A picture is now starting to emerge regarding the liver-bile acid-microbiome axis. Increasing levels of the primary bile acid cholic acid (CA) causes a dramatic shift toward the Firmicutes, particularly *Clostridium* cluster XIVa and increasing production of the harmful secondary bile acid deoxycholic acid (DCA). During progression of cirrhosis, the microbiome, both through their metabolism, cell wall components (LPS) and translocation lead to inflammation. Inflammation suppresses synthesis of bile acids in the liver leading to a positive-feedback mechanism. Decrease in bile acids entering the intestines appears to favor overgrowth of pathogenic and pro-inflammatory members of the microbiome including *Porphyromonadaceae* and *Enterobacteriaceae*. Decreasing bile acid concentration in the colon in cirrhosis is also associated with decreases in *Clostridium* cluster XIVa, which includes bile acid 7α-dehydroxylation bacteria which produce DCA. Rifaximin treatment appears to act by suppressing DCA production, reducing endotoxemia and harmful metabolites without significantly altering microbiome structure. The study of the interaction between bile acids and gut microbiota in the context of liver disease is essential because the human liver is the only organ in the body that produces all 14 enzymes required for de novo synthesis of the primary bile acids. The "classical" or "neutral" pathway of bile acid synthesis begins with cholesterol 7α-hydroxylase (CYP7A1), which produces both the dihydroxy bile acid chenodeoxycholic acid (CDCA; 3α, 7α) and the trihydroxy bile acid cholic acid (CA; 3α, 7α, 12α). The "acidic" pathway produces mostly CDCA and is initiated by mitochondrial steroid 27 hydroxylase (CYP27A1) catalyzed side-chain oxidation, which is followed by cleavage of a three-carbon side chain resulting in the C-24 bile acids. CA synthesis requires...
sterol 12α-hydroxylase (CYP8B1) followed by side-chain oxidation and cleavage. The classical pathway produces the majority of the two primary bile acids in healthy humans. In the diseased liver, the classical pathway is down-regulated and the acidic pathway produces most of the primary bile acids. Bile acids are conjugated to glycine or taurine before being actively transported across the gallbladder. Eating stimulates gallbladder contraction and emptying of the contents into the small intestine. Bile salts function to solubilize fats and fat-soluble vitamins before they are actively transported across the ileum, entering the portal circulation, and return to the liver in what is termed the entero-hepatic circulation (EHC). The EHC is 95% efficient, with roughly 600–800 mg bile salts escaping into the large bowel daily.

The large bowel is the most densely populated natural environment known, containing roughly 10^{14} bacterial cells. Diversity in this environment is almost entirely at lower taxonomic levels (genus, species, strain). Phylum level diversity in the gut is remarkably low, with two major divisions, the Bacteroidetes and Firmicutes predominating, followed by Actinomycetes. Greater than 99% of the functional genes associated with the human body are microbial, and most of these are found in bacteria colonizing the large bowel. The lumen of the large bowel is a highly anaerobic environment and microbes that occupy this ecological niche must carry out fermentative metabolism in order to generate ATP and grow. The main endogenous substrate used by the large bowel microbiota consists of sloughed intestinal epithelial cells (100–200 g/day) and bile components, whereas, exogenous substrates include resistant starch, plant polysaccharides and proteins. From these substrates gut bacteria produce mostly short chain fatty acids (acetate, propionate, and butyrate). During EHC bile acids enter the colon where they are metabolized by the large bowel microbiota. CA and CDCA are 7α-dehydroxylated exclusively by a small population of anaerobic bacteria to form deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. Human gut bacteria carrying out bile acid 7α-dehydroxylation have been shown to belong to the genus Clostridium, which are gram-positive anaerobic spore forming members of the Firmicutes.

Animal model studies demonstrate that increased bile acid levels in the colon select against the Bacteroidetes and Actinobacteria and favor the Firmicutes including bile acid 7α-dehydroxylating bacteria in vivo. Indeed, Islam et al. reported a marked increase in Firmicutes including bile acid 7α-dehydroxylating bacteria in colon, C. hiranonis, C. ileoanaeae and C. wodellii, which are each nested within a phylogenetic tree containing members of Blautia, Ruminococcaceae and Lachnospiraceae all of which are in clostridial rRNA cluster XIVa (Fig. 1). We have determined the levels of bile acid 7α-dehydroxylating bacteria in control C57BL/6 mice fed a normal chow diet (NC) and in mice fed a NC diet plus 17β w/w cholic acid. After 2 weeks of feeding, the ceca were removed and immediately serially diluted in anaerobic brain infusion broth containing (24 °C)-cholic acid. We observed a 1000-fold increase in the levels of bile acid 7α-dehydroxylating bacteria (p = 0.001) with cholic acid feeding consistent with the hypothesis that increased primary bile acid substrate supports a larger population of these bacteria in the intestines (Fig. 2). The current publication by Kakiyama et al. (2011) reported a marked increase in members of clostridial cluster XIVa following CA feeding in rats and significant increase in DCA with higher input of CA. We have detected bile acid 7α-dehydroxylation activity in Clostridium scindens, C. hiranonis, C. ileoanaeae and C. wodellii, which are each nested within a phylogenetic tree containing members of Blautia, Ruminococcaceae and Lachnospiraceae all of which are in clostridial rRNA cluster XIVa.

We have previously elucidated a multi-step bile acid 7α-dehydroxylation pathway in these bacteria that allow them to use primary bile acids as an electron acceptor allowing for increased ATP formation and growth. However, members of Clostridium cluster XIVa lack the bai operon and thus do not convert CA to DCA, so for most of these microbes, energetic considerations can be ignored. Energies may be ancillary to production of and resistance to DCA, a potent antimicrobial agent that reduces competition for growth substrates. Is the expansion of the bile acid 7α-dehydroxylating bacteria population due mainly to selection for bile tolerance coupled with reduced competition for growth substrates, or does metabolism of bile acid determine population size of members with the bai operon? We know that growth of these microbes in vitro is not dependent on the presence of bile acids. However, competition for resources in vivo is fierce in the colon, and their low levels (0.0001% of the microbiota) indicate a specialized niche. Inhibition of the bile acid 7α-dehydroxylation pathway without inhibition of the organism itself either pharmacologically or through genetic-knockout of the bile acid 7α-dehydroxylation pathway will be necessary to determine the in vivo role of the bile acid 7α-dehydroxylation pathway, particularly in the presence of exogenously added DCA.

At least in rodents, bile acid 7α-dehydroxylating bacteria are capable of regulating bile acid synthesis in the liver by removing an FXR-antagonist, tauo-β-muricholic acid, in the ileum. In humans, other members of the microbiome are capable of shrinking the bile acid pool through inhibition of the organism itself either pharmacologically or through genetic-knockout of the bile acid 7α-dehydroxylation pathway.
Veillonellaceae, Alcaligenaceae and Porphyromonadaceae. Indeed, we have previously shown a positive correlation between levels of Alcaligenaceae and Porphyromonadaceae and cognitive impairment in cirrhotic patients who develop hepatic encephalopathy. Kakiyama et al. (2013) observed a positive correlation between fecal levels of CDCA and members of the Enterobacteriaceae. Previously, Bajaj et al. (2012) found a positive linkage between Enterobacteriaceae, endotoxemia and inflammation, which was also positively correlated with development of HE.

Lipopolysaccharide (LPS) or endotoxin, a component of the cell wall of gram-negative bacteria varies between species. This structural variance leads to differing degrees of inflammation in the host. Quantitatively, species of Bacteroides are among the most predominant genera in the human GI tract, while genera within the Enterobacteriaceae, Porphyromonadaceae and Alcaligenaceae are only minor members of the gut microbiome of healthy humans, while several members of these families are human pathogens. However, LPS from members of the Enterobacteriaceae, for instance, show potency on the order of 4–50-fold that of LPS from members of the Bacteroidetes in TNF-α assays. Indeed, animal models of non-alcoholic fatty liver disease (NAFLD) found a positive correlation between Porphyromonadaceae and exacerbation of hepatic steatosis and inflammation through TLR4 and TLR9 activation of TNF-α. Porphyromonas gingivalis LPS has been shown to induce TNF-α through TLR-2 and TLR-4. Therefore, the data presented by Kakiyama et al. (2013) confirm previous observations that increase in specific gram-negative taxa, particularly Enterobacteriaceae and Porphyromonadaceae could lead to inflammation, which contributes to cirrhosis and its complications. Interestingly, we have previously shown that while the stool microbiome between HE and non-HE cirrhotic patients were not significantly

Figure 1. Reference species tree of the clostridia and related Clostridiales bacteria. Phylogenetic tree obtained from analysis. Maximum likelihood and Bayesian analyses of 20 concatenated single-copy proteins from 99 genomes, showing showing Clostridium scindens as distantly related to most other Clostridium species and being deeply embedded in a group of bacteria mainly from the Lachnospiraceae and Ruminococcaceae families. Streptococcus sanguinis SK36 was included as an outgroup. Numbers on nodes indicate support from bootstrap support and posterior probability (numbers below 50 or 0.5 not shown, indicated by a dash); black circles indicate support of 100 and 1, with all other values explicitly written.
The study of secondary BA formation as a potential marker for disease progression in human cirrhosis is important because while the 7α-dehydroxylation can be construed as a “functional test” for microbiota, the production of these secondary BAs does not provide an energy advantage to the human host, but to the bacteria that achieve this conversion. Due to their membrane-destabilizing properties and potential for worsening intestinal permeability, the decrease in secondary BAs in advancing cirrhosis, actually may be protective. This hypothesis is also supported by the reduction in the secondary/primary BA ratio after rifaximin in early cirrhotic patients. Therefore this initial study of BAs in the modulation of the fecal microbiome in worsening cirrhosis raises several important questions that need to be answered in future studies in order to delineate this complex liver-bile acid-microbiome interaction within the gut milieu.

Disclosure of Potential Conflicts of Interest
No potential conflict of interest was disclosed.
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