



The Role of the Gut Microbiota in Bile Acid Metabolism

Oscar Ramírez-Pérez, Vania Cruz-Ramón, Paulina Chinchilla-López, Nahum Méndez-Sánchez.

Liver Research Unit, Medica Sur Clinic & Foundation, Mexico City, Mexico.

ABSTRACT

The gut microbiota has been considered a cornerstone of maintaining the health status of its human host because it not only facilitates harvesting of nutrients and energy from ingested food, but also produces numerous metabolites that can regulate host metabolism. One such class of metabolites, the bile acids, are synthesized from cholesterol in the liver and further metabolized by the gut microbiota into secondary bile acids. These bioconversions modulate the signaling properties of bile acids through the nuclear farnesoid X receptor and the G protein-coupled membrane receptor 5, which regulate diverse metabolic pathways in the host. In addition, bile acids can regulate gut microbial composition both directly and indirectly by activation of innate immune response genes in the small intestine. Therefore, host metabolism can be affected by both microbial modifications of bile acids, which leads to altered signaling via bile acid receptors, and by alterations in the composition of the microbiota. In this review, we mainly describe the interactions between bile acids and intestinal microbiota and their roles in regulating host metabolism, but we also examine the impact of bile acid composition in the gut on the intestinal microbiome and on host physiology.

Key words. Bile acids. Gut microbiota. Dysbiosis. Health and disease.

INTRODUCTION

The human intestinal tract harbors a diverse and complex microbial community that plays a key role in human health. It has been estimated that our gut contains more than 1,000 phylotypes that contain 100-fold more genes than are found in the human genome.¹ This gut microbiota (GM) is composed of bacteria, archaea, viruses, and fungi, which are divided into six divisions/phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Acinetobacteria*, *Fusobacteria*, and *Verrucomicrobia*.^{2,3} The vast majority of bacteria comprising the GM are obligate anaerobes with lesser numbers of facultative anaerobes, archaea, and yeast species. *Firmicutes* and *Bacteroidetes* make up more than 90% of the overall GM. The most frequent genera of obligate anaerobes include *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus*, and *Ruminococcus*. The genera of facultative anaerobic bacte-

ria include *Escherichia*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, and *Proteus*.^{4,5}

THE MARRIAGE: IN SICKNESS AND IN HEALTH

It is well established that a healthy GM is important for the overall health of the host, because the composition of the GM has an immense impact on human well-being, including host metabolism, physiology, nutrition, and immune function.⁶ The composition and function of the GM differs according to geographical location, age, sex, and the mother's microbiota. The simple community of microbes present at birth gradually develops into a diverse ecosystem during host growth. Over time, many host-bacterial associations have developed into beneficial relationships.⁷ Symbiotic bacteria metabolize indigestible compounds, supply essential nutrients, defend against colonization by

opportunistic pathogens, and contribute to the formation of the intestinal architecture.⁸ Some studies have shown that a number of factors play a role in shaping the normal GM, including the mode of delivery at birth (cesarean or vaginal), diet during infancy (breast milk or formula) and in adulthood (meat based or vegan/vegetarian), plus the use or presence of antibiotic-like molecules derived from the environment or the gut commensal community.

In contrast, an imbalance in the equilibrium of the GM (dysbiosis) can predispose to a range of different types of diseases at different ages, ranging from allergies in childhood to inflammatory bowel disease (IBD) in young adults.⁹ Dysbiosis can result from exposure to diverse environmental factors, including diet, drugs, toxins, and pathogens, and is negatively associated with the host's health, leading to host susceptibility to diseases such as diabetes, IBD, and metabolic syndrome.¹⁰

GUT MICROBIOTA

It has long been known that humans are colonized by a range of microorganisms, most of which are present in the intestinal tract where they form a complex microbial community known as the intestinal or gut microbiota (GM).¹¹ The establishment of the GM occurs during childhood, and the nature of the microbiota in the human intestine during the early stages of life plays a key role in the maturation and modulation of the host immune system and in the promotion of various physiological processes in the human intestine, including the regulation of intestinal barrier integrity and the secretion of mucus.¹²⁻¹⁴ In adult life, the GM performs a variety of functions for the maintenance of human health, for example, assisting in food degradation, releasing nutrients, promoting the dif-

ferentiation of certain host tissues, reducing the risk of intestinal colonization by pathogens, and modulating the immune system.¹⁵

BILE ACIDS AND GUT MICROBIOTA

Bile acids (BA) are amphipathic molecules synthesized in the liver from cholesterol, which are stored in the gallbladder and released into the small intestine after food intake¹⁶ (Figure 1). They have many fundamental roles but one of their major functions is to facilitate the emulsification of dietary fats and to assist the intestinal absorption of lipids and lipophilic vitamins.¹⁷ Recently, it has been recognized that BA are signaling molecules for a variety of activities mediated through the farnesoid X receptor (FXR) and the G protein-coupled membrane receptor 5 (TGR5). These receptors mediate the signaling cascade and activate expression of genes involved in the metabolism of BA, lipids, and carbohydrates and in energy expenditure and inflammation, predominantly in enterohepatic tissues but also in peripheral organs.^{18,19}

The human BA pool consists of the primary colic and chenodeoxycholic acids and the secondary deoxycholic and lithocolic acids.²⁰ It is known that synthesis of BA is a multi-step process that involves diverse enzymes located in the endoplasmic reticulum, mitochondria, cytosol, and peroxisomes of cells. BA synthesis from cholesterol can occur via two pathways: the classical pathway, which occurs in hepatocytes and is known as the neutral route, and the alternative pathway, which occurs in the gut.²¹ The classical pathway mediates synthesis of primary BA and represents more than 90% of BA synthesis, which is why it is considered the main route of BA synthesis.²² The alternative way is responsible for synthesis of secondary BA

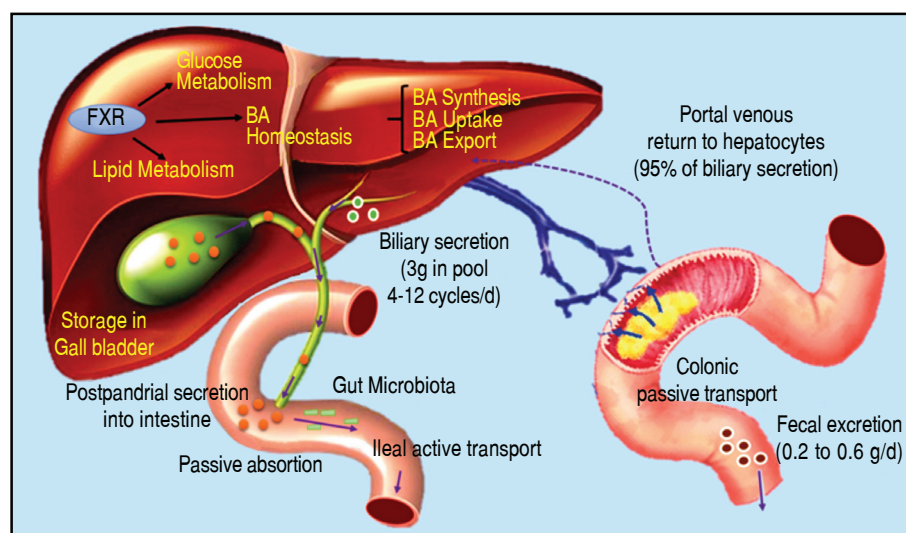


Figure 1. Bile acid synthesis and enterohepatic circulation. The human bile acid (BA) pool consists of approximately 3 g of BA. Food intake stimulates the gallbladder to release BA into the small intestine. Humans produce on average about 0.5 g BA per day by synthesis in the liver, and secrete approximately 0.5 g/day. Conjugated BA are efficiently reabsorbed from the ileum by active transport, and a small amount of unconjugated BA is reabsorbed by passive diffusion in the small and large intestines. The first-pass extraction of BA from the portal blood by the liver is very efficient.

and mediates less than 10% of BA synthesis under normal physiological conditions.²³

In this context, it is important to review the enterohepatic circulation of BA to understand the key role of these molecules. Conjugated BA are secreted across the canalicular membrane into the bile and stored in the gall bladder. After a meal, the duodenum secretes cholecystokinin, which stimulates the contraction of the gallbladder and thereby releases BA into the intestinal tract. Within the small intestine, micellar BA act as effective detergents to facilitate the solubilization of monoacylglycerols and fatty acids, and the digestion and absorption of dietary lipids and fat-soluble vitamins. Finally, BA are reabsorbed in the ileum and conveyed back to the liver through the portal blood for re-secretion into the bile.²⁴⁻²⁸

Hepatic BA transport requires active transport systems because BA cannot cross the hepatocyte membrane. Most circulating BA are taken up by hepatocytes via Na⁺-dependent cotransport systems. The Na⁺-dependent taurocholate transporter has been identified as the major BA uptake transporter in the basolateral membrane of hepatocytes.²⁹ The results of studies in mice by Fretland, *et al.*³⁰ suggested a role for the microsomal epoxide hydrolase (mEH) in regulating basolateral Na-dependent BA uptake, however, a study by Zhu, *et al.* demonstrated that a point mutation that resulted in significantly decreased mEH expression in a human individual led to hypercholanemia, a condition in which BA levels are increased in plasma in the absence of hepatocyte injury, suggesting impaired basolateral BA uptake rather than intrahepatic BA accumulation.³¹ It has been estimated that more than 25% of BA uptake by hepatocytes is regulated through Na⁺-independent transporters (Organic anion transporters: OATP1A2, OATP1B1 and OATP1B3).³² This pathway is primarily responsible for the uptake of unconjugated BA.

However, FXR, a nuclear receptor activated by BA, plays an important role in BA homeostasis by controlling the expression of genes for proteins including the nuclear receptor small heterodimer partner.³³

Equally importantly, it has been observed that the GM is involved in the biotransformation of BA through deconjugation, dehydroxylation, and re-conjugation of these molecules.³⁴ Moreover, it has been recognized that BA has antimicrobial activity that can damage bacterial cell membranes and thus inhibit bacterial overgrowth.³⁵ BA can also regulate the overgrowth and composition of the intestinal microbiota through FXR and TGR-5 to protect the liver and intestine against inflammation³⁶ (Figure 2). A study in humans by David, *et al.* showed that an animal-based diet rapidly altered the GM, increasing the number of bile-tolerant microorganisms (*B. wadsworthia* and *Bacteroides*) and decreasing the number of Firmicutes.³⁷ The results of this study suggested a relationship between dietary fat, BA, and the overgrowth of microorganisms in IBDs such as Crohn's disease.³⁸

GUT MICROBIOTA, OBESITY, AND BARIATRIC SURGERY

A healthy GM is crucial for normal metabolic function and host homeostasis. Alterations in the composition of the GM may be related to obesity by modifying the reservoir metabolism and the mechanism of appetite.³⁹ Some studies have shown a close relationship between obesity and the composition of the GM. In 2005, Ley, *et al.* demonstrated that metabolic dysfunction was related to changes in the *Bacteroidetes/Firmicutes* ratio. They analyzed 5,088 bacterial 16S rRNA gene sequences from the distal intestinal microbiota of genetically obese ob/ob mice, lean ob/+ and wild-type siblings, and their ob/+ mothers, all fed with a

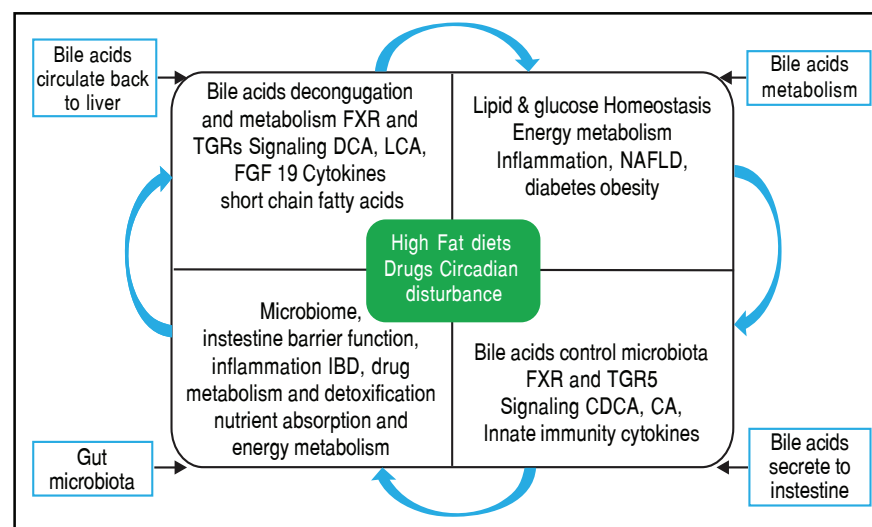


Figure 2. Bidirectional interactions between bile acid synthesis and gut microbiota. The relationship between bile acids and the gut microbiota is close and complementary. Bile acids control gut bacteria overgrowth and protect against inflammation while the gut microbiota plays a role in biotransformation of bile acids and affects bile acid composition and metabolism via Farnesoid X Receptor and G protein-coupled membrane receptor 5 signaling in the liver.

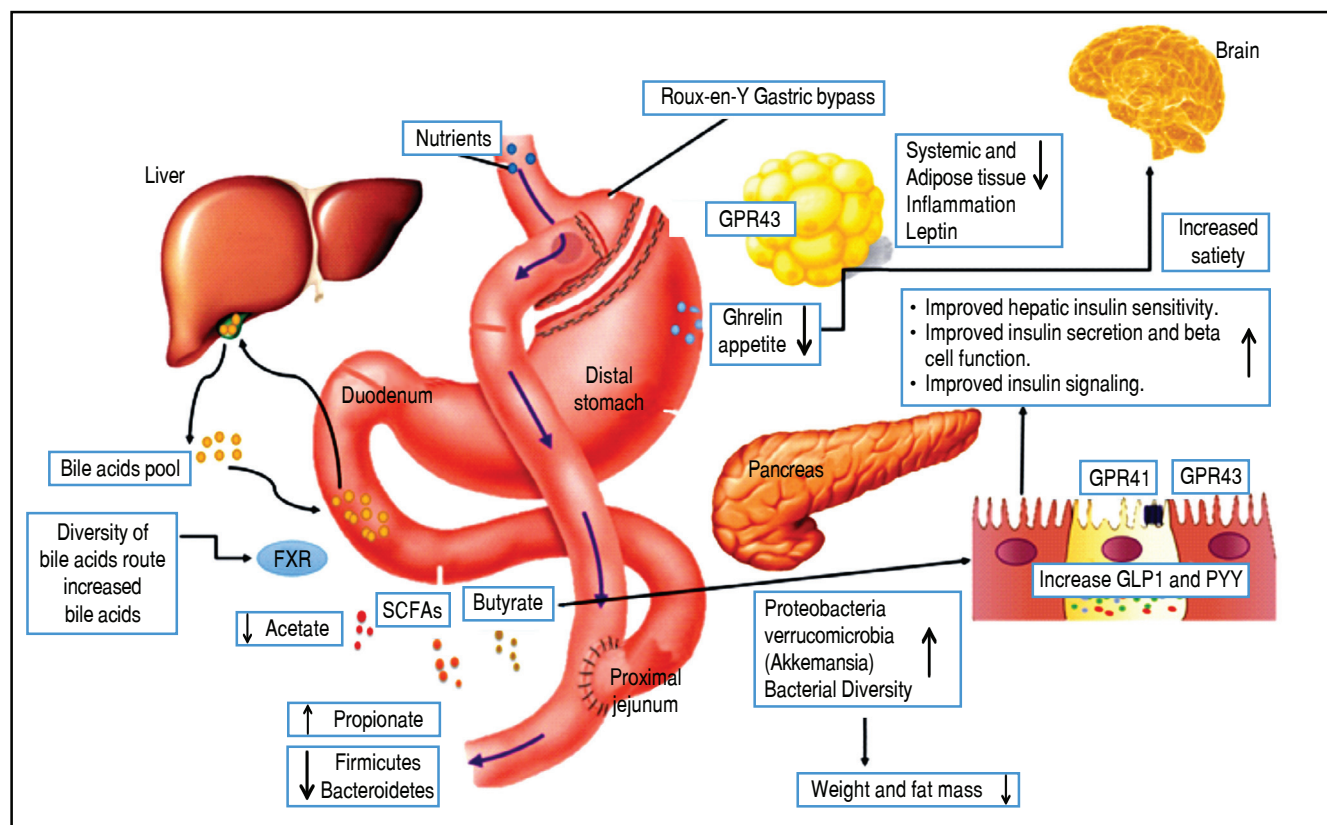


Figure 3. Effects of Roux-en-Y Gastric Bypass surgery on the gut microbiota and its metabolic outcomes. RYGB induces various environmental, systemic, and anatomical changes that might directly or indirectly affect the composition of the gut microbiota.

polysaccharide-rich diet. They observed that compared the lean mice, regardless of lineage, the ob/ob animals had a 50% reduction in the amount of *Bacteroidetes* and a proportional increase in *Firmicutes*. Based on these results, they suggested that obesity could affect the diversity of the GM.⁴⁰ In contrast, a study in humans by Walter, *et al.* concluded that the strong relationship between microbiome changes and obesity that is observed in mice does not apply in humans, because no significant differences in the *Bacteroidetes/Firmicutes* ratio were observed between obese and non-obese individuals.³⁸

However, there are several well-described interactions between the GM and the host. One of these is the gut-brain axis, which indirectly influences the nature of the commensal organisms, gastrointestinal motility and secretion, and intestinal permeability, and directly, via signaling molecules released into the gut lumen from cells in the lamina propria, modifying levels of plasma peptides: mainly glucagon-like peptide 1 (GLP-1) and peptide YY (PYY).⁴¹⁻⁴³ Analysis of the importance of these hormones has focused on obese patients undergoing bariatric surgery, who show increased levels of GLP-1 and PYY post-prandially. Significantly, inhibiting the PYY and GLP-1

responses resulted in the return of appetite and increased food intake. Therefore, it is likely that elevated levels of PYY and GLP-1 play a key role in the sustained weight loss observed following gastric bypass surgery.⁴⁴⁻⁴⁷

Bariatric surgery, a range of minimally invasive laparoscopy procedures used to treat severe cases of obesity including gastric banding, Roux-en-Y gastric bypass (RYGB), gastric sleeve, and biliopancreatic diversion,⁴⁸⁻⁴⁹ has shown success in changing the GM, resulting in a greater abundance of *Gammaproteobacteria* and *Verrucomicrobia* (Akkermansia) together with a reduced abundance of *Firmicutes*^{50,51} and importantly, increased GLP-1 and PYY levels. These are promising results for the use of bariatric surgery to treat obese patients and their metabolic complications; however, more studies are necessary to assess the safety of these procedures^{52,54} (Figure 3).

CONCLUSION

The GM can be modified by age, diet, drugs, and disease. BAs appear to be a principal regulator of the GM. In addition, the size of the BA pool has been shown to be a function of microbial metabolism in the intestines, al-

though the majority of these studies have been performed in mice. Consequently, there is a lack of evidence for this link in humans, which makes it clear that further studies are necessary to identify new therapeutic targets for maintaining human intestinal health.

ABBREVIATIONS

- **BA:** bile acids.
- **FXR:** Farnesoid X Receptor.
- **GLP-1:** glucagon-like peptide 1.
- **GM:** gut microbiota.
- **IBD:** inflammatory bowel disease.
- **mEH:** microsomal epoxide hydrolase.
- **OATP:** Organic anion transporter.
- **PYY:** peptide YY.
- **RYGB:** Roux-en-Y gastric bypass.
- **TGR5:** G protein-coupled membrane receptor 5.

REFERENCES

1. Qin J, Li R, Raes JA, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; 7285: 59-65.
2. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev* 2010; 3: 859-904.
3. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; 4: 837-48.
4. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; 7402: 207-14.
5. Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 2014; 3: 332-8.
6. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol* 2015; 29: 8787-803.
7. Rogier EW, Frantz AL, Bruno ME, Wedlund L, Cohen DA, Stromberg AJ, Kaetzel CS. Lessons from mother: long-term impact of antibodies in breast milk on the gut microbiota and intestinal immune system of breastfed offspring. *Gut Microbes* 2014; 5: 663-8.
8. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; 5: 313-23.
9. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, et al. Human gut microbiome viewed across age and geography. *Nature* 2012; 486: 222-7.
10. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and non-alcoholic fatty liver disease. *Gastroenterology* 2017; 7: 1679-94.
11. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature* 2012; 489: 220-30.
12. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li HL, DLieber A, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Sci Transl Med* 2016; 343: 343-82.
13. Yassour M, Vatanen T, Siljander H, Hamalainen AM, Harkonen T, Ryhanen SJ, Franzosa EA, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med* 2016; 343: 343-81.
14. Macpherson AJ, de Agüero MG, Ganai-Vonarburg SC. How nutrition and the maternal microbiota shape the neonatal immune system. *Nat Rev Immunol* 2017; 8: 508-17.
15. Planer JD, Peng Y, Kau AL, Blanton LV, Ndao IM, Tarr PI, Wagner BB, et al. Development of the gut microbiota and mucosal IgA responses in twins and gnotobiotic mice. *Nature* 2016; 7606: 263-6.
16. Shiffka SJ, Kane MA, Swaan PW. Planar bile acids in health and disease. *Biochim Biophys Acta* 2017; 11: 2269-76.
17. Begley M, Sleator RD, Gahan CG, Hill C. Contribution of three bile-associated loci, bsh, pva and btlB, to gastrointestinal persistence and bile tolerance of listeria monocytogenes. *Infect Immun* 2005; 2: 894-904.
18. Cariou B, Harmelen KV, Duran-Sandoval D. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. *J Biol Chem* 2006; 281: 11039-49.
19. Abdelkarim M, Caron S, Duhem C. The farnesoid X receptor regulates adipocyte differentiation and function by promoting peroxisome proliferator-activated receptor-gamma and interfering with the Wnt/beta-catenin pathways. *J Biol Chem* 2010; 285: 36759-67.
20. Long SL, Gahan CGM, Joyce SA. Interactions between gut bacteria and bile in health and disease. *Mol Aspects Med* 2017; 56: 54-65.
21. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; 72: 137-74.
22. Arrese M, Trauner M, Sacchiero RJ, Crossman MW, Shneider BL. Neither intestinal sequestration of bile acids nor common bile duct ligation modulate the expression and function of the rat ileal bile acid transporter. *Hepatology* 1998; 28: 1081-7.
23. Axelsson M, Aly A, Sjövall J. Levels of 7 alpha-hydroxy-4-cholesten-3-one in plasma reflect rates of bile acid synthesis in man. *FEBS Lett* 1988; 239: 324-8.
24. Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev* 2003; 83: 633-71.
25. Duez H, van der Veen JN, Duhem C, Pourcet B, Touvier T, Fontaine C, Derudas B, et al. Regulation of bile acid synthesis by the nuclear receptor Rev-erbalpha. *Gastroenterology* 2008; 2: 689-98.
26. Hofmann AF. Biliary secretion and excretion in health and disease: Current concepts. *Ann Hepatol* 2007; 1: 15-27.
27. Wagner M, Trauner M. Transcriptional regulation of hepatobiliary transport systems in health: implications for a rational approach to the treatment of intrahepatic cholestasis. *Ann Hepatol* 2005; 2: 77-9.
28. Meier PJ, Stieger B. Bile salt transporters. *Annu Rev Physiol* 2002; 64: 635-61.
29. Ananthanarayanan M, von Dippe P, and Levy D. Identification of the hepatocyte Na⁺-dependent bile acid transport protein using monoclonal antibodies. *J Biol Chem* 1988; 17: 8338-43.
30. Fretland AJ, Omiecinski CJ. Epoxide hydrolases: biochemistry and molecular biology. *Chem Biol Interact* 2000; 129: 41-59.
31. Zhu QS, Xing W, Qian B, Von Dippe P, Shneider BL, Fox VL, Levy D. Inhibition of human m-epoxide hydrolase gene expression in a case of hypercholanemia. *Biochim Biophys Acta* 2003; 1638: 208-16.
32. Trauner M and Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev* 2003; 2: 633-67.

33. Wagner M, Zollner GM. Nuclear receptors as new perspective for the management of liver diseases. *Gastroenterology* 2011; 4: 1120-34.
34. Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 2014; 30: 332-8.
35. Kurdi P, Kawanishi K, Mizutani K, Yokota A. Mechanism of growth inhibition by free bile acids in lactobacilli and bifido bacteria. *J Bacteriol* 2006; 188: 1979-86.
36. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol Hepatol* 2017; 10: 747-56.
37. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; 505: 559-63.
38. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett* 2014; 588: 4223-33.
39. Sze MA, Schloss PD. Looking for a signal in the noise: revisiting obesity and the microbiome. *Mbio* 2016; 4: e01018-16.
40. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; 31: 11070-5.
41. Rhee SH, Poehoullakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat Rev Gastroenterol Hepatol* 2009; 5: 306-14.
42. Cani P, Knauf C. How gut microbes talk to organs: the role of endocrine and nervous routes. *Mol Metab* 2016; 9: 743-52.
43. Fetissov SO. Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour. *Nat Rev Endocrinol* 2016; 13: 11-25.
44. Cani PD, Everard A, Duparc T. Gut microbiota, enteroendocrine functions and metabolism. *Curr Opin Pharmacol* 2013; 6: 935-40.
45. Zhang LS, Davies SS. Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. *Genome Med* 2016; 8: 46.
46. Nohr MK, Pedersen MH, GilleA, Egerod KL, Engelstoft MS, Husted AS, Sichlau RM, et al. GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* 2013; 154: 3552-64.
47. Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 2014; 30: 332-8.
48. De Silva A, Bloom S. Gut hormones and appetite control: a focus on PYY and GLP1 as therapeutic targets in obesity. *Gut Liver* 2012; 1: 10-20.
49. Chavez-Tapia NC, Tellez-AvilaFI, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Cervera J, Uribe M. Bariatric surgery for non-alcoholic steatohepatitis in obese patients. *Cochrane Database Syst Rev* 2010; 3: 1-30.
50. Aguilar-Olivos NE, Almeda-Valdes P, Aguilar-Salinas CA, Uribe M, Méndez-Sánchez N. The role of bariatric surgery in the management of nonalcoholic fatty liver disease and metabolic syndrome. *Metabolism* 2016; 8: 1196-207.
51. Zhang H, Di Baise JK, Zuccolo A. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci USA* 2009; 106: 2365-70.
52. Aron-Wisniewsky J, Clement K. The effects of gastrointestinal surgery on gut microbiota: potential contribution to improved insulin sensitivity. *Curr Atheroscler Rep* 2014; 16: 454.
53. João Cabrera E, Valezi AC, Delfino VD, Lavado EL, Barbosa DS. Reduction in plasma levels of inflammatory and oxidative stress indicators after Roux-en-Y gastric bypass. *Obes Surg* 2010; 1: 42-9.
54. Poitou C, Perret C, Mathieu F, Truong V, Blum Y, Durand H, Alili R, et al. Bariatric surgery induces disruption in inflammatory signaling pathways mediated by immune cells in adipose tissue: a RNA-Seq study. *PLoS One* 2015; 5: e0125718.

Correspondence and reprint request:

Prof. Nahum Méndez-Sánchez, M.D., MSc, PhD.
Liver Research Unit, Medica Sur Clinic & Foundation,
Puente de Piedra 150, Col. Toriello Guerra, ZP 14050,
Mexico City, Mexico. E-mail: nmendez@medicasur.org.mx