post-operative increases in GSIS after VSG. Reductions in fasting plasma insulin, leptin and cholesterol after VSG was partially attributed to TGR5 signaling as this was not found in $Tgr5^{-/-}$ mice. VSG reduced hepatic monocyte chemotacticprotein-1 (MCP-1) and TNFα expression relative to sham-WM in $Tgr5^{+/+}$ but not $Tgr5^{-/-}$. MCP-1 and TNFα were elevated in VSG KO compared to VSG WT and thus it was concluded that TGR5 may contribute to VSG-induced improvements in GTT by promoting improved hepatic insulin signaling through decreases in inflammation [61].

At 4 months post surgery, fasting total BA concentrations were elevated in VSG-WT and VSG-KO relative to control groups and this was primarily due to an increase in conjugated BAs. An increase in the 12α-hydroxylated BA/non-12α-hydroxylated BA ratio has been associated with increased insulin resistance in humans [62]. This ratio was 2-fold higher in VSG KO compared to VSG-WT and plasma DCA and TDCA were elevated 4-fold in VSG KO relative to VSG-WT. VSG resulted in a decreased BA hydrophobicity index for both WT and KO mice but this effect was blunted in KO mice. There were increased amounts of hydrophilic muricholates in VSG-WT compared to VSG-KO suggesting that TGR5 signaling contributes to this shift in BA profile.

Examination of hepatic protein expression was measured and whereas there were no differences between the mouse groups for SHP, Cyp7a1 and Cyp27a1, there was a significant decrease in the expression of Cyp8b1 in the VSG-WT group. Cyp8b1 is a critical enzyme for CA biosynthesis and therefore, TGR5-dependent reduction of Cyp8b1 in VSG-WT may be responsible for the increase in the 12α-OH:non-12α-OH ratio.

Assessment of the microbial populations at the phyla level revealed that the relative abundance of cyanobacteria was 10-fold higher in VSG-WT compared to sham controls. The relative abundance of cyanobacteria in VSG-KO remained unchanged relative to controls, suggesting that TGR5 contributes to this postoperative change in microbiota [61].

BAs and circadian rhythm dysregulation in diabetes

Feeding restriction, nutrient intake [63] and the central circadian clock all control BA production, expression of metabolic hormones such as glucagon and insulin [64] as well as lipid and glucose homeostasis [65,66]. Misalignment of the circadian clock has been associated with metabolic syndrome in humans [67].

Molecular clocks are present in most cell types and they consist of autoregulated feedback loops of rhythmically expressed genes that oscillate within a 24 h period. A detailed description of hepatic circadian clock signaling is given in the legend of Fig. 5. From Fig. 5 it can be seen that the circadian rhythm of HMGCR via SREBP2 engenders corresponding changes in oxysterol production, which are known to regulate LXR and increase transcription of *Cyp7a1*. Thus, circadian control of cholesterol synthesis may be linked to the regulation of *Cyp7a1* transcription [68].

The circadian rhythm of BAs has been extensively studied in mice [15]. Primary, conjugated BAs exhibited two peaks in the dark phase corresponding to presumably two episodes of food ingestion. Unconjugated BAS peaked in the light phase, suggesting that active deconjugation of BAs by the gut microbiota occurred when mice were fasting [15]. Notably, BAs which are controlled by circadian rhythms also modulate activation of LXR via FXR to cause a decrease in transcription of *Cyp7a1* (Fig. 2). RORα, in contrast, is a positive regulator of sterol-12α-hydroxylase (*Cyp8b1*) for CA synthesis [69]. Both PPARα and the co-activator, peroxisome proliferators-activated receptor gamma co-activator 1-α (PGC1α) modulate *Bmal1* transcription [64,70]. *Ppar*α is also a target gene of BA modulated FXR (Fig. 2).

Sleep disruption is a well-documented cause of circadian clock misalignment [66,70]. In a recent study, B6xC57 mice were maintained on either normal chow or HFD and then sleep disrupted for 6 h/day for 5 days [72]. Measurements were made of expression of genes for hepatic metabolism, core clock, and regulators of BAs. WAT weight was unchanged with sleep disruption in chow-fed mice and was significantly increased in HFD mice and further increased in sleep-disrupted HFD mice. Core clock genes, *Clock, Bmal1* and *Per1, 2* were suppressed at all times but did not change in phase.

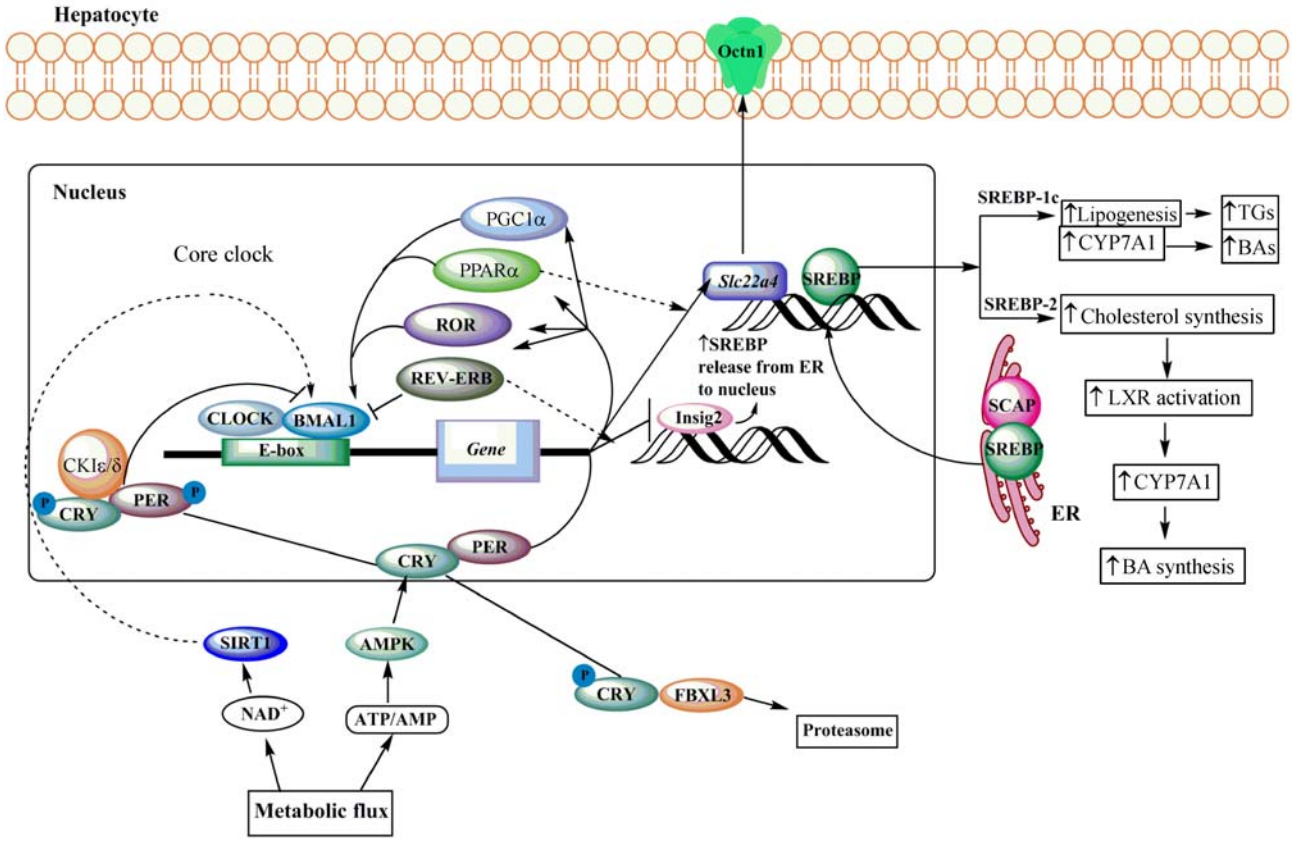

Fig. 5 The circadian control of BA synthesis, lipogenesis and the Octn1 transporter. In hepatocytes, there exists a circadian core clock that consists of an autoregulated feedback loop of rhythmically expressed genes that oscillate within a 24 h period. Two important genes, *Clock* and *Bmal1* comprise the forward segment of the clock loop. CLOCK and BMAL1 proteins form a heterodimer which then bind to the DNA response element, E-box, to cause the transcription of *Per* and *Cry* genes. PER and CRY proteins then re-enter the nucleus and inhibit CLOCK:BMAL1 protein activity to reduce their own transcription. There is further regulation of the feedback loop by the nuclear hormone receptors, *Rev-erbα* which negatively regulates and *RORα* that positively regulates *Bmal1* transcription [70]. REV-ERBα effects on BA metabolism include feeding independent circadian control of SREBP2, SREBP1c, the transcription factors for enzymes involved in lipogenesis and cholesterol-7α-hydroxylase (CYP7A1), the rate-limiting enzyme for BA synthesis in the liver. Transcription of a key protein, Insig2 is blocked by REV-ERBα and this causes increased release of SREBP from the ER and allows it to translocate into the nucleus. The circadian rhythm of SREBP2 causes corresponding changes in oxysterol production which are known to regulate LXR and increase transcription of *Cyp7a1* [68]. Both PPARα and the co-activator, peroxisome proliferator-activated receptor gamma co-activator 1-α (PGC1α) modulate *Bmal1* transcription [64,70]. PPARα is a positive regulator of the Octn1 transporter via control of transcription of the *Slc22a4* gene [71]. In addition, certain nutrient-sensitive signaling pathways such as SIRT1 and AMPK couple metabolic flux to the circadian cycle [70]. Abbreviations: circadian locomotor output cycles kaput (*Clock*), brain and muscle Arnt-like 1 (*Bmal1*), period (*Per*), cryptochrome (*Cry*), reverse-erythroblastosis α (*Rev-erbα*), retinoic acid-related orphan receptor α (*RORα*), cholesterol-7α-hydroxylase (CYP7A1), sterol response element binding protein (SREBP). liver X receptor (LXR, peroxisome proliferator-activated receptor α (PPARα), peroxisome proliferator-activated receptor gamma co-activator 1-α (PGC1α), insulin-induced gene-2 (Insig2), SREBP-cleavage activating protein (SCAP), adenosine monophosphate-activated protein kinase (AMPK), nicotinamide adenine dinucleotide (NAD), sirtuin 1 (SIRT1), casein kinase 1(CK1), F-box/LRR repeat protein 3 (FBLX3), organic cation transporter novel type-1 (Octn1).

REV-ERBα was suppressed by sleep disruption. Expression of *Cyp7a1* and an important transcriptional regulator of *Cyp7a1,* hepatocyte nuclear receptor 4α(*Hnf4α*) peaked at the same time but amplitudes were significantly reduced by sleep disruption. Arhythmic expression of *Srebp1c* was also observed with sleep disruption. Several enzymes involved in lipid metabolism were suppressed due to sleep disruption. These included HMGCR, FAS, and ACC. FFAs were elevated in both the liver and serum while TGs were decreased in the serum. Therefore, sleep disruption caused changes in lipid homeostasis. Liver BAs were decreased consistent with the depressed expression of *Cyp7a1.* However, intestinal BAs pool size was increased and the total BA pool was also increased. Therefore, sleep disruption altered BA homeostasis and enterohepatic circulation. Overall, in this study, even short-term circadian disruption was enough to impair BA and lipid homeostasis in mice.

Circadian rhythms are thought to affect the efficacy and/or toxicity of drugs [73,74] and a recent study identified

soluble carrier protein family 22 member 4 (*Slc22a4*) which encodes the organic cation transporter novel type-1 (Octn1) as a PPARα-regulated gene and that its intestinal expression was regulated by BA circadian oscillation (middle of Fig. 5) [71]. BAs have been shown to repress the expression of PPARα target genes by interfering with the recruitment of the transcription factor coactivator, cAMP response element binding protein (CBP) and its analog p300 to PPARα/RXRα complexes in mice fed high CA diets [75]. In the study described here, oligonucleotide microarray analysis using RNA isolates from CA treated intestinal cells from WT or PPARα null mice revealed 58 PPARα target genes that were repressed by the CA treatment and among these, the gene *Slc22a4 for the Octn1* transporter was identified as being repressed by CA. This repressive effect of CA on the PPARα/RXRα-induced expression of *Slc22a4* was verified by CA treatment of the intestinal cell line, Caco-2. A significant circadian oscillation of BAs were observed in both WT and PPARα$^{-/-}$ mice with the greatest accumulation during the dark/feeding phase and an oscillation of mRNA for *Slc22a4* was observed with a peak in the light phase and then decreased after the start of the dark phase, nearly anti-phase to BA accumulation. The expression of PPARα protein was detected at all time points with no obvious circadian oscillation. However, the proteins levels of Octn1 peaked before the start of the dark phase and exhibited circadian oscillation. PPARα mice had a similar BA circadian rhythm but the amplitude of the rhythms for *Slc22a4* and Octn1 was significantly decreased, suggesting that PPARα acted as a positive regulator of *Slc22a4* in intestinal epithelial cells and that PPARα-mediated expression of *Slc22a4* is repressed in a BA dependent way. The next experiment was to test whether the circadian oscillation of Octn1 affected the pharmacokinetics of the anti-neuropathic pain medication often prescribed for diabetics, gabapentin. Incubation of WT jejunal segments with 5 μmol/L radiolabeled gabapentin showed time-dependent variations in drug uptake into the issue. Further verification of this was done using 39 serum concentrations of orally administered gabapentin from 39 different mice. Thus, in mice, increased accumulation of BAs due to feeding of CA influenced the uptake of drugs used to treat diabetic patients [71]. T2D patients have been reported to have increased amount of 12-α-hydroxylated BAs such as CA and its derivatives [40].

Conclusions

In this review, we have discussed the role of BAs in glucose and lipid metabolism and ways in which they can be manipulated to achieve early glycemic control for T2D patients. Early glycemic control can be effectively initiated through the use of insulin treatment for T1D, but this approach has not been recommended for T2D. The importance of early glycemic control is due to the potential development of "metabolic memory," a term used to describe the upregulation of glucose utilizing pathways that result in (1) the accumulation of AGEs which are linked to cardiovascular complications such as retinal disorders, end-stage renal disease, and accelerated atherosclerosis, (2) increased activity in the polyol pathway which produces increased amount of NADH/ROS in the mitochondria to cause dysfunction and also increased DHAP which leads to more insulin resistance, (3) increased amounts of fructose (also a product of the polyol pathway) that can enter the hexosamine pathway and produce excess *O*-GlcNAc products, including those which affect insulin signaling and lipogenesis. The changes associated with metabolic memory are not always reversible and can only be avoided by aggressive early glycemic control [2]. It is therefore important to seek out ways to achieve rapid control of hyperglycemia perhaps by utilizing some of the approaches discussed in this review which involve the use and/or manipulation of BA pool size, composition, transport, hormone signaling capabilities, bariatric surgery, and circadian rhythms.

While the use of animal models may show us a potential solution, there is also still a need for clinical trials to assess the true effectiveness of some of the approaches discussed in this review. In addition, the growing and evolving field of metabolomics will perhaps need to expand its repertoire to include quantification of the metabolites resulting from metabolic memory pathways such as glycosylated proteins and AGEs.

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Compliance with ethics guidelines

Cynthia Rajani and Wei Jia declare that they have no financial conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval by a relevant institutional review board or ethics committee.

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