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## Bile acid dysregulation, gut dysbiosis, and gastrointestinal cancer

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### Abstract

Because of increasingly widespread sedentary lifestyles and diets high in fat and sugar, the global diabetes and obesity epidemic continues to grow unabated. A substantial body of evidence has been accumulated which associates diabetes and obesity to dramatically higher risk of cancer development, particularly in the liver and gastrointestinal tract. Additionally, diabetic and obese individuals have been shown to suffer from dysregulation of bile acid (BA) homeostasis and dysbiosis of the intestinal microbiome. Abnormally elevated levels of cytotoxic secondary BAs and a pro-inflammatory shift in gut microbial profile have individually been linked to numerous enterohepatic diseases including cancer. However, recent findings have implicated a detrimental interplay between BA dysregulation and intestinal dysbiosis that promotes carcinogenesis along the gut–liver axis. This review seeks to examine the currently investigated interactions between the regulation of BA metabolism and activity of the intestinal microbiota and how these interactions can drive cancer formation in the context of diabetes. The precarcinogenic effects of BA dysregulation and gut dysbiosis including excessive inflammation, heightened oxidative DNA damage, and increased cell proliferation are discussed. Furthermore, by focusing on the mediatory roles of BA nuclear receptor farnesoid x receptor, ileal transporter apical sodium dependent BA transporter, and G-coupled protein receptor TGR5, this review attempts to connect BA dysregulation, gut dysbiosis, and enterohepatic carcinogenesis at a mechanistic level. A better understanding of the intricate interplay between BA homeostasis and gut microbiome can yield novel avenues to combat the impending rise in diabetes-related cancers.

### Keywords

Gut–liver axis; gut dysbiosis; intestinal microbiota; bile acids; farnesoid X receptor; G-protein-coupled BA receptor 1 (TGR5); apical sodium dependent bile acid transporter; polymorphism; inflammation; bacterial translocation; diabetes; obesity; metabolic syndrome; gastrointestinal carcinogenesis

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## Introduction

As the worldwide obesity epidemic continues to grow, the prevalence of type II diabetes is also rising to a projected 439 million of individuals globally by 2030.<sup>1</sup> Of all obesity-related chronic conditions, diabetes is most strongly associated because of their similar symptomatic manifestations. Type II diabetes and obesity are both characterized by insulin resistance, glucose intolerance, hypoadiponectinemia, endoplasmic reticulum (ER) stress, and low-grade inflammation. Over 11% of the 34% of U.S. obese adults are reported to be diabetic in 2011.<sup>2</sup> Obesity is generally thought to stem from a combination of genetic and environmental factors. Increased dietary consumption of fat and refined carbohydrates along with decreased physical activity contributes to excessive weight gain while underlying genetic dispositions may lead to differential clinical progression. It has become clear that modest weight reduction can improve glycemic control and alleviate insulin resistance as obesity is considered a modifiable risk factor for diabetes.<sup>2</sup> Furthermore, the comorbidity of diabetes and obesity has been linked to liver and colon cancer risk, although the precise mechanisms remain unresolved. Meta-analysis of cohort studies has identified increased risk in obese individuals (relative risk (RR)=1.89, 95% confidence interval (CI): 1.51–2.36) for developing liver cancer compared to normal weight individual while meta-analysis of prospective studies correlated a larger waist circumference and waist–hip ratio with increased risk for colon cancer.<sup>3,4</sup> Similarly, diabetics are more than twice as likely to be diagnosed with cancer of the liver, pancreas, endometrium, and to a lesser extent, colon, breast, and bladder.<sup>5</sup>

Many possible underlying mechanisms including hyperinsulinemia, hyperglycemia, and inflammation have been proposed to explain the increased cancer incidence. Insulin, produced by b-cells in the pancreas, is released to promote cellular absorption of blood glucose and many factors including excess weight and increased plasma triglyceride (TG) levels can raise circulating levels of insulin. Chronic insulin elevation results in resistance, which then increases the biological activity of insulin-like growth factor (IGF-1), an endocrine and paracrine hormone regulating tissue growth and metabolism.<sup>6</sup> Epidemiological studies have linked IGF-1 to several cancer types, including hepatocellular carcinoma (HCC) and colorectal cancer (CRC).<sup>6,7</sup> Moreover, excess adiposity leads to the derangement of other peptide hormones such as resistin, leptin, adiponectin, and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), contributing to the metabolic abnormalities commonly observed in obese and diabetic individuals. Indeed, increased leptin and reduced adiponectin have been identified as risk factors for the progression of liver steatosis, fibrosis, and tumorigenesis as well as CRC formation.<sup>8–11</sup>

Both bile acids (BAs) and the intestinal microbiota have been extensively studied in the context of various health conditions, particularly obesity and type II diabetes-associated HCC and CRC.<sup>12–15</sup> Although the exact mechanism of how gut microbes and BAs affect one another remains unclear, it is evident that the introduction of intestinal microbes increased liver cholesterol and altered BA profiles in germ-free mice.<sup>16,17</sup> Conversely, dietary BA supplementation can modulate gut microbial profile in animal models.<sup>18,19</sup> This review will focus on the current understanding of the complex interplay between BA

homeostasis and gut microbial profiles in regards to obesity and diabetes-associated liver and colon carcinogenesis.

## Method

This review article covers information obtained from peerreviewed papers published in the past 15 years. We researched various combinations of key words in PubMed that were accessible through University of California-Davis Institutional database subscriptions. The key words used in the search were as listed: *intestinal microbiome, intestinal microbiota, gut–liver axis, GI health, bile acid, liver cancer, colon colorectal cancer, DNA damage, inflammation, gastric bypass, obesity, diabetes, FXR, TGR5, ASBT, polymorphism, genetic variation, prebiotics, probiotics, bile acid dysregulation, gut dysbiosis, oxidative stress.*

## BA homeostasis in the gut–liver axis

BAs are the primary facilitators of lipid absorption in the gastrointestinal (GI) tract. They are synthesized by cholesterol catabolism in the liver through both the classical (CYP7A1 and CYP8B1) and the acidic pathway (CYP27A1 and CYP8B1), which differ in the modification order of the sterol ring and side chain oxidation.<sup>20</sup> BAs synthesized in the liver are subsequently conjugated for storage in the gallbladder. Upon ingestion of fat and protein, cholecystokinin, a peptide hormone in the small intestine will stimulate the release of bile containing digestive enzymes and primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA), from the gall bladder. These BAs will then activate farnesoid x receptor (FXR) in the liver which induces the expression of small heterodimer partner (SHP) to inhibit the activity of liver receptor homolog-1 responsible for upregulating the rate-limiting BA synthesis enzyme CYP7A1.<sup>21,22</sup> Intestinal FXR activity on the other hand, induces the expression of fibroblast growth factor 19 (human FGF19/rodent Fgf15) which binds hepatic fibroblast growth factor receptor 4 and activates c-Jun N-terminal kinase 1/2 (JNK1/2) and extracellular signal-regulated kinase 1/2 (ERK1/2) to inhibit BA synthesis. BAs are actively reabsorbed from the ileum by the ileal BA transporters and circulated back to the liver through the hepatic portal vein. This highly efficient process ensures that a majority of synthesized BAs are recycled with only 1-2% being converted into secondary BAs, deoxycholic acid (DCA), and lithocholic acid (LCA), by bacterial 7 $\alpha$ -dehydroxylation in the terminal ileum and colon and excreted in feces. The regulation of BA circulation between the liver and intestines is summarized in Figure 1.

## BA related molecular players

### BA nuclear receptor FXR

In addition to modulating BA synthesis, FXR also regulates the expression of several transporters including apical sodium dependent BA transporter (ASBT), fatty acidbinding protein subclass 6, and organic solute transporter  $\alpha$  and  $\beta$  to control the absorption of not only BAs but also lipids, vitamins, and xenobiotics.<sup>23</sup> Interestingly, recent studies have implicated FXR in the interplay between obesity-associated BA dysregulation and gut dysbiosis to potentially promote carcinogenesis in the liver and colon. Within the context of obesity and diabetes, FXR can regulate glucose and lipid homeostasis through actions at

various sites along the gut-liver axis. Agonist activation or hepatic overexpression of FXR significantly lowered blood glucose levels in both diabetic and wild type<sup>24</sup> mice.<sup>24,25</sup> FXR stimulation also decreased blood low density lipoprotein levels and inhibited fatty acid and TG synthesis in mice fed a high sugar and fat diet<sup>22</sup>. These combined effects of FXR protected mice against body weight gain, liver and muscle fat deposition, and reversed insulin resistance.<sup>26</sup>

At the metabolic level, FXR functions to repress hepatic gluconeogenesis, lipogenesis, and fatty acid synthesis genes. Consequently, FXR stimulation promotes glycogen synthesis and enhances insulin sensitivity in obese mice.<sup>25,26</sup> FXR activity in pancreatic  $\beta$ -cell lines and human islets can regulate transcription factor Kruppel-like factor 11 to increase insulin gene expression and protein kinase B-dependent phosphorylation and translocation of glucose transporter 2 at the plasma membrane of hepatocytes.<sup>27</sup> By stimulating pancreatic insulin secretion and hepatic glucose uptake, FXR can effectively delay the pathological progression of insulin resistance, hyperglycemia, and glucosuria in diabetic mice. Consistently, FXR knockout (KO) mice exhibited glucose intolerance, insulin insensitivity, elevated serum TG, cholesterol, and BA levels resulting in greater hepatic fat accumulation compared to wild type (WT) mice.<sup>22</sup> Hepatic FXR expression is conversely regulated by glucose levels in streptozotocin-induced diabetic rats.<sup>28</sup> Chromatin immunoprecipitation in mice also revealed that long-term high-glucose exposure increased histone acetylation and demethylation on the FXR-target *Cyp7a1* gene promoter region leading to elevated basal expression and consequently, a larger BA pool with altered composition.<sup>29</sup> These observations strongly support the existence of crosstalk between the cellular mechanisms regulating glucose, lipid, and BA homeostasis in the liver and intestines with FXR serving mediator.

A link between FXR and enterohepatic cancer was firmly established when FXR KO mice were found to have markedly elevated hepatic inflammatory and oxidative stress markers compared to WT mice and a striking 100% incidence rate of spontaneous liver tumors between 13 and 15 months of age.<sup>23,30</sup> BA-containing diet further exacerbated inflammation and oxidative stress in FXR KO mouse liver supporting that BA dysregulation subjects hepatocytes to higher oxidative stress.<sup>31</sup> Hepatocyte-specific overexpression of SHP failed to alter liver tumor incidence or size in FXR KO mice, but did result in lower neoplasia grade, decreased cell proliferation, and increased apoptosis.<sup>32</sup> Moreover, FXR stimulation can down-regulate lipopolysaccharide (LPS)-induced, nuclear factor kappa-light-chain-enhancer of activated B cells (NF $\kappa$ B)-mediated hepatic inflammation by suppressing the expression of proinflammatory mediators in human HCC cells and mouse primary hepatocytes.<sup>33</sup> FXR KO mice displayed higher hepatic mRNA levels of inducible nitric oxide synthase, prostaglandin-endoperoxide synthase 2 (COX-2), chemokine ligand 10, and interferon type II (IFN- $\gamma$ ) which resulted in exaggerated inflammation and necrosis after LPS exposure at a dose that failed to elicit measurable liver injury or inflammation in WT mice.<sup>33</sup> The HCC in FXR KO mice was associated with sustained oncogenic Wnt/B-catenin signaling through Wnt4 and dishevelled induction, E-cadherin repression, and glycogen synthase kinase-3B inactivation as the mice aged.<sup>34</sup>

Furthermore, microarray analysis of FXR KO mouse liver revealed altered gene expression profiles related to metabolism, inflammation, and fibrosis compared to WT liver recapitulating human HCC progression.<sup>35</sup> Liver tumor bearing FXR KO mice showed elevated levels of interleukin 6 (IL-6) and signal transducer and activator of transcription 3 (STAT3) due to diminished expression of suppressor of cytokine signaling 3, a direct FXR target gene.<sup>32</sup> STAT3 activation in conjunction with elevated TNF $\alpha$  and IL-6 levels has been shown to potentiate HCC formation.<sup>36</sup> Additionally, FXR can epigenetically silence the promoter of gankyrin, a proteasome subunit responsible for the degradation of retinoblastoma, p53, hepatic nuclear factor 4 alpha, and CCAAT (a sequence of DNA nucleotides)/enhancer-binding tumor suppressor proteins. The loss of FXR in mice increased gankyrin expression to promote tumorigenesis. Interestingly, long-lived little mice with high basal FXR expression do not develop liver cancer with age or carcinogen administration due to insufficient gankyrin induction.<sup>37</sup> FXR activation in human hepatocytes and hepatoma cells protected against cytotoxicity induced by cisplatin and other DNA-damaging agents.<sup>38</sup> These findings support that in addition to its metabolic regulation, FXR also functions to modulate oxidative stress, inflammation, and cell proliferation to inhibit cancer development.

Evidence also exists to suggest that FXR may act as a modulator of intestinal inflammation and a link between BA homeostasis and the intestinal microbiome. In the small intestine, FXR negatively regulates the expression of transporters involved in BA reabsorption while inducing the production and secretion of FGF19/Fgf15 to inhibit hepatic BA synthesis.<sup>23</sup> Colon inflammation in Crohn's disease patients and rodent colitis models is correlated with reduced FXR mRNA levels. The progression of colon inflammation is exacerbated in FXR KO mice while treatment with FXR agonist attenuated colonic tissue damage and immune cell activation.<sup>39</sup> Conversely, FXR stimulation protected WT mice from chemical-induced colitis by reducing epithelial permeability, ulceration, and inflammatory cell infiltration. Moreover, FXR agonist-treated WT mice and differentiated enterocyte-like cells displayed lower pro-inflammatory cytokines and better preserved epithelial barrier function.<sup>40</sup>

In addition to its beneficial effects on intestinal function and inflammation, a connection between FXR and intestinal microbes was observed when ampicillin-treated mice had inhibited ileal expression of FXR, SHP, and FGF19/Fgf15. Expression of FXR and its target genes levels were rescued by combination treatment with CA, but not taurocholic acid, in ampicillin-treated mice suggesting that enterobacteria can enhance BA-mediated FXR activity via taurocholic acid deconjugation.<sup>41</sup> Furthermore, intestinal inflammation in mice down-regulated FXR expression in a toll-like receptor 9 (TLR9)-dependent manner since the FXR promoter contains a response element to interferon regulatory factor 7, a TLR9-regulated factor.<sup>42</sup> These preliminary findings suggest a possible role of intestinal FXR as a mediator between BA homeostasis, the gut microbiome, and host immunity to prevent excessive inflammation and maintain GI health.

Examination of FXR in human HCC samples and cell lines has yielded further evidence to support its protective role against cancer formation. Marked reduction in FXR levels and activity were observed in human HCC samples compared to normal liver tissue. This reduction resulted from inhibition of hepatic nuclear factor 1 alpha activity on the FXR gene

promoter by elevated pro-inflammatory mediators.<sup>34,35</sup> The 3' untranslated region of FXR mRNA was found to be a target of miR-421 and FXR downregulation by miR-421 promoted proliferation, migration, and invasion in human HCC cells.<sup>43</sup> Decreased FXR levels in HCC cells also correlated with overexpression of active Ras resulting in strong activation of ERK1/2, a common characteristic of malignant cells.<sup>44,45</sup> However, additional studies are required to determine whether dysregulated FXR activity increases the risk of HCC and CRC.

One possible underlying cause of FXR insufficiency in humans is genetic variation in the gene itself resulting in diminished expression or function. Indeed, sequencing analysis of FXR in intrahepatic cholestasis of pregnancy patients revealed four functional heterozygous variants, three of which demonstrated functional defects in either translation efficiency or signaling activity.<sup>46</sup> Additionally, FXR polymorphism identification analysis of European-, African-, Chinese-, and Hispanic-Americans identified a common, hypomorphic single nucleotide polymorphism (SNP) with population allelic frequencies ranging from 2.5% (European Americans) to 12.1% (Chinese Americans). The *in vitro* transactivation activity of this hypomorphic SNP was lower relative to that of WT allele and human carriers of this allele showed significantly reduced hepatic SHP levels.<sup>47</sup> Furthermore, the global FXR haplotype distribution between inflammatory bowel disease and healthy individuals was significantly different which emphasizes the link between FXR-mediated BA signaling and intestinal inflammation.<sup>48</sup> Since chronic inflammation is widely considered a predisposition to cancer development, enhancement of FXR signaling appears to be a promising clinical target to not only normalize the BA dysregulation seen in obese and diabetic individuals but also combat chronic hepatic and intestinal inflammation.

### BA transporter ASBT

The appropriate circulation of BAs between the liver and small intestine is crucial to the maintenance of BA homeostasis and consequently, normal GI physiology. The ileum is where approximately 90% of secreted BAs are actively reabsorbed into the bloodstream by ASBT for transport back to the liver through the hepatic portal vein.<sup>49,50</sup> Because of its predominantly ileal expression and central role in enterohepatic cycling of BAs, ASBT is another potential participant in the interplay between BA dysregulation and gut dysbiosis. In Caco-2 cells, 25-hydroxycholesterol and CDCA treatments greatly reduced ASBT promoter activity and mRNA levels through the actions of FXR, SHP, retinoic acid receptor, and retinoid x receptor (RXR).<sup>51,52</sup> Mice fed a cholesterol-enriched diet exhibited down-regulation of ASBT at both the mRNA and protein levels, decreased ileal BA uptake, and elevated fecal BA excretion.<sup>53</sup> Interestingly, exposure of Caco-2 cells to pro-inflammatory factor IL-1B also caused a 65% reduction in ASBT mRNA level.<sup>54</sup> Elevated levels of cholesterol in the intestinal lumen and pro-inflammatory mediators in the intestinal epithelium appear to down-regulate ASBT activity, thereby disrupting enterohepatic BA circulation. Consequently, a greater amount of unabsorbed BAs remain in the intestines where they can be transformed by intestinal microbes into toxic, hydrophobic BAs.<sup>55</sup> Indeed, ASBT KO mice had a 10 - to 20-fold increase in fecal BA excretion and an 80% reduction in BA pool size compared to WT mice despite up-regulated BA synthesis.<sup>56</sup>

Paralleling its upstream regulator FXR, ASBT activity can also be modulated by the gut microbiome. Pharmacological inhibition of ASBT in diabetic fatty rats significantly raised fecal BA concentrations and non-fasting plasma total glucagon-like peptide 1 (GLP-1) while decreasing hemoglobin A1c and blood glucose. However, ASBT inhibition also reduced FXR mRNA levels in both the liver and small intestine, likely as compensation for the disrupted BA circulation.<sup>57</sup> Interestingly, ASBT deficiency or inhibition in mice lowered serum glucose, insulin, and TG as a result of diminished sterol regulatory element-binding protein 1 c expression.<sup>58</sup> Based on these results, ASBT inhibition appears as a possible clinical intervention for the management of obesity and diabetes. However, it remains to be determined whether such beneficial effects also occur in humans and whether they outweigh the adverse effects of disrupted BA cycling.

An association between ileal ASBT activity and intestinal microbes was established when ampicillin-treated mice showed markedly decreased BA fecal excretion and elevated BA concentrations in hepatic portal blood. The ampicillin-treated mice displayed significantly higher ileal ASBT mRNA and brush-border membrane protein levels, elevated total BAs, and reduced intestinal enterobacteria-biotransformed BAs. These observations indicate negative regulation of ileal ASBT expression by the gut microbiome.<sup>59</sup> This modulation of ASBT activity by intestinal microbes was shown to occur in part through a proteasomal degradation pathway since proteasome inhibition attenuated the CA-induced reduction of ileal ASBT protein level in ampicillin-treated mice.<sup>60</sup> Consistent murine findings showed Caco-2 cells infected with *E. coli* had drastically diminished ASBT activity due to decreased transport Vmax and protein level at the plasma membrane.<sup>61</sup> This suggests that certain species of intestinal microbiome can alter the enterohepatic circulation of BAs by influencing ileal ASBT.

Furthermore, hypomorphic genetic variants in human ASBT have also been linked to increased cancer risk of the lower GI tract. One particular human ASBT SNP was associated with a twofold higher risk of colorectal adenomas potentially due to malabsorption of BAs leading to elevated colonic levels of toxic BAs.<sup>55,62</sup> Another sequencing study revealed that carriers of several minor ASBT variants had significantly reduced ileal ASBT expression at both the mRNA and protein levels.<sup>63</sup> Lastly, three ASBT nonsynonymous SNPs exhibited partially impaired to near complete loss of BA transport compared to WT allele *in vitro*.<sup>64</sup> Being required for normal enterohepatic BA circulation, ASBT appears to be not only influenced by ileal FXR, cholesterol levels, and microbiota activity but also genetic variation.

### **G-coupled protein receptor TGR5**

Although investigation into the mechanisms through which BAs function in the liver and intestines have been predominantly focused on nuclear receptors such as FXR, there is accumulating evidence for the participation of G-protein coupled BA receptor 1 (GPBAR1/TGR5) in mediating the systemic actions of BAs. Highly expressed in the liver, intestine, and brown adipose tissue, TGR5 complements FXR in regulating BA homeostasis, glucose metabolism, and enterohepatic inflammation.<sup>65</sup> Therefore, examination of the regulatory roles of TGR5 and its potential contribution to BA dysregulation, gut dysbiosis and



ultimately, liver and colon carcinogenesis is pertinent. TGR5 activation at the plasma membrane initiates the canonical G-protein signal transduction resulting in initiation of downstream pathways that remain poorly defined.<sup>66</sup>

Nevertheless, TGR5 was implicated in modulating BA and energy metabolism when TGR5 KO mice on a highfat diet experienced a 21–25% reduction in BA pool size and greater body fat accumulation accompanied by body weight gain relative to WT mice.<sup>50</sup> Intriguingly, TGR5 can stimulate GLP-1 release and suppress hepatic glycogenolysis resulting in improved glucose tolerance, hepatic and pancreatic functions in obese mice.<sup>67,68</sup> Treatment of enteroendocrine cells with a TGR5 agonist also enhanced GLP-1 secretion.<sup>68</sup> Administration of BA sequestrant to diet-induced obese mice caused markedly higher energy expenditure, body weight reduction, and improved glycemic control.<sup>69,70</sup> These findings in mice were replicated in monkeys with TGR5 agonist triggering co-secretion of peptide YY (PYY) and GLP-1 from distal GI L-cells to produce sustained improvements in glucose tolerance.<sup>71</sup> From these studies, TGR5 appears to control BA and glucose metabolism through its up-regulation of PYY and GLP-1. However, it is unknown whether if TGR5 expression or function is inhibited in obese and diabetic individuals.

Complementary to FXR, TGR5 can prevent excessive inflammation in the liver and intestines to preserve normal physiology. In mouse macrophages and Kupffer cells, TGR5 activation suppressed I $\kappa$ B $\alpha$  phosphorylation, p65 translocation, NF $\kappa$ B DNA binding, and transcriptional regulation activity by stabilizing the interaction between I $\kappa$ B $\alpha$  and  $\beta$ -arrestin 2. Consistently, TGR5 KO mice exhibited more severe LPS-induced liver necrosis and inflammation relative to WT mice whereas TGR5 agonist inhibited LPS-induced NF $\kappa$ B-mediated expression of pro-inflammatory mediators in WT mouse liver.<sup>72</sup> Loss of TGR5 in mice also increased susceptibility to diethylnitrosamine-induced acute liver injury and cancer due to increased hepatocyte death, compensatory proliferation, and expression of inflammatory cytokines.<sup>73</sup> Moreover, TGR5 KO mice displayed abnormal hydrophobic BA composition, severe hepatocyte necrosis, prolonged cholestasis, exacerbated inflammatory response, and delayed regeneration compared to WT mice post partial hepatectomy.<sup>74</sup> Treatment of non-alcoholic fatty liver disease (NAFLD) mice with dual FXR/TGR5 agonist decreased intrahepatic inflammation and improved histological features.<sup>75</sup> Furthermore, TGR5 KO mice developed abnormal colonic mucous cell morphology with an altered molecular architecture of epithelial tight junctions resulting in increased intestinal permeability and microbial translocation.<sup>76</sup> TGR5 stimulation reduces TNF $\alpha$  production by Crohn's disease-associated macrophages after LPS exposure by inhibiting c-FOS phosphorylation and NF $\kappa$ B activation.<sup>77</sup> Taken together, these findings implicate TGR5 as another important player in protecting the liver and intestines against excessive inflammation.

In addition to regulating energy homeostasis and inflammation, altered TGR5 expression and activity can also influence signaling pathways associated with cancer formation in cells of the liver, intestines, and beyond. Treatment of human gastric carcinoma cells with BAs activated epidermal growth factor receptor (EGFR) and ERK1/2 proliferative signaling in a TGR5-dependent manner.<sup>78,79</sup> DCA exposure redistributed TGR5 to plasma membrane microdomains to transactivate EGFR resulting in downstream ERK1/2 activation.<sup>80</sup> Low

concentrations of CDCA can stimulate human endometrial cancer cell growth by upregulating cyclin D1 expression through TGR5-mediated recruitment of cAMP response element-binding protein to the cyclinD1 gene promoter.<sup>81</sup> In human HCC cells, TGR5 inhibition decreased BA-induced caspase 8 activation by interfering with the JNK-mediated recruitment of caspase 8 to the death-inducing signaling complex.<sup>30</sup> Furthermore, TGR5 stimulation inhibited cell proliferation and migration by suppressing STAT3 phosphorylation, transcription, and DNA binding activity.<sup>73</sup> These results implicate TGR5 as a regulator of survival and growth pathways in liver and GI cells in response to BAs. Thus, within the context of BA dysregulation, overstimulation of TGR5 may encourage carcinogenesis.

Indeed, a significant positive correlation was found between BA concentrations and the grades of intestinal atrophy and metaplasia with GI cancer developing more frequently in individuals with high BA concentrations.<sup>82</sup> Strong TGR5 staining was present in 12% of human intestinal metaplasia cases but none in normal gastric epithelium cases and moderate to strong membranous and cytoplasmic TGR5 staining was present in 52% of intestinal but only 25% of diffuse subtype adenocarcinomas.<sup>79</sup> Sequencing of primary sclerosing cholangitis patients identified six TGR5 variants, five of which were found to exhibit reduced or abolished function because of altered localization or activity.<sup>83</sup> Akin to FXR and ASBT, TGR5 appears to be another intersection between BA and glucose homeostasis, inflammation, and cell proliferation in the liver and intestines.

## Relationship between diabetes, BA dysregulation, and gut dysbiosis

### Crosstalk between BA homeostasis and the intestinal microbiota

Beyond the previously discussed molecular players, BAs also interact with other receptors such as constitutive androstane receptor, pregnane x receptor, and vitamin D receptor to regulate glucose and lipid homeostasis as well as innate immunity.<sup>84</sup> Although BAs are critical for regulating lipid absorption and glucose homeostasis, they can exert harmful effects when their levels become dysregulated. In abnormally high concentrations, hydrophobic secondary BAs are cytotoxic, leading to DNA damage and cell death.<sup>85</sup> BAs also have antimicrobial and amphipathic properties that are regarded as important regulators for the gut microbe environment with DCA being the most potent of all BAs at physiological concentrations in the human colon.<sup>14</sup> DCA at a concentration of 0.5mM can effectively inhibit intestinal bacteria growth in cell culture, indicating that BA can regulate intestinal microbe composition through environmental stress.<sup>86</sup> The human intestines house 10–100 trillion microbes, providing efficient metabolic capability to process indigestible dietary sources.<sup>87</sup> Since the gut microbiome profile is largely dependent on the host diet and surrounding intestinal environment, there is a degree of variability among individuals, even in twins. However, shared microbial genes are identifiable to construct a “core microbiome” at the genetic level.<sup>88,89</sup> Disturbances to the gut microbiome with phylum-level changes can result in altered nutrient acquisition, energy extraction, and metabolism.<sup>90</sup>

The crosstalk between BAs and the gut microbiome was established in the mid-1960s and continues to be a major research focus. A direct relationship was observed between the intestinal microbiota and BA levels using nuclear magnetic resonance spectroscopy (NMR)

after microbial colonization in germ-free mice.<sup>16</sup> These intestinal microbes stimulated multiple pathways in xenobiotic metabolism and pharmacokinetics in the liver, kidney, plasma, urine, and colon.<sup>16</sup> CYP8B1, required for the production of CA, helps to control the hydrophobicity of the BA pool by regulating the CA/CDCA ratio in conjunction with CYP7A1 and CYP27A1. Together, these enzymes are regulated by FXR, in a negative feedback manner. However, overexpression of FXR in germ-free mice did not lead to down-regulation of Cyp8b1, Cyp7a1, or Cyp27a1.<sup>16</sup> Therefore, the regulation of these genes by FXR is more complex than previously thought with possible participation by the gut microbiome. In addition to regulating intestinal Fgf15, gut microbes also regulated hepatic Cyp7a1 through intestinal FXR as well as endogenous FXR antagonists, tauro-conjugated alpha, and beta-muricholic acid as demonstrated in a mice feeding study.<sup>17</sup> Conventionally raised mice had reduced expression level of FXR antagonists and Cyp7a1 and increased expression of Cyp8b1 allowing for greater intestinal FXR activity compared to germ-free mice.<sup>16,17</sup> Since gut microbe colonization did not affect liver FXR, it is postulated that the intestinal microbiome acts mainly in the ileum to create a more hydrophobic BA profile by suppressing Cyp7a1 expression through increased induction of intestinal Fgf15.<sup>17</sup>

### Diabetes associated shifts in BA and gut microbe profiles

Previous studies have shown that consumption of a high-fat diet increased BA secretion and altered the gut microbial profiles in obese animal models and in patients with type 2 diabetes.<sup>86,91</sup> The human gut contains a variety of species with different segments of the GI tract varying in bacterial density and diversity.<sup>89</sup> For instance, the ileum contains around  $10^7$  colony-forming units per gram of bacteria with a dominance of gram-negative aerobes and obligate anaerobes while the colon houses  $10^{12}$  colony-forming units per gram of bacteria with a dominance of anaerobes.<sup>89,92</sup> Gram-positive firmicutes and actinobacteria and gram-negative bacteroidetes are the most prominent phyla with each taxon differing in their ability to extract and utilize dietary and host-derived resources, leading to variations in host lipid, glucose, and amino acid metabolism. It was found that differences in body composition between obese (*ob*) twins and lean (*ln*) twins were attributable in part to variations in bacterial fermentation of short-chain fatty acids, metabolism of branched-chain amino acids, and transformation of BAs.<sup>93</sup> Co-housing *ln* mice with *ob* mice prevented diet-induced obesity in the latter by facilitating an ingress of bacteroidetes from the *ln* to *ob* mouse gut and altered several BA concentrations to resemble those in *ln* cage mates and *ln* control. Carbohydrate fermentation and BA metabolism in co-housed *ob* mice were phenotypically rescued. Notably, a high fat diet with minimal fruits and vegetables selected against microbial profiles associated with leanness and prevented successful ingress by *ln*-microbes.<sup>93</sup>

The association between the intestinal microbiota and diet-induced weight gain in both mice and human models mentioned earlier suggests that BA may be interacting with the intestinal microbes in the pathogenesis of obesity and diabetes. Studies have found that increasing levels of the primary BA cause a shift toward firmicutes, particularly genus *Clostridium* cluster XIVa, which raises production of hydrophobic secondary BAs.<sup>18,19</sup> Firmicutes increased significantly from 54.1% in the control group to 93.4% in the high CA group at the expense of bacteroidetes and other minority bacterial populations. Potentially pathogenic

proteobacteria, specifically gammaproteobacteria *E. coli*, also expanded in the gut of rats fed the high CA diet, decreasing bacterial densities and diversity.<sup>18</sup> Metagenome sequencing of the fecal microbiome of obese individuals was consistent with these murine study findings.<sup>94,95</sup> Weight loss studies further showed that a decrease in firmicutes and an increase of bacteroides were correlated with percent body weight loss, but not caloric intake.<sup>96,97</sup> Even though most studies showed a shift toward firmicutes in obese and diabetic individuals, others have shown opposite results: a shift toward bacteroidetes or no difference between these two phyla.<sup>98,99</sup> Nevertheless, several bacterial species and families have been associated with biological markers of obesity and diabetes. *E. coli* and *Lactobacillus*, for example, are linked to leptin variation independent of weight changes while the Coriobacteriaceae family is correlated with changes in hepatic TG, glucose, and glycogen levels.<sup>16,100</sup>

Because various signaling pathways may be altered in the diseased state, crosstalk between the gut microbiome and host may also be disrupted.<sup>101</sup> The most studied human gut bacteria responsible for 7 $\alpha$ -dehydroxylation of BAs belong to the genus *Clostridium*, which are grampositive anaerobic members of the Firmicutes family. They are capable of regulating BA synthesis in the liver by removing FXR-antagonist tauro-beta-muricholic acid in the ileum and converting primary BAs into secondary BAs.<sup>102</sup> Rats fed with CA displayed increased BA levels in the colon which selected against bacteroidetes and actinobacteria in favor of firmicutes, leading to greater generation of DCA and LCA, which can be cytotoxic at higher concentrations than the physiological range of 0.046–0.210 and 0–0.03 mM, respectively.<sup>14,19,103</sup> Conversely, cirrhotic patients were found to have a collapse of *Clostridium* XIVa family because of a smaller BA pool.<sup>19</sup> This direct relationship between the size of the BA pool and colonization by the *Clostridium* genus supports an intimate interaction between the intestinal microbiome and BA metabolism. The harmful effects of BA dysregulation and gut dysbiosis in the gut–liver axis are summarized in Figure 2.

## Gastric bypass alters BA synthesis and intestinal microbe composition

Rou-En-Y-Gastric Bypass (RYGB) is an increasingly popular bariatric surgical procedure for the treatment of obese and diabetic patients because of its resulting weight loss, improved insulin resistance and glucose intolerance, as well as reduction in other comorbidities. A systematic review and meta-analysis conducted for reviews from January 1990 to April 2006 showed that a striking 86.6% of type II diabetes mellitus patients who underwent gastric bypass surgery experienced resolution or dramatically alleviated symptoms of improved glycemic index and insulin levels.<sup>104</sup> The mechanisms underlying these beneficial outcomes remain subjects of intense investigation. Leptin circulation, pH changes, GLP-1 secretion, as well as BA synthesis and gut microbiome shifts are all postulated as potential mechanisms.<sup>105–109</sup> Due to the nature of the procedure, gastric bypass patients were thought to have poorer reabsorption rate, thus increasing BA synthesis and improving their glucose tolerance. Several studies reported a positive correlation between the increased postprandial GLP-1 and insulin secretion and the significant increase in serum BA in obese individuals compared to lean controls.<sup>110,111</sup> In an attempt to elucidate the effect of RYGB on BA synthesis, a similar technique was used in rats to study the molecular changes during intestinal adaptation. A decrease in mRNA expression of

Cyp7a1 and Cyp27a1 and major BA exporters such as bile salt export pump and organic anion-transporting polypeptide was observed in rats subjected to bile diversion surgery.<sup>112</sup> Ileal FXR and Fgf15 were also reduced at the mRNA level, but no changes were observed for hepatic FXR, which is consistent with previous results from ileal interposition.<sup>112</sup> Causing increased serum BA levels and reduced in ER stress markers, bile diversion improved glucose tolerance and liver steatosis in diet-induced obese rats.<sup>112</sup> RYGB on obese patients, similarly, had restored blunted BA mobilization, suggesting a possible weight loss mechanism postsurgery.<sup>113</sup>

An altered intestinal lumen microbiota can also contribute to the weight loss seen after RYGB. Several studies reported that intestinal microbes quickly adapt to the starvation-like, less acidic GI environment. The transfer of the gut microbial community from RYGB mice to germ-free mice resulted in similar weight loss and adiposity observed in RYGB patients.<sup>114</sup> Microbiota analysis via next generation sequencing revealed that a notable increase in the relative abundance of *Escherichia* and *Faecalibacterium prausnitzii* across the fecal content of mice, rats, and humans after receiving RYGB independent of calorie restriction.<sup>100,114</sup> *F. prausnitzii* abundance was also associated with reduced low-grade inflammation and reduction in colitis.<sup>115</sup> Furthermore, plasma leptin levels negatively correlated with *E. coli* levels and positively correlated with *Lactobacillus* levels. A marked decrease in blood bacterial DNA was also noted suggesting that RYGB restricted bacterial translocation in the intestines. Meanwhile, the role of Bifidobacteria in regulating GI health remains unclear. After RYGB, *Bifidobacterium* count increases and negatively correlated with adiponectin, a marker for insulin sensitivity and inflammation in the liver.<sup>100,101</sup> Additional investigation at the genetic and molecular levels is needed to elucidate specific microbial and metabolomic mechanisms from which the beneficial results of RYGB are derived. Nevertheless, the aforementioned findings further associate BA homeostasis and the gut microbiome in the pathogenesis of obesity and diabetes.

## BA dysregulation and gut dysbiosis in the gut–liver axis

### Effects on inflammation, immunity, and metabolism

A growing body of evidence has linked various inflammatory signaling pathways including TLR/MyD88, NFκB, and COX2 as bridging factors between pathogen-triggered inflammation and carcinogenesis. More recently, BA dysregulation and gut dysbiosis were also implicated in modulating the inflammatory process. Serum BA levels have increasingly served as biomarkers for liver diseases, obesity, and diabetes.<sup>116</sup> BAs not only regulate hepatic *de novo* lipogenesis, TG export, and plasma turnover, but also hepatic gluconeogenesis and insulin sensitivity through the actions of FXR and TGR5.<sup>15</sup> For instance, ursodeoxycholic acid (UDCA), a weak FXR and TGR5 binding ligand, improved hepatic ER stress and insulin sensitivity in diabetic mice, while norUDCA, a short chained homologue of UDCA, lowered hepatic TG levels.<sup>15</sup> BA-activated FXR inhibited bacterial overgrowth and mucosal injury in the distal small intestine through promotion of innate defense mechanisms to prevent epithelial barrier deterioration and bacterial translocation, thus reducing the risk for HCC and CRC.<sup>117,118</sup>

Conversely, BA dysregulation causes increased bacterial translocation by disrupting barrier function in the small intestine leading to systemic infection.<sup>117,119,120</sup> High concentration of DCA and CDCA can damage tissue by solubilizing the cell membrane and acting as immunosuppressive agents.<sup>120</sup> Indeed, BAs, especially DCA over 500  $\mu\text{M}$ , can increase chloride secretion and intestinal permeability, inhibit mucosal healing, and eventually elicit intestinal inflammation in the colon often seen in irritable bowel disease (IBD) patients.<sup>119,120</sup> Similar dose-dependent findings were also found in mice feeding studies. The physiological DCA concentration under a high-fat diet (1–3 mM) can impair gut barrier function whereas DCA concentration under a low-fat diet (0–1 mM) did not disrupt gut permeability.<sup>103,121</sup> Secondary BA UDCA and DCA exhibited opposing roles, although higher than physiological concentrations of UDCA were necessary to produce significant protective effects against DCA-induced gut permeability, especially in the colon. A high-fat diet was reported to decrease the ratio of UDCA and DCA, even though the overall fecal BA pool is larger.<sup>121</sup> The enterohepatic circulation of toxic BAs such as DCA increased the senescence-associated secretory phenotype in mouse hepatic stellate cells, induced greater levels of inflammatory and tumor-promoting factors, and promoted non-alcoholic steatohepatitis and HCC development.<sup>122</sup>

Furthermore, the integrity of the intestinal mucosal barrier and immunological activities are governed by a network of nuclear receptors including FXR. Increases in intestinal FXR and TNF $\alpha$  expression were observed in mice fed a high fat diet.<sup>121</sup> Elevated BA not only up-regulated numerous pro-inflammatory mediators and NF $\kappa\beta$ , but also shifted intestinal microbe composition toward endotoxin-producing species. Endotoxic microbes are capable of aggravating glucose tolerance and insulin resistance to elicit increased metabolic inflammation and gut permeability.<sup>123,124</sup> Low grade inflammation and harmful metabolites generated by endotoxic microbes in the gut permitted microbial translocation into the enterohepatic circulation. Bacterial translocation through compromised tight junctions in the small intestine was found to precede intestinal bacterial overgrowth in acute liver injury caused by cholestasis, alcohol toxicity, and obesity in mice.<sup>117</sup> Given that plasma endotoxin levels correlate with the severity of liver disease and that KO mice lacking toll-like receptor 2 and TNF $\alpha$  signaling pathways are resistant to liver injury and fibrosis, bacterial translocation appears to play a role in the progression of chronic liver disease.<sup>117,125,126</sup> Although bacterial LPS may not be involved in the onset of gut barrier dysfunction, it translocates through the intestinal mucosa in the presence of a leaky gut.<sup>121</sup> Consistent with murine findings, elevated bacterial and antigen uptake in the intestines of obese and diabetic individuals resulted from impaired tight junctions.<sup>119</sup>

It is uncertain whether microbial profile shift is the cause or consequence of increased intestinal permeability, but it is clear that the intestinal microbiome significantly impacts intestinal and colonic health. Individuals suffering from gut dysbiosis, characterized by a reduction in beneficial, anti-inflammatory *Bifidobacterium* and *Lactobacillus* and elevation in aerobic, pro-inflammatory *Enterobacter* and *Clostridium* species, are at higher risk for liver diseases.<sup>127</sup> Hydrophobic, taurine-conjugated BAs enhanced the growth of sulfate-reducing gut bacteria, antigen and bacterial translocation resulting in ulcerative colitis and CRC.<sup>128–130</sup> One study positively correlated fecal levels of CDCA with members of the

Enterobacteriaceae family while another found a positive linkage between Enterobacteriaceae, endotoxemia, and hepatic inflammation.<sup>19,131</sup> On the other hand, *F. prausnitzii* has been consistently associated with anti-inflammatory properties and a marked decrease in *F. prausnitzii* is seen in IBD patients compared to healthy individuals.<sup>91,100,115</sup>

Intestinal microbes not only play a role in gut permeability regulation and innate immunity, but also influence the expression of genes involved in regulating inflammation and energy homeostasis. A significant increase in gramnegative proteobacteria and the disappearance of Bifidobacteria were observed in the gut of mice under a high-fat diet.<sup>132</sup> Inflammasome, a multiprotein oligomer that activates pro-inflammatory cytokines, has recently been proposed to mediate liver injury progression. In the gut, inflammasome protects against gram-negative bacterial infection whereas in the liver, it aggravates hepatic steatosis and inflammation through activation of TLR4 and TLR9, driving tumorigenesis.<sup>133</sup> LPS can induce inflammasome through activation of caspase-11 and specific toll-like receptors.<sup>134</sup> Interestingly, mice infused with LPS for 4 weeks displayed a similar phenotype to those under a high-fat diet exhibiting increased weight gain, insulin resistance, hepatic TG content, and adipose tissue inflammation.<sup>135</sup>

Furthermore, circulating LPS correlated positively with insulin levels, adipose macrophage infiltration, and fasting blood glucose in diabetic patients.<sup>136</sup> Impaired tight junctions as previously discussed and chylomicron-facilitated transport are two mechanisms proposed for how LPS promotes inflammation in the liver and intestines. FXR KO mice with dysregulated BAs and increased inflammation also exhibit impaired glucose tolerance and decreased insulin sensitivity in a manner similar to diabetic mice and patients with type 2 diabetes mellitus.<sup>25,35,137</sup> The role of FXR in the regulating glucose homeostasis was demonstrated from multiple murine studies and a Phase 2 clinical study for type 2 diabetes mellitus and NAFLD in which FXR stimulation improves glucose tolerance, hepatic steatosis, and insulin sensitivity.<sup>23,26,137</sup> The lack of FXR up-regulation by elevated BA levels in mice fed a high-fat diet may suggest a disrupted feedback system in BA synthesis.

Intriguingly, the intestinal microbiota may be a potential contributor to the development of NAFLD by modulating lipid and glucose metabolism through FXR and TGR5 signaling. NAFLD is characterized by an accumulation of TG in hepatocytes, which increase inflammation and progresses into fibrosis, cirrhosis, and ultimately HCC.<sup>111</sup> The condition is associated with low-grade chronic inflammation, a common feature in obese individuals with high visceral adiposity.<sup>6</sup> Hepatocyte apoptosis resulting from increased fat in the liver and insulin resistance are common pathological signs of NAFLD.<sup>138,139</sup> Elevated serum free fatty acid can impair insulin signaling, increase hepatic glucose production while hepatic fatty acid overload induces inflammatory cytokines, fibrogenic cytokines, and oxidative stress. Hepatic BA accumulation due to hepatic steatosis can up-regulate NF $\kappa$ b signaling along with major inflammatory markers IL-6, IL-8, and TNF $\alpha$  in the progression of liver carcinogenesis.<sup>140</sup> Similar to the liver and the intestines, adipose tissue in obese and diabetic patients also exhibit increased expression of proinflammatory cytokines. Hypertrophic adipocytes and macrophage infiltration exacerbate this chronic inflammatory microenvironment and enhance the survival and proliferation of neoplastic liver and intestinal cells.<sup>141</sup>

## Effects on genome integrity

As previously mentioned, obese and diabetic individuals suffer from abnormally elevated BA levels, particularly hydrophobic secondary BA such as DCA and LCA. A growing body of evidence exists to support that hydrophobic secondary BA can increase intracellular production of reactive oxygen and nitrogen species resulting in elevated oxidative stress and DNA damage. Insights into the mechanism of BA induced-genotoxicity were obtained when treatment of human Barrett's esophagus tissues with a combination of BAs led to significant elevation in oxidative stress biomarker 8-hydroxy-deoxyguanosine and reactive oxygen species (ROS).<sup>142,143</sup> Moreover, esophageal cells treated with DCA showed increased generation of reactive nitrogen species and DNA damage which were inhibited by co-treatment with nitric oxide scavenger or pretreatment with nitric oxide synthase inhibitor.<sup>143,144</sup> DCA-treated colon cancer cells exhibited higher ROS generation, membrane blebbing, activation of mitochondrial apoptotic pathway, and formation of apoptotic bodies compared to untreated cells.<sup>145</sup> DCA exposure also resulted in micronuclei formation accompanied by a dose-dependent response in NF $\kappa$ b activation that was attenuated by cotreatment.<sup>146</sup>

The above studies clearly demonstrate that hydrophobic BAs induce cellular oxidative stress through elevated reactive oxygen and nitrogen species production with similar results observed between human liver and colon cell lines. Interestingly, human HCC cells treated with hydrophobic primary CDCA also displayed cell cycle arrest, caspase-9-like activity, poly ADP-ribose polymerase cleavage, dose-dependent sites of DNA lesions, and extensive nuclear fragmentation.<sup>147,148</sup> These cells also showed activation of ERK1/2 leading to the phosphorylation and stabilization of myeloid cell leukemia sequence 1 (MCL-1) in a mitogenactivated protein kinase-dependent (MEK-dependent) manner which conferred greater resistance to chemotherapeutic drugs.<sup>147</sup> These findings strongly support that excessive levels of hydrophobic BA can exert a carcinogenic effect on enterohepatic tissues by promoting genomic instability through oxidative injury. Figure 3 provides an overview of the implicated carcinogenic consequences of BA dysregulation, gut dysbiosis, and insulin resistance.

## Potential preventative measures

### Synthetic compounds

Several synthetic drugs such as colestevlam and colestimide, designed to sequester BAs, have demonstrated efficacy in improving insulin resistance and glucose tolerance.<sup>149–151</sup> BA receptor agonists also appeared to be promising in the management of obesity and diabetes related symptoms.<sup>152,153</sup> Specifically, synthetic retinoid such as acyclic retinoid (ACR) was investigated for its beneficial effect on obesity-related liver tumorigenesis, since RXR $\alpha$ , a heterodimer partner of FXR, was found to be repressed in early stages of HCC due to phosphorylation by the Ras/MAPK signaling pathway. Results indicate that ACR inhibited the Ras/MAPK pathway, ameliorated liver steatosis, improved insulin sensitivity, and decreased inflammation. Ultimately, ACR appears promising in restoring RXR $\alpha$  function in the liver, making it an effective chemoprevention drug against HCC progression.<sup>36</sup>



### Prebiotics, probiotics, and synbiotics

With the intestinal microbiota implicated as one of the key players in the progression of liver and colon carcinogenesis in obese and diabetic patients, selective modification of the gut microbial composition has been extensively researched as a viable alternative or additive to current treatment plans. Prebiotics promote the growth of beneficial bacteria while probiotics are live microorganisms, administered exogenously, which provide a benefit beyond nutrition. Most prebiotics exist in the form of non-digestible carbohydrates and exert protective effects against liver and colon cancer.<sup>154–157</sup> Dietary fibers shortened GI transit time to reduce the length of exposure to toxic metabolites such as hydrophobic BAs and bacterial toxins while aiding in their incorporation in feces for excretion.<sup>158</sup> In addition, these fibers enhanced the growth of beneficial bacteria such as Bifidobacteria, lowered intestinal pH, and inhibited the growth of harmful bacteria.<sup>158</sup>

An increase in bifidobacteria is the signature of prebiotic treatment using inulin-type fructans or galactooligosaccharides.<sup>159</sup> Other resistant dietary fibers such as pomegranate peel extract and natural phytoalexin resveratrol can alter the gut microbiome in favor of bifidobacteria while lowering inflammatory markers in the colon and visceral adipose tissue.<sup>160,161</sup> Short chain fatty acids (SCFAs), the fermented end products of dietary fibers, are recognized for their ability to inhibit growth and promote apoptosis in colon and liver cancer cells.<sup>162</sup> SCFAs can also activate various drug metabolizing enzymes to decrease DNA mutation and reduce cancer risk.<sup>162</sup> Complementing SCFAs with fish oil selectively reduced unsaturated fatty acid accumulation in the liver, improving hepatic fat oxidation and inflammation.<sup>163</sup>

Furthermore, probiotics have been suggested as a tool to manage inflammatory bowel disease.<sup>164</sup> Although no general consensus exists for the beneficial effects of probiotics in obese, diabetic, and NAFLD patients, reduced hepatic total fatty acid content and serum alanine aminotransferase levels were noted in rodent models treated with probiotics.<sup>165,166</sup> A synergistic effect of probiotics and blueberry husks in preventing colon carcinomas and subsequent liver tumors has been observed in rats.<sup>167</sup> Blueberry husks alone significantly decrease the number of colonic ulcers and dysplastic lesions while probiotics alone improved liver function by decreasing parenchymal infiltration and bacterial translocation. Thus, the combination treatment of probiotics and blueberry husks delayed colonic carcinogenesis and prevented hepatic injuries. The combined treatment also reduced endotoxin-producing enterobacteriaceae and increased beneficial, anti-inflammatory lactobacilli.<sup>167</sup> Synbiotics, mixtures of probiotics and prebiotics, were noted to lower colon cancer risk through improved insulin resistance, reduced inflammation, and preservation of gut barrier integrity in rats fed a high-fat diet.<sup>168</sup> In terms of gut microbial ecology, synbiotic treatment significantly increased fecal *Lactobacillus* species at the expense of potentially pathogenic *E. coli* and *Staphylococcus*, thus lowering the endotoxin level in cirrhotic patients.<sup>169</sup>

### Dietary changes and natural derivatives

Simple dietary changes can also aid in reversing BA dysregulation and intestinal microbiome derangement. Vitamin B6 can improve colon health by significantly reducing

hydrophobic LCA levels in the colon, creating a reduced LCA to DCA ratio. LCA was 20-fold more toxic than DCA toward liver and colon cancer cells, and vitamin B6 helped with detoxifying enzymes and decreased DNA damage.<sup>170</sup> Moreover, various naturally derived products are also under investigation including the antioxidant tempol which reduced obesity and improved insulin resistance in mice fed a high-fat diet by activating bile salt hydrolase to increase intestinal tauro-beta-muricholic acid, an FXR antagonist.<sup>102</sup> Tempol appears to exert its antiobesity effects through FXR since inhibition of intestinal FXR promoted diet-induced obesity and insulin resistance. Tempol was also able to shift the gut microbial profile from firmicutes toward bacteroidetes dominance.<sup>102</sup> Plant-based products such as burdock powder and genistein also showed moderate efficacy in normalizing BA homeostasis and the gut microbiome in animal models.<sup>102,171</sup> The proposed beneficial effects on BA dysregulation and gut dysbiosis by pharmacological and dietary intervention are described in Figure 4.

## Conclusion

Great strides have been made in our understanding of the intricate relationship between BAs and the intestinal microbiome. There is growing interest in the roles of BAs and gut microbes in the pathophysiology of chronic liver and GI diseases. However, the precise mechanisms of how BA dysregulation and gut dysbiosis exacerbate the chronic symptoms of obesity and diabetes to promote liver and colon cancer remain poorly defined. Substantial experimental data suggest that in addition to chronic hyperinsulinemia and derangement of peptide hormones, gut dysbiosis and BA dysregulation, frequently observed in obese and diabetic patients, are also contributors to inflammation and injury of the liver and colon. The NIH Common Fund Human Microbiome Project should help provide some definitive answers to many of these partially explained findings on the role of the gut microbes in metabolic disorders. Even though our current knowledge on how BAs and gut microbial affect liver and intestinal health has substantially expanded, the molecular pathways of their interplay in enterohepatic diseases require further elucidation.

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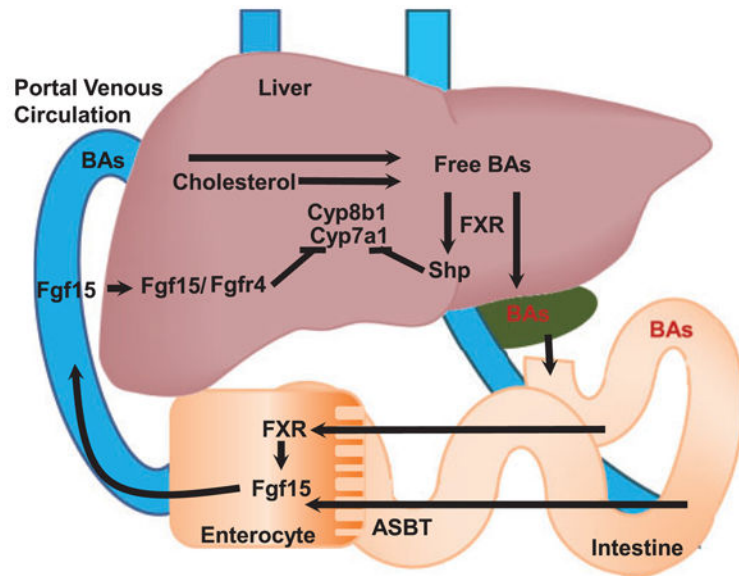


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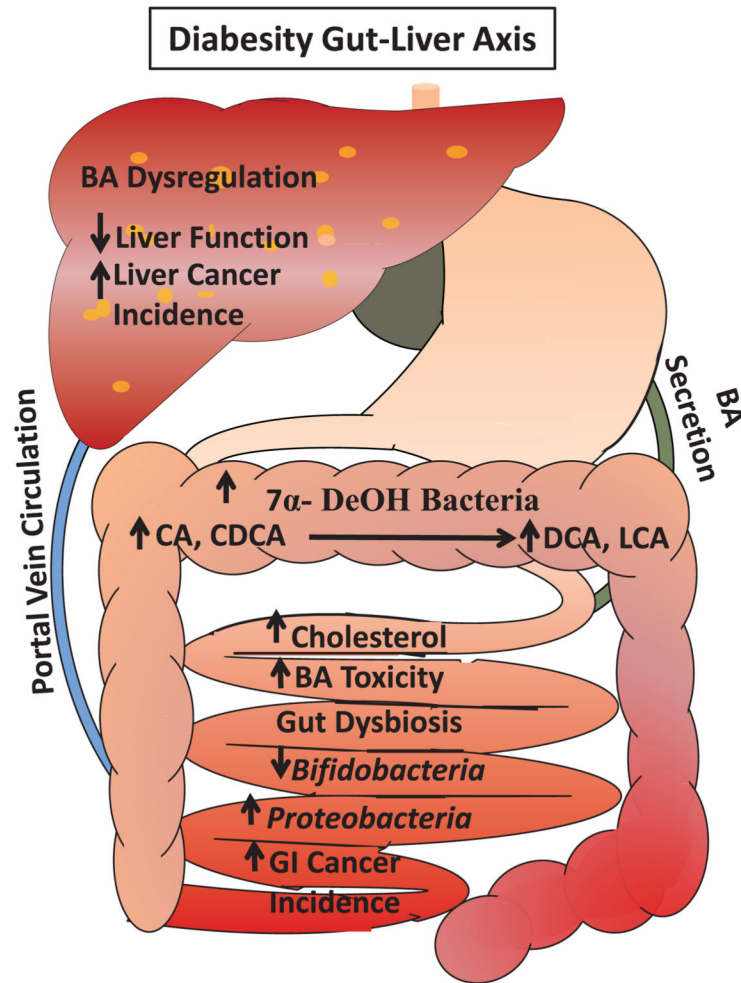
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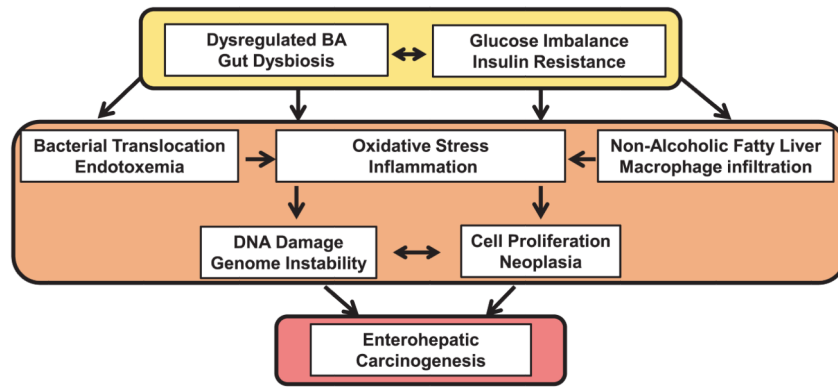
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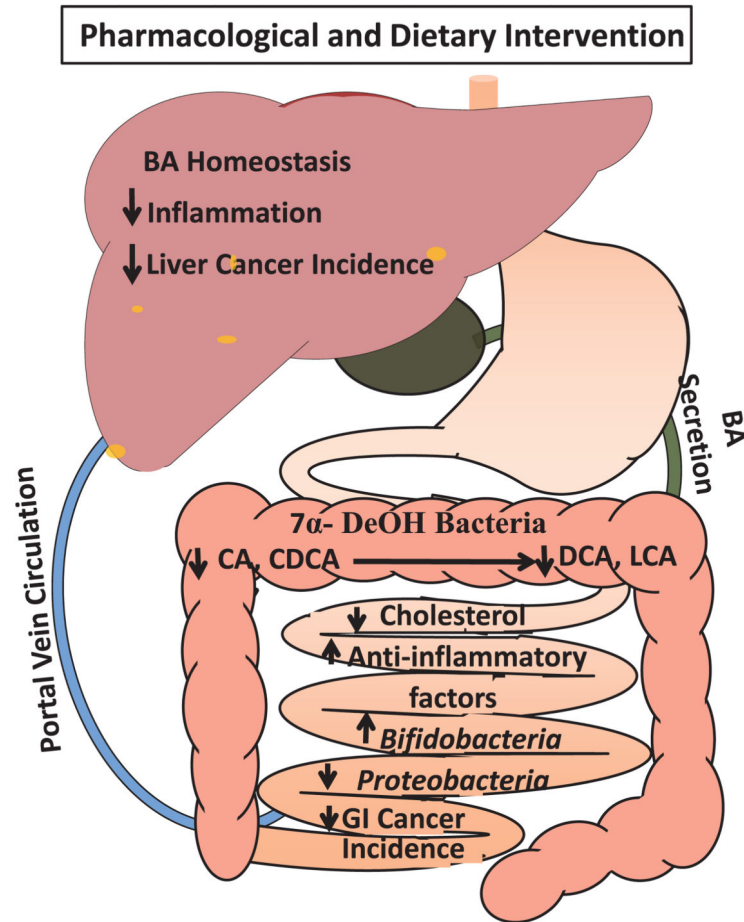
**Figure 1. Overview of enterohepatic circulation of bile acids. (A color version of this figure is available in the online journal)**



**Figure 2.** The proposed mechanisms by which dysregulated bile acid and gut dysbiosis induce carcinogenesis. (A color version of this figure is available in the online journal.)



**Figure 3. Overview of the interplay between bile acid dysregulation and gut dysbiosis in diabetes. (A color version of this figure is available in the online journal.)**



**Figure 4. Beneficial effects of normalized bile acid homeostasis and gut microbiota. (A color version of this figure is available in the online journal.)**