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COMMENTARY

Linking Sex Differences in Non-Alcoholic Fatty Liver Disease to Bile Acid Signaling, Gut Microbiota, and High Fat Diet

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Sex differences in liver cancer and metabolic diseases have been recognized; males have higher prevalence and a higher death rate than females.[1] Non-alcoholic fatty liver disease has emerged as a major health concern worldwide. Non-alcoholic fatty liver disease covers a spectrum of liver diseases from simple steatosis to non-alcoholic steatohepatitis, which is characterized by hepatic inflammation and fibrosis. Some non-alcoholic steatohepatitis patients develop to hepatocellular carcinoma and require liver transplant.

Bile acids are steroid-derived amphipathic molecules that act as detergents for absorption of nutrients, steroids, and drugs in the intestine, and also as serve as signaling molecules that activate nuclear farnesoid X receptor (FXR) and the membrane G-protein bile acid receptor-1 (Gpbar-1, aka TGR5).[2] Bile acid signaling plays a critical role in regulation of metabolic homeostasis via the gut-to-liver axis. Bile acids activate FXR to regulate lipid, glucose, and energy metabolism. Deficiency of FXR impairs bile acid and lipoprotein metabolism and aggravates Western high fat diet (WD)—induced obesity and diabetes. In this issue of *The American Journal of Pathology*, Jena et al[3] reported a comprehensive study of WD-induced dysbiosis in both male and female $Fxr^{-/-}$ mice compared to wild-type mice.

In the intestine, activation of FXR induces fibroblast growth factor 15 (FGF15, or FGF19 in humans) to inhibit bile acid synthesis in the liver. Intestinal bacteria bile salt hydrolases de-conjugate taurine-conjugated bile acids followed by bacteria 7α-dehydroxylase activity, which converts the primary bile acids, cholic acid and chenodeoxycholic acid, to secondary bile acids, deoxycholic acid and lithocholic acid, respectively. In humans, taurine- and glycine-conjugated cholic acid, chenodeoxycholic acid, and deoxycholic acid are the major bile acids in a highly hydrophobic bile acid pool, whereas in mice taurine chenodeoxycholic acid is converted to Tα-muricohlic acid and Tβ-muricholic acid, and taurine cholic acid (TCA) and Tα/β-MCAs are major bile acids in a highly hydrophilic bile acid pool.

Bile acids control gut bacteria overgrowth to protect intestinal barrier function. Among all bile acids, deoxycholic acid has the highest bactericidal activity. Ablation of the *Fxr* gene in mice ($Fxr^{-/-}$) impairs bile acid and lipoprotein metabolism and aggravates WD-induced hepatic steatosis.[4] WD increases biliary secretion of bile acids and reshapes the gut microbiota in obesity by increasing *Firmicutes* and decreasing *Bacteriodetes*.[5] Feeding a cholic acid—containing diet increases the gut *Firmicutes* to *Bacteriodetes* ratio, which is also increased in obese mice.[6] Dietary saturated fats increase TCA and promote bile-tolerant and sulfur-producing *Bilophila wadsworthia* to increase pro-inflammatory cytokines and colitis.[7] Consistently, an animal based—diet increases abundance of *B. wadsworthia* and *Bacteroides*, and decreases abundance of *Firmicutes*.[8] Obese and non-alcoholic fatty liver disease patients also have reduced *Firmicutes* and increased *Proteobacteria*,[9] which causes dysbiosis and contributes to liver inflammation.[10] These studies suggest a link between dietary fats, bile acids, and gut microbiota.

Jena et al[3] reported a comprehensive study of WD-induced dysbiosis in both male and female $Fxr^{-/-}$ mice compared to wild-type mice. They found that male $Fxr^{-/-}$ mice had more severe hepatic inflammation and steatosis than female mice,

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and WD-feeding aggravates hepatic steatosis more in male than female $Fxr^{-/-}$ mice. To study the role of the gut microbiota in hepatic inflammation, they treated control diet− and WD-fed $Fxr^{-/-}$ mice with different antibiotics, Abx (ampicillin, neomycine, metronidazole, and vancomycin), vancomycin, and polymyxin B. Abx eliminated hepatic neutrophils and lymphocytes in control diet, but not in WD-fed $Fxr^{-/-}$ mice. Vancomycin and polymyxin B reduced hepatic lymphocytes. These antibiotics have differential effect on reducing inflammatory and fibrogenic gene mRNA expression in control diet− and WD-fed mice. Gut microbiome analysis showed that $Fxr^{-/-}$ mice had reduced *Firmicutes* and increased *Proteobacteria*, which could be reversed by Abx, but *Proteobacteria* and *Bacteroides* persisted in WD-fed $Fxr^{-/-}$ mice. Interestingly, they found that reducing hepatic inflammation by antibiotics was associated with decreased free and conjugated secondary bile acids, and also caused changes in gut microbiota. The same group has recently reported that the sex differences in WD-induced hepatic steatosis, insulin resistance, bile acids, and microbiota profiles are all FXR-dependent.[11] Male WD-fed $Fxr^{-/-}$ mice had more severe insulin resistance that may contribute to higher severity of hepatic steatosis compared to female mice. *Fxr* deficiency increases TCA and T-βMCA, and the gut microbiota profiles in male WD-fed $Fxr^{-/-}$ mice are distinctly different from control diet−fed $Fxr^{-/-}$ mice. WD also shifts the gut microbiota in $Fxr^{-/-}$ mice by decreasing *Firmicutes* and increasing *Proteobacteria*.[9] Analysis of the gut microbiota revealed differential effects of WD on several metabolic pathways in male and female $Fxr^{-/-}$ mice. All these data provide convincing evidence that bile acid/FXR signaling plays a critical role in mediating sex difference in dysbiosis and non-alcoholic fatty liver disease. How the gut microbiota differentially alters metabolic pathways in male and female mice, and how high fat diet influences the gut microbiomes and metabolic pathways remains to be studied in detail.

Growth hormone/STAT5 signaling has been shown to cause sexual dimorphic expression of the male dominant enzyme oxysterol 7α-hydroxylase (Cyp7b1) and regulate bile acid synthesis and lipid metabolism.[12] A recent study reports that the incidence of streptozotocin (STZ)-high fat diet (HFD)-induced hepatocellular carcinoma is significantly higher in male mice than female mice.[13] Interestingly, metagenomic analysis showed differences in gut bacteria involved in bile acid metabolism between normal male and female mice. STZ-HFD treatment amplified the observed sex differences in gut microbiota. At the phylum level, female mice have a higher ratio of *Firmicutes* to *Bacteroidetes* than male mice. STZ-HFD treatment reduced *Firmicutes* and *Bacteroidetes*, and markedly increased *Proteobacteria* in female mice, whereas STZ-HFD treatment increased *Firmicutes*, decreased *Bacteroidetes*, and increased *Proteobacteria* much less in male mice. STZ-HFD treatment caused more intrahepatic retention of hydrophobic bile acids (TCA, taurine chenodeoxycholic acid, and taurine lithocholic acid) in male mice compared to female mice. Interestingly, STZ-HFD treatment strongly reduced Cyp7b1 mRNA expression, consistent with a much higher increase of TCA in male than female mice, and an altered gut microbiota between male and female mice. In diabetic patients, bile acid pool size and 12α-hydroxylated bile acids to non-12α-hydroxylated bile acids ratio in serum is increased. It is known that insulin and glucagon, and fasting and refeeding regulates bile acid synthesis.[14] These recent advances suggest that the sex disparity in diabetes, non-alcoholic steatohepatitis, and liver cancer is linked to sex differences in regulation of bile acid metabolism, which is influenced by insulin, glucocorticoid, and growth hormone levels in males and females. Future study is needed to show how hormones integrate diet, bile acid signaling, and gut microbiota in the gut-to-liver axis to determine sex disparities in metabolic diseases.

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