OPEN ACCESS
CellPress

Cell Reports
Report

Microbiota Modification with Probiotics Induces Hepatic Bile Acid Synthesis via Downregulation of the Fxr-Fgf15 Axis in Mice

Chiara Degirolamo,[1,2] Stefania Rainaldi,[2] Fabiola Bovenga,[1,2] Stefania Murzilli,[2] and Antonio Moschetta[1,3,*]
[1]National Cancer Research Center, IRCCS Istituto Oncologico "Giovanni Paolo II," Bari 70124, Italy
[2]Fondazione Mario Negri Sud, Santa Maria Imbaro, 66030 Chieti, Italy
[3]Department of Interdisciplinary Medicine, "Aldo Moro" University of Bari, Bari 70124, Italy
*Correspondence: a.moschetta@oncologico.bari.it
http://dx.doi.org/10.1016/j.celrep.2014.02.032

SUMMARY

Gut microbiota influences host health status by providing trophic, protective, and metabolic functions, including bile acid (BA) biotransformation. Microbial imprinting on BA signature modifies pool size and hydrophobicity, thus contributing to BA enterohepatic circulation. Microbiota-targeted therapies are now emerging as effective strategies for preventing and/or treating gut-related diseases. Here, we show that gut microbiota modulation induced by VSL#3 probiotics enhances BA deconjugation and fecal excretion in mice. These events are associated with changes in ileal BA absorption, repression of the enterohepatic farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis, and increased hepatic BA neosynthesis. Treatment with a FXR agonist normalized fecal BA levels in probiotic-administered mice, whereas probiotic-induced alterations in BA metabolism are abolished upon FXR and FGF15 deficiency. Our data provide clear in vivo evidence that VSL#3 probiotics promote ileal BA deconjugation with subsequent fecal BA excretion and induce hepatic BA neosynthesis via downregulation of the gut-liver FXR-FGF15 axis.

INTRODUCTION

The human gastrointestinal tract is home to a complex and dynamic microbial ecosystem containing tens of trillions of microorganisms and encoding at least 150-fold more genes than the human genome (Gareau et al., 2010; Leser and Mølbak, 2009). The gut microbial community is instrumental in host energy metabolism and immune functions and greatly contributes to a wide range of processes including protection against pathogens, vitamin synthesis and ion absorption, carbohydrate and protein fermentation, bile acid (BA) biotransformation, and immune system modulation (Guarner and Malagelada, 2003; Hamer et al., 2012; Kinross et al., 2008; Ridlon et al., 2006; Round and Mazmanian, 2009; Tsai and Coyle, 2009).

Composition of gut microbiota is modulated by numerous extrinsic factors such as diet, age, medication, and stress. Disturbances of gut microbiota, also known as dysbiosis, have often been associated with several diseases including inflammatory bowel disease (IBD), obesity, type 2 diabetes, and cancer (Muegge et al., 2011). Accumulating evidence indicates that gut microbiota composition is readily changeable and, accordingly, that the plasticity of microbiome favors the development of gut microbiota-targeted therapy including antibiotics, prebiotics, and probiotics. Probiotics are live microorganisms that provide beneficial effects to the host when adequately administered, and their therapeutic potential has been documented in the maintenance treatment of ulcerative colitis, pouchitis, and IBD (Jijon et al., 2004; Gionchetti et al., 2000).

The integrated metabolism of the BA pools is a good example of the complex transgenomic biochemical interactions between host and enteric microbiome symbionts (Turroni et al., 2008). Primary BAs, cholic acid (CA), and chenodeoxycholic acid (CDCA) in humans (beta-muricholic acid [β-MCA] in mice) are sterol compounds synthesized from cholesterol in the liver, conjugated with taurine and glycine, and then secreted into the small intestine. During the transit to the large intestine, primary BAs undergo deconjugation, oxidation of hydroxyl groups at C-3, C-7, and C-12, and 7α/β-dehydroxylation reactions mediated by enteric anaerobic bacteria enzymes, yielding secondary BAs such as deoxycholic acid (DCA), lithocholic acid (LCA), and β-muri-deoxycholicacid (βMDCA) (Begley et al., 2006; Lefebvre et al., 2009; Patel et al., 2010; Ridlon et al., 2006). Bile salt hydrolase (BSH) catalyzes the "gateway" reaction in the bacterial metabolism of conjugated BAs and is a prerequisite for the subsequent 7α/β-dehydroxylation (Batta et al., 1990; Jones et al., 2008). BA pool size and composition are strongly influenced by gut microbiota, and BA biotransformation has important biological consequences; indeed, BAs participate in the regulation of dietary lipid absorption and act as signaling molecules, modulating cholesterol and triglyceride metabolism and glucose and energy homeostasis (Houten et al., 2006; Ridlon et al., 2006).

Previous animal studies have reported that changes in BA signature were associated with both acute (i.e., antibiotic treatment) and chronic (germ-free condition) gut microbiota depletion

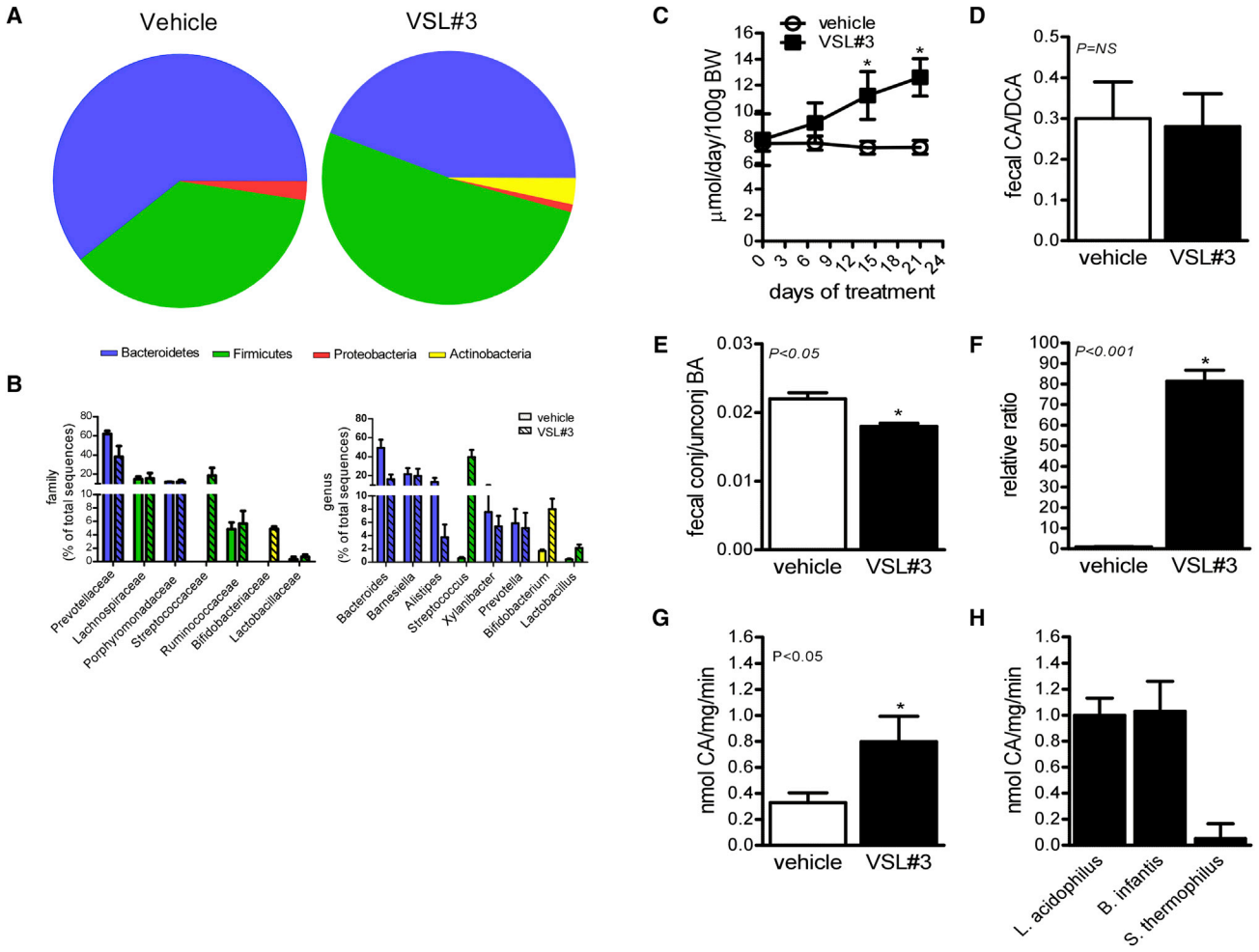

Figure 1. Colonization of Gut Microbiota with Probiotic Bacteria Enhances Fecal BA Excretion and BA Deconjugation

Phylum- (A) and family- and genus- (B) level modifications of fecal microbiota upon VSL#3 treatment are shown as mean percentage of the total sequences. (C) Mice receiving VSL#3 (filled squares) exhibited increased fecal BA excretion compared to vehicle-treated mice (open circles). Data points represent means ± SEM (n = 6 mice/group for each time point). Although the fecal CA/DCA ratio (D) was unchanged, the conjugated/unconjugated BA ratio (E) decreased in VSL#3 mice (black bars) compared to vehicle (white bars). These metabolic changes were associated with increased fecal BSH expression (F) and enzyme activity (G) in mice receiving probiotic bacteria with *L. acidophilus* and *B. infantis* displaying much higher BSH activity compared to *S. thermophilus* (H). An asterisk indicates significant differences between groups ($p < 0.05$).

(Claus et al., 2008; Martin et al., 2007; Wostmann, 1973; Swann et al., 2011) and linked to the development of diet-induced dysbiosis (Devkota et al., 2012). Although CA has been identified as a host factor regulating composition of cecal microbiota (Islam et al., 2011), recent mouse studies have suggested a direct connection between FXR function and gut microbiota (Sayin et al., 2013; Hu et al., 2014). Moreover, levels of tauro-β-muricholic acid (TβMCA) have recently been associated with alterations of the FXR signaling pathway together with *Lactobacillus* abundance and bile-acid-deconjugating activity in feces (Li et al., 2013). Nevertheless, it is unclear whether colonization of gut microbial community with lactic-acid-producing bacteria (namely probiotics) can modify BA pool size and composition thus influencing BA enterohepatic circulation. In this study, we provide evidence that probiotic modulation of gut microbiota composition increases fecal BA deconjugation and excretion along with induction of hepatic BA neosynthesis.

RESULTS

VSL#3 Administration Modifies Gut Microbiota while Enhancing BA Deconjugation and Fecal Excretion

We first explored the phylum-, family-, and genus-level modifications of gut microbiota upon VSL#3 mixture administration. The bacterial population of vehicle-treated mice in our animal facility was dominated by Bacteroidetes (60.1%) and Firmicutes (37%) with minor populations such as Proteobacteria (2.36%). Twenty-one day administration of VSL#3 resulted in a significantly higher abundance of Firmicutes (51.47%) and Actinobacteria (3.31%) at the expense of Bacteroidetes (44.22%) and Proteobacteria (1%) (Figure 1A). Significant expansion of the

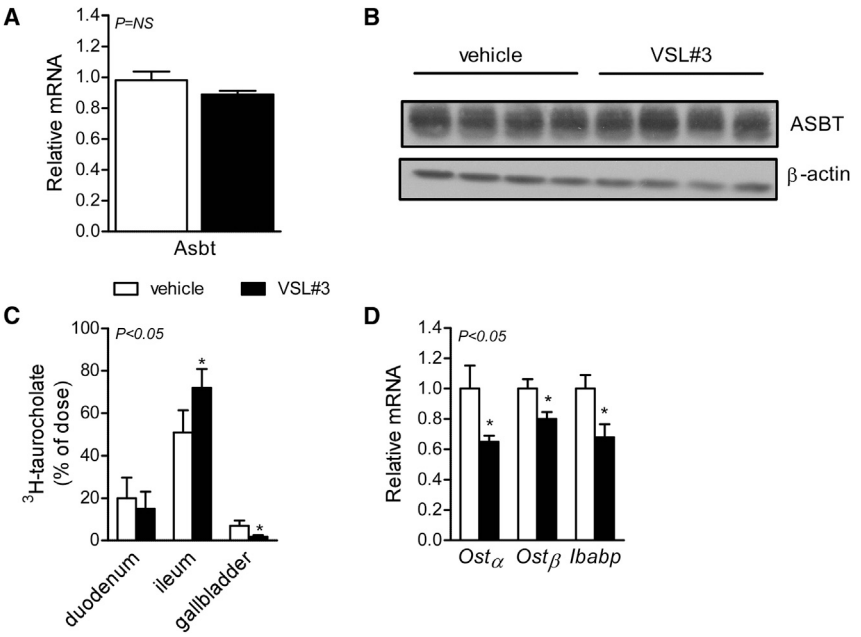

Figure 2. VSL#3 Probiotics Influence Ileal BA Absorption without Modifying Asbt Function

(A) ASBT mRNA.

(B) Protein levels.

(C) Metabolic flux study was performed to assess ileal [^{3}H]-TCA absorption in vehicle and VSL#3-treated mice.

(D) Ileal *Ostα*, *Ostβ*, and *Ibabp* expression level.

Data are expressed as means ± SEM (n = 6 mice/group). Cyclophillin was used as a housekeeping gene to normalize data and vehicle-treated mice were used as calibrators. An asterisk indicates significant differences between groups ($p < 0.05$).

Streptococcaceae, Bifidobacteriaceae, and Lactobacillaceae families, at the expense of Prevotellaceae, contributed to the higher abundance of Firmicutes and Actinobacteria (Figure 1B) in VSL#3-treated mice. Moreover, within the aforementioned families, the increased percentage of sequences attributed to the genus Streptococcus, Lactobacillus, and Bifidobacterium indicated that 21 day treatment with VSL#3 was sufficient to colonize gut microbiota with probiotic bacteria, as previously reported by Pagnini et al. (2010). Enteric bacterial enzymes shape BA pool size and composition by mediating deconjugation and 7α-dehydroxylation of primary BAs. To study the extent of BA biotransformation upon probiotic colonization, we measured fecal BA excretion and composition. VSL#3 administration enhanced fecal BA loss (Figure 1C) while leaving the fecal CA/DCA ratio unchanged (Figure 1D) and decreasing the fecal conjugated/unconjugated BA ratio (Figure 1E). These metabolic changes in fecal BA composition (Table S1) were accompanied by a significant increase of both BSH transcript (Figure 1F) and enzymatic activity (Figure 1G) in the feces collected from VSL#3-treated mice. The BA-deconjugating ability of probiotic bacteria, mostly Lactobacilli and Bifidobacteria, has been extensively documented (Begley et al., 2006; Tanaka et al., 1999; Tannock et al., 1989). To explore whether the increased fecal BSH activity measured in VSL#3-treated mice was conferred by the bacterial strains contained in the VSL#3 mixture, three representative strains, namely, *L. acidophilus*, *B. infantis*, and *S. thermophilus* were cultured and assessed for BSH activity. As shown in Figure 1H, BSH activity was detected in both *L. acidophilus* and *B. infantis* but not in *S. thermophilus*. Finally, it has been reported that probiotic strains do not exhibit 7α-dehydroxylase activity (Takahashi and Morotomi, 1994); accordingly, the fecal CA/DCA ratio was similar between treatment groups (Figure 1D).

VSL#3-Mediated Enhanced Fecal Loss Is Independent from Asbt

Enhanced fecal BA loss has been reported when ileal BA absorption is disrupted (Dawson et al., 2003) or in the case of so-called "malabsorption syndrome" as observed in humans (Oelkers et al., 1997). In order to exploit the molecular mechanism by which VSL#3-treated animals displayed higher levels of fecal BA, we measured the mRNA and protein levels of the apical sodium bile acid transporter (ASBT), responsible for the ileal BA uptake with a preference for conjugated over unconjugated BAs (Alrefai and Gill, 2007; Craddock et al., 1998). As shown in Figures 2A and 2B, no differences between vehicle and VSL#3-treated animals were detected in either *Asbt* mRNA or protein levels. We then hypothesized that, even in the presence of unchanged ASBT levels, VSL#3 treatment might modify intraluminal and transepithelial BA transport. As illustrated in Figure 2C, mice receiving VSL#3 displayed an increased intraluminal retention of radiolabeled [^{3}H]-TCA, which was paralleled by decreased absorption, serum disposal, and biliary secretion as shown by the lower radiolabeled content in the gallbladder. These findings were associated with an appreciable reduction in mRNA levels of bile acid transporters (organic solute transporter alpha and beta, *Ostα* and *Ostβ* and the ileal bile acid binding protein *Ibabp*) relevant to both ileal intracellular binding and basolateral secretion into portal circulation (Figure 2D).

VSL#3 Treatment Impacts BA Enterohepatic Circulation and Induces Cyp7a1 and Cyp8b1 Expression Levels in the Liver

Along with the VSL#3-mediated increase in fecal BA excretion, gallbladder BA and TCA/TβMCA ratio also increased (Figures 3A and 3B, Table S1), whereas no differences were measured in hepatic BA content (Figure 3C). Small intestine luminal BA content was higher upon VSL#3 treatment, presumably as a result of increased biliary output (Figure 3D). Finally, BA pool size and serum levels appeared unchanged (Figures 3E and 3F). It has been previously reported that enhanced fecal BA loss, either driven by ASBT genetic deletion or induced by BA sequestrant administration, is accompanied by enhanced hepatic BA neosynthesis (Dawson et al., 2003; Herrema et al., 2010; Jung et al., 2007; Out et al., 2011). As indicated in Figure 3G,

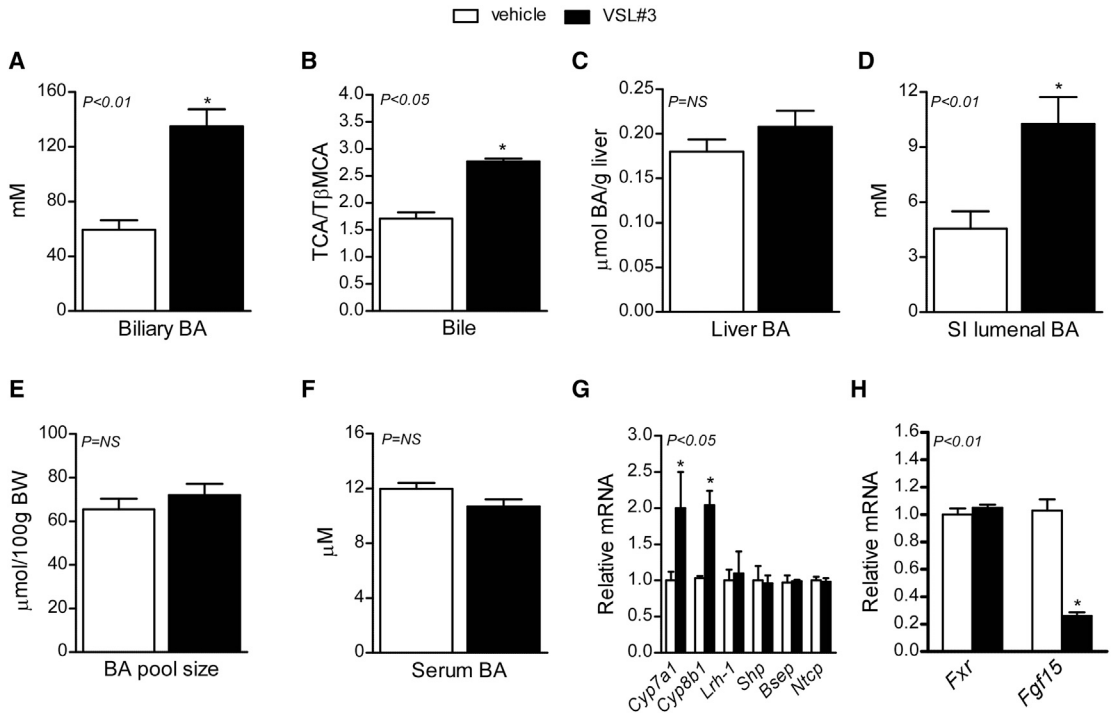

Figure 3. VSL#3 Probiotics Modify BA Homeostasis and Induce Hepatic BA Synthesis via Fxr-Fgf15 Axis Downregulation

(A–F) Bile acid (A) and TCA/TβMCA ratio (B) in gallbladder bile were increased upon VSL#3 treatment. BA content in liver (C), small intestine lumen (D), pool size (E), and serum (F).

(G and H) Hepatic (G) and ileal (H) gene expression profile in vehicle and VSL#3-treated mice.

Data are expressed as means ± SEM (n = 6 mice/group). Cyclophillin was used as a housekeeping gene to normalize data, and vehicle-treated mice were used as calibrators. An asterisk indicates significant differences between groups ($p < 0.05$ and $p < 0.01$). Bsep, bile salt export pump; Cyp7a1, cholesterol-7alpha-hydroxylase; Cyp8b1, sterol-12alpha-hydroxylase; Fgf15, fibroblast growth factor 15; Fxr, farnesoid X receptor; Lrh1, liver receptor homolog- 1; Ntcp, sodium-dependent taurocholic-cotransporting polypeptide; Shp, small heterodimer partner; TCA, tauro-cholic acid; TβMCA, tauro-β-muricholic acid.

VSL#3-treated mice exhibited increased mRNA levels of cholesterol-7α-hydroxylase (*Cyp7a1*) and sterol-12α-hydroxylase (*Cyp8b1*), rate-limiting enzymes in BA synthesis (Chiang, 2009; Jelinek et al., 1990; Schwarz et al., 2001). BA synthesis is tightly regulated, and the feedback suppression of *Cyp7a1* and *Cyp8b1* gene transcription by the farnesoid X receptor (*Fxr*) is the one of most important mechanisms in the maintenance of BA homeostasis (Sinal et al., 2000). Previous studies have shown that FXR activation is the major mechanism in BA synthesis suppression, inducing small heterodimer partner (*Shp*) and fibroblast growth factor 15/19 (*Fgf*15/19), which cooperate to suppress *Cyp7a1* and *Cyp8b1* (Goodwin et al., 2000; Inagaki et al., 2005; Lu et al., 2000). As shown in Figures 3G and 3H, *Cyp7a1* and *Cyp8b1* upregulation was driven by repression of the ileal FGF15 signal with no changes in *Shp* and liver receptor homolog-1 (*Lrh-1*) mRNA levels. These data underline that reduction in ileal BA absorption via VSL#3 decreased intracellular BA levels with downregulation of FXR transcriptional activity, as shown by reduction of mRNA levels of its target genes *Fgf15* (Figure 3H), *Ostα* and *Ostβ*, and *Ibabp* (Figure 2D). Moreover, our findings support the notion that deconjugated BA are less efficient at being absorbed in the ileum and activating intra-enterocyte FXR transcriptional machinery compared to conjugated ones, as previously reported by Parks et al. (1999). Of note, ileal *Fxr* transcript levels were unaffected by VSL#3 treatment. Finally, in agreement with no changes in BA pool size, hepatic bile salt export pump (*Bsep*) and sodium-dependent taurocholic-cotransporting polypeptide (*Ntcp*) mRNA levels were unaltered (Figure 3G).

The Functional FXR/Fgf15 Axis Is Required for VSL#3-Mediated Increase in Fecal BA Excretion while FXR Reactivation Normalizes the Phenotype

To fully characterize the role of FXR/FGF15 axis in probiotic-induced alterations of BA metabolism, we treated FXR-deficient mice with vehicle or VSL#3. As illustrated in Figures 4A and 4B, 21-day treatment with VSL#3 was sufficient to increase Firmicutes abundance (from 40.47% to 50.04%) at the expense of Bacteroidetes (from 57.86% to 46.9%) and to determine alterations at family and genus level similar to those found in wild-type animals (Figures 1A and 1B). Interestingly, enrichment of gut flora with lactic-acid-producing bacteria was able to lower colonic pH (from 6.9 to 6.3), as assessed in colonic luminal contents from VSL#3-treated animals (data not shown). To determine whether FXR deficiency would prevent VSL#3-induced BA metabolism modifications and to evaluate whether the VSL#3 phenotype would arise from reduced Fgf15 expression only (Figure 3H), we measured fecal BA levels in $FXR^{-/-}$ and

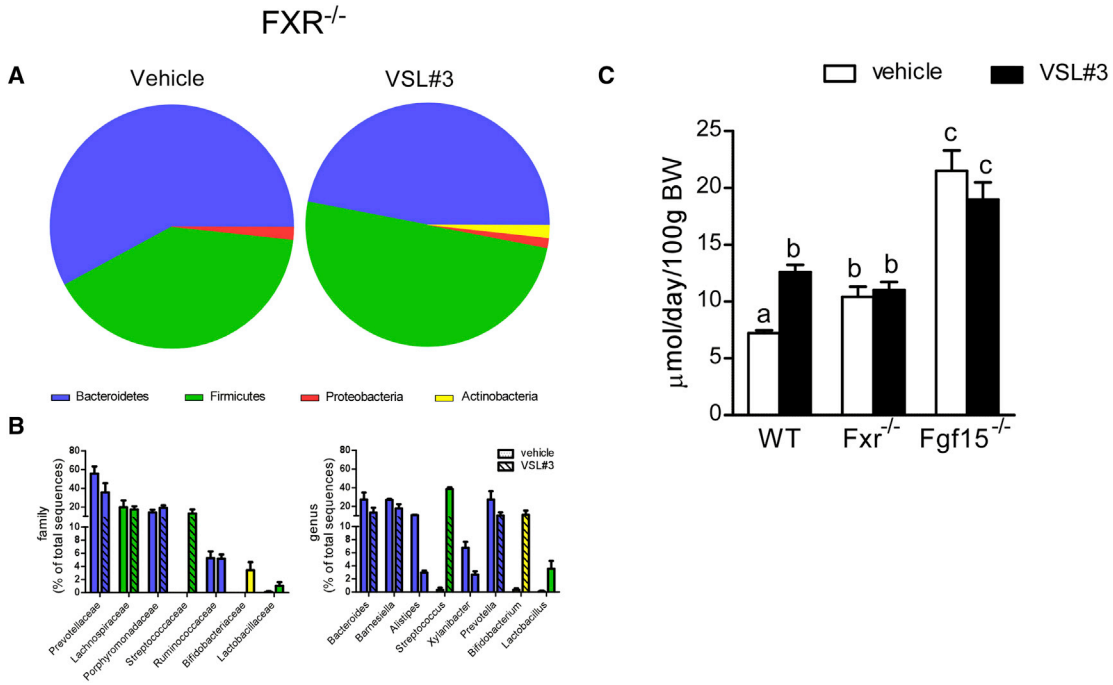

Figure 4. FXR and Fgf15 Deficiency Abolish VSL#3 Probiotic-Mediated Increase of Fecal BA Excretion

(A and B) Phylum- (A), family- and genus- (B) level modifications of fecal microbiota of FXR-deficient mice upon VSL#3 treatment are shown as a mean percentage of the total sequences. Data are expressed as means ± SEM (n = 6 mice/group).

(C) Fecal BA excretion in wild-type, FXR-, and Fgf15-deficient mice after 21 days of VSL#3 treatment.

Fgf15$^{-/-}$ mice that exhibited a constitutive elevated fecal BA excretion due to hepatic CYP7A1 derepression. In both FXR$^{-/-}$ and Fgf15$^{-/-}$ animals, fecal BA loss was similar between groups (Figure 4C). Of note, biliary and fecal BA composition (Table S1) appeared unmodified by VSL#3 probiotics upon FXR deficiency. Indeed, no changes in any hepatic or intestinal FXR target genes were detected in FXR$^{-/-}$ mice (Figures S1A and S1B). Collectively, the data suggest that a functional FXR transcriptional activity is required for VSL#3 probiotic administration to promote fecal BA excretion and induce hepatic BA neosynthesis. Finally, to test the hypothesis that reactivation of FXR transcriptional machinery would normalize the phenotype, we cotreated wild-type mice with a synthetic FXR agonist (GW4064) for 2 days prior to necropsy. As indicated in Figure S2, GW4064 was able to reverse VSL#3-induced alterations in BA levels in feces (Figure S2A) and intestinal lumen (Figure S2B), whereas leaving hepatic BA levels unchanged (Figure S2C).

DISCUSSION

Gut microbiota and BA metabolism are mutually linked, as changes in BA signature as well as modulation of the enterohepatic FXR/Fgf15/Cyp7a1 axis are found in germ-free, gnotobiotic, and antibiotic-treated animals (Claus et al., 2008; Martin et al., 2007; Wostmann, 1973; Swann et al., 2011; Sayin et al., 2013; Hu et al., 2014). Bacterial metabolism mediates primary BA deconjugation and subsequent conversion to secondary BAs, is partially responsible for BA fractional turnover rate, determines the amount of secondary BAs that may be detrimental on colonic epithelium architecture, and primes BA ability to function as efficient FXR ligands.

In this report, we show that colonization of gut flora with VSL#3 probiotics, widely employed in the therapeutic management of IBD, promotes increased BA deconjugation and fecal excretion. These events are associated with increased hepatic synthesis and biliary output and repression of the enterohepatic FXR/Fgf15 axis and, conversely, are reversed upon FXR agonist administration. We provide evidence that enrichment of gut flora with the BSH-retaining species (mostly Lactobacilli and Bifidobacteria) produces significant alterations in BA homeostasis only in the presence of a functional FXR transcriptional activity because VSL#3 phenotype is abrogated in both FXR$^{-/-}$ and Fgf15$^{-/-}$ animals. In agreement with our data, an earlier report suggested that TCA- or TβMCA-deconjugating bacterial strains promote an increased fecal BA loss (Chikai et al., 1987) in germ-free rats compared to those strains that deconjugate neither (i.e., *E. coli*). One may hypothesize that, by increasing BSH activity and, accordingly, unconjugated BA content in the intestinal lumen, VSL#3 administration may negatively influence BA reuptake thus negatively regulate intraenterocyte FXR transcriptional activity (Parks et al., 1999). Previous studies employing in situ perfused intestinal segments demonstrated that ileal bile acid transport is a high-capacity system accounting for reabsorption of biliary BA output and is the major route for conjugated BAs (Aldini et al., 1994; Marcus et al., 1991). In our study, *Asbt* mRNA and protein levels appeared unaltered by VSL#3 treatment, whereas, by performing a metabolic flux study using [^{3}H]-TCA, an increased intraluminal retention of radiolabeled compound

along with significantly reduced levels in the gallbladder (that might correspond to lower intracellular BA absorption and serum disposal) were measured. VSL#3 administration by limiting the availability of efficient FXR ligands (i.e., conjugated BAs) induces a repression of the FXR/Fgf15 axis thus upregulating the *Cyp7a1* and *Cyp8b1* genes. Moreover, the metabolic phenotype of VSL#3-treated mice is reminiscent of the findings of Herrema et al. (2010) (increases in both fecal BA excretion and hepatic synthesis with no change in BA pool size) in lean mice receiving the bile acid sequestrant colesevelam. More importantly, VSL#3-induced alterations of BA metabolism were abolished in FXR- and Fgf15-deficient mice thus suggesting that a functional FXR transcriptional activity is required for probiotic bacteria to promote fecal BA loss and induce hepatic BA synthesis.

Probiotic formulations are currently employed as therapeutic options in patients diagnosed with IBD, diarrhea, pouchitis, and ulcerative colitis. As variations in the gut microbial capacity for BA modification may be a significant factor in IBD onset or progression, the ability of VSL#3 to modulate BSH activity could be relevant in pathological conditions such as colitis, bacterial translocation secondary to intrahepatic cholestasis, or colon carcinogenesis.

In summary, the findings of this study expand our current knowledge of microbiota-induced changes of BA metabolism, providing evidence that VSL#3 administration enhances fecal BA excretion and hepatic BA synthesis and requires a functional FXR/Fgf15 enterohepatic axis.

EXPERIMENTAL PROCEDURES

Animals and Treatments

Wild-type C57BL/6J male mice were obtained from Charles River Laboratories. Wild-type C57BL/6J mice were treated daily with GW4064 (75 mg/kg/body weight, Sigma-Aldrich) or vehicle (arabic gum) by gavage for 2 days prior to necropsy. Pure strain C57BL/6J $FXR^{-/-}$ and C57BL/6J/129Sv $Fgf15^{-/-}$ male mice and their wild-type littermates were kindly provided by Drs. D.J. Mangelsdorf and S.A. Kliewer (UT Southwestern Medical Center, Dallas, TX). Mice were housed in a temperature-controlled room (22°C–23°C) under a 12 hr light/12 hr dark cycle and fed standard rodent chow and water ad libitum. Mice were given a daily oral gavage of saline or VSL#3 mixture (50×10^9 cfu/day) for 21 days, and fecal samples were collected to measure fecal bile acid excretion, bacterial gene expression, and enzymatic activity. Total DNA isolated from feces was used as a template for the amplification of the 16S rRNA V5-V6 region, and subjected to GS-FLX multiplex pyrosequencing to measure the relative abundance at phylum, family, and genus level between saline- and VSL#3-treated animals as previously described (De Filippo et al., 2010). After 21 day treatment, the mice were sacrificed, and tissues were weighed, snap-frozen in liquid nitrogen, and stored at −80°C until processing. All experiments were approved by the Ethical Committee of the Fondazione Mario Negri Sud (Chieti, Italy) and certified by the Italian Ministry of Health in accordance with internationally accepted guidelines for animal care.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.02.032.

ACKNOWLEDGMENTS

We thank Nicola Celli, Carmine Di Filippo, and Giuseppe Di Tullio for technical assistance. We thank Drs. Steven A. Kliewer and David J. Mangelsdorf (UT Southwestern, USA) for supplying FXR- and FGF15-null mice, Dr. Paul Dawson (Wake Forest University, USA) for providing ASBT mouse antibody, and Dr. Claudio De Simone for providing VSL#3 probiotic formulation. The work was funded by the Italian Association for Cancer Research (AIRC, IG 14732), the Italian Ministry of University and Education (Finanziamenti per la Ricerca di Base IDEAS RBID08C9N7; PRIN2010FHH32M-002), the Italian Ministry of Health (Young Researchers Grant GR-2008-1143546; GR-2010-2314703), and the University of Bari (IDEA GRBA0802SJ-2008). S.M. is a fellow of CariSPAQ (L'Aquila, Italy). S.R. is a fellow of the University of L'Aquila.

Received: August 22, 2012
Revised: January 16, 2014
Accepted: February 22, 2014
Published: March 20, 2014

REFERENCES

Aldini, R., Roda, A., Montagnani, M., Polimeni, C., Lenzi, P.L., Cerre, C., Galletti, G., and Roda, E. (1994). Hepatic uptake and intestinal absorption of bile acids in the rabbit. Eur. J. Clin. Invest. *24*, 691–697.

Alrefai, W.A., and Gill, R.K. (2007). Bile acid transporters: structure, function, regulation and pathophysiological implications. Pharm. Res. *24*, 1803–1823.

Batta, A.K., Salen, G., Arora, R., Shefer, S., Batta, M., and Person, A. (1990). Side chain conjugation prevents bacterial 7-dehydroxylation of bile acids. J. Biol. Chem. *265*, 10925–10928.

Begley, M., Hill, C., and Gahan, C.G. (2006). Bile salt hydrolase activity in probiotics. Appl. Environ. Microbiol. *72*, 1729–1738.

Chiang, J.Y. (2009). Bile acids: regulation of synthesis. J. Lipid Res. *50*, 1955–1966.

Chikai, T., Nakao, H., and Uchida, K. (1987). Deconjugation of bile acids by human intestinal bacteria implanted in germ-free rats. Lipids *22*, 669–671.

Claus, S.P., Tsang, T.M., Wang, Y., Cloarec, O., Skordi, E., Martin, F.P., Rezzi, S., Ross, A., Kochhar, S., Holmes, E., and Nicholson, J.K. (2008). Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. Mol. Syst. Biol. *4*, 219.

Craddock, A.L., Love, M.W., Daniel, R.W., Kirby, L.C., Walters, H.C., Wong, M.H., and Dawson, P.A. (1998). Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter. Am. J. Physiol. *274*, G157–G169.

Dawson, P.A., Haywood, J., Craddock, A.L., Wilson, M., Tietjen, M., Kluckman, K., Maeda, N., and Parks, J.S. (2003). Targeted deletion of the ileal bile acid transporter eliminates enterohepatic cycling of bile acids in mice. J. Biol. Chem. *278*, 33920–33927.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G., and Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Natl. Acad. Sci. USA *107*, 14691–14696.

Devkota, S., Wang, Y., Musch, M.W., Leone, V., Fehlner-Peach, H., Nadimpalli, A., Antonopoulos, D.A., Jabri, B., and Chang, E.B. (2012). Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature *487*, 104–108.

Gareau, M.G., Sherman, P.M., and Walker, W.A. (2010). Probiotics and the gut microbiota in intestinal health and disease. Nat. Rev. Gastroenterol. Hepatol. *7*, 503–514.

Gionchetti, P., Rizzello, F., Venturi, A., Brigidi, P., Matteuzzi, D., Bazzocchi, G., Poggioli, G., Miglioli, M., and Campieri, M. (2000). Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology *119*, 305–309.

Goodwin, B., Jones, S.A., Price, R.R., Watson, M.A., McKee, D.D., Moore, L.B., Galardi, C., Wilson, J.G., Lewis, M.C., Roth, M.E., et al. (2000). A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol. Cell *6*, 517–526.

Guarner, F., and Malagelada, J.R. (2003). Gut flora in health and disease. Lancet *361*, 512–519.

Hamer, H.M., De Preter, V., Windey, K., and Verbeke, K. (2012). Functional analysis of colonic bacterial metabolism: relevant to health? Am. J. Physiol. Gastrointest. Liver Physiol. *302*, G1–G9.

Herrema, H., Meissner, M., van Dijk, T.H., Brufau, G., Boverhof, R., Oosterveer, M.H., Reijngoud, D.J., Müller, M., Stellaard, F., Groen, A.K., and Kuipers, F. (2010). Bile salt sequestration induces hepatic de novo lipogenesis through farnesoid X receptor- and liver X receptor alpha-controlled metabolic pathways in mice. Hepatology *51*, 806–816.

Houten, S.M., Watanabe, M., and Auwerx, J. (2006). Endocrine functions of bile acids. EMBO J. *25*, 1419–1425.

Hu, X., Bonde, Y., Eggertsen, G., and Rudling, M. (2014). Muricholic bile acids are potent regulators of bile acid synthesis via a positive feedback mechanism. J. Intern. Med. *275*, 27–38.

Inagaki, T., Choi, M., Moschetta, A., Peng, L., Cummins, C.L., McDonald, J.G., Luo, G., Jones, S.A., Goodwin, B., Richardson, J.A., et al. (2005). Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab. *2*, 217–225.

Islam, K.B., Fukiya, S., Hagio, M., Fujii, N., Ishizuka, S., Ooka, T., Ogura, Y., Hayashi, T., and Yokota, A. (2011). Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology *141*, 1773–1781.

Jelinek, D.F., Andersson, S., Slaughter, C.A., and Russell, D.W. (1990). Cloning and regulation of cholesterol 7 alpha-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. J. Biol. Chem. *265*, 8190–8197.

Jijon, H., Backer, J., Diaz, H., Yeung, H., Thiel, D., McKaigney, C., De Simone, C., and Madsen, K. (2004). DNA from probiotic bacteria modulates murine and human epithelial and immune function. Gastroenterology *126*, 1358–1373.

Jones, B.V., Begley, M., Hill, C., Gahan, C.G., and Marchesi, J.R. (2008). Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. Proc. Natl. Acad. Sci. USA *105*, 13580–13585.

Jung, D., Inagaki, T., Gerard, R.D., Dawson, P.A., Kliewer, S.A., Mangelsdorf, D.J., and Moschetta, A. (2007). FXR agonists and FGF15 reduce fecal bile acid excretion in a mouse model of bile acid malabsorption. J. Lipid Res. *48*, 2693–2700.

Kinross, J.M., von Roon, A.C., Holmes, E., Darzi, A., and Nicholson, J.K. (2008). The human gut microbiome: implications for future health care. Curr. Gastroenterol. Rep. *10*, 396–403.

Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., and Staels, B. (2009). Role of bile acids and bile acid receptors in metabolic regulation. Physiol. Rev. *89*, 147–191.

Leser, T.D., and Mølbak, L. (2009). Better living through microbial action: the benefits of the mammalian gastrointestinal microbiota on the host. Environ. Microbiol. *11*, 2194–2206.

Li, F., Jiang, C., Krausz, K.W., Li, Y., Albert, I., Hao, H., Fabre, K.M., Mitchell, J.B., Patterson, A.D., and Gonzalez, F.J. (2013). Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. Nat. Commun. *4*, 2384.

Lu, T.T., Makishima, M., Repa, J.J., Schoonjans, K., Kerr, T.A., Auwerx, J., and Mangelsdorf, D.J. (2000). Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol. Cell *6*, 507–515.

Marcus, S.N., Schteingart, C.D., Marquez, M.L., Hofmann, A.F., Xia, Y., Steinbach, J.H., Ton-Nu, H.T., Lillienau, J., Angellotti, M.A., and Schmassmann, A. (1991). Active absorption of conjugated bile acids in vivo. Kinetic parameters and molecular specificity of the ileal transport system in the rat. Gastroenterology *100*, 212–221.

Martin, F.P., Dumas, M.E., Wang, Y., Legido-Quigley, C., Yap, I.K., Tang, H., Zirah, S., Murphy, G.M., Cloarec, O., Lindon, J.C., et al. (2007). A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. Mol. Syst. Biol. *3*, 112.

Muegge, B.D., Kuczynski, J., Knights, D., Clemente, J.C., González, A., Fontana, L., Henrissat, B., Knight, R., and Gordon, J.I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science *332*, 970–974.

Oelkers, P., Kirby, L.C., Heubi, J.E., and Dawson, P.A. (1997). Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). J. Clin. Invest. *99*, 1880–1887.

Out, C., Hageman, J., Bloks, V.W., Gerrits, H., Sollewijn Gelpke, M.D., Bos, T., Havinga, R., Smit, M.J., Kuipers, F., and Groen, A.K. (2011). Liver receptor homolog-1 is critical for adequate up-regulation of Cyp7a1 gene transcription and bile salt synthesis during bile salt sequestration. Hepatology *53*, 2075–2085.

Pagnini, C., Saeed, R., Bamias, G., Arseneau, K.O., Pizarro, T.T., and Cominelli, F. (2010). Probiotics promote gut health through stimulation of epithelial innate immunity. Proc. Natl. Acad. Sci. USA *107*, 454–459.

Parks, D.J., Blanchard, S.G., Bledsoe, R.K., Chandra, G., Consler, T.G., Kliewer, S.A., Stimmel, J.B., Willson, T.M., Zavacki, A.M., Moore, D.D., and Lehmann, J.M. (1999). Bile acids: natural ligands for an orphan nuclear receptor. Science *284*, 1365–1368.

Patel, A.K., Singhania, R.R., Pandey, A., and Chincholkar, S.B. (2010). Probiotic bile salt hydrolase: current developments and perspectives. Appl. Biochem. Biotechnol. *162*, 166–180.

Ridlon, J.M., Kang, D.J., and Hylemon, P.B. (2006). Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. *47*, 241–259.

Round, J.L., and Mazmanian, S.K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. *9*, 313–323.

Sayin, S.I., Wahlström, A., Felin, J., Jäntti, S., Marschall, H.U., Bamberg, K., Angelin, B., Hyötyläinen, T., Oresic, M., and Bäckhed, F. (2013). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab. *17*, 225–235.

Schwarz, M., Russell, D.W., Dietschy, J.M., and Turley, S.D. (2001). Alternate pathways of bile acid synthesis in the cholesterol 7alpha-hydroxylase knockout mouse are not upregulated by either cholesterol or cholestyramine feeding. J. Lipid Res. *42*, 1594–1603.

Sinal, C.J., Tohkin, M., Miyata, M., Ward, J.M., Lambert, G., and Gonzalez, F.J. (2000). Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell *102*, 731–744.

Swann, J.R., Want, E.J., Geier, F.M., Spagou, K., Wilson, I.D., Sidaway, J.E., Nicholson, J.K., and Holmes, E. (2011). Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc. Natl. Acad. Sci. USA *108* (*Suppl 1*), 4523–4530.

Takahashi, T., and Morotomi, M. (1994). Absence of cholic acid 7 alpha-dehydroxylase activity in the strains of Lactobacillus and Bifidobacterium. J. Dairy Sci. *77*, 3275–3286.

Tanaka, H., Doesburg, K., Iwasaki, T., and Mierau, I. (1999). Screening of lactic acid bacteria for bile salt hydrolase activity. J. Dairy Sci. *82*, 2530–2535.

Tannock, G.W., Dashkevicz, M.P., and Feighner, S.D. (1989). Lactobacilli and bile salt hydrolase in the murine intestinal tract. Appl. Environ. Microbiol. *55*, 1848–1851.

Tsai, F., and Coyle, W.J. (2009). The microbiome and obesity: is obesity linked to our gut flora? Curr. Gastroenterol. Rep. *11*, 307–313.

Turroni, F., Ribbera, A., Foroni, E., van Sinderen, D., and Ventura, M. (2008). Human gut microbiota and bifidobacteria: from composition to functionality. Antonie van Leeuwenhoek *94*, 35–50.

Wostmann, B.S. (1973). Intestinal bile acids and cholesterol absorption in the germfree rat. J. Nutr. *103*, 982–990.