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Gut Microbiota, Intestinal Permeability, Obesity-Induced Inflammation, and Liver Injury

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Obesity and its metabolic complications are major health problems in the United States and worldwide, and increasing evidence implicates the microbiota in these important health issues. Indeed, it appears that the microbiota function much like a metabolic “organ,” influencing nutrient acquisition, energy homeostasis, and, ultimately, the control of body weight. Moreover, alterations in gut microbiota, increased intestinal permeability, and metabolic endotoxemia likely play a role in the development of a chronic low-grade inflammatory state in the host that contributes to the development of obesity and associated chronic metabolic diseases such as nonalcoholic fatty liver disease. Supporting these concepts are the observations that increased gut permeability, low-grade endotoxemia, and fatty

liver are observed in animal models of obesity caused by either high-fat or high-fructose feeding. Consistent with these observations, germ-free mice are protected from obesity and many forms of liver injury. Last, many agents that affect gut flora/permeability, such as probiotics/prebiotics, also appear to affect obesity and certain forms of liver injury in animal model systems. Here the authors review the role of the gut microbiota and metabolic endotoxemia-induced inflammation in the development of obesity and liver injury, with special reference to the intensive care unit setting. (*JPEN J Parenter Enteral Nutr.* 2011;35:14S-20S)

Keywords: obesity; gut microbiota; liver injury

Introduction

The trillions of microorganisms normally residing in the human gut are collectively referred to as the microbiota. Dominated by anaerobic bacteria, the microbiota vastly outnumber human cells and contain 100 times more genes (microbiome) than the human genome.^{1,2} There is accumulating evidence to suggest that the microbiota function much like a metabolic “organ,” influencing nutrient acquisition, energy homeostasis, and, ultimately,

the control of body weight.³ In addition, there is emerging evidence indicating that the indigenous gut microbiota affect intestinal permeability and may play a role in the development of a chronic low-grade inflammatory state in the host that contributes to the development of chronic metabolic diseases such as atherosclerosis, insulin resistance, nonalcoholic fatty liver disease (NAFLD), and dyslipidemia.⁴ This chronic subclinical inflammation associated with obesity may occur, at least in part, as a result of the development of “metabolic endotoxemia” and may be an important determinant of both the long-term complications of obesity and the generally worse outcomes of critically ill obese patients.⁵ This article reviews the role of the gut microbiota and metabolic endotoxemia-induced inflammation in the development of obesity and obesity-related hepatic steatosis and liver injury.

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The Development of the Gut Microbiota

The gastrointestinal tract of a fetus is sterile until birth. Following vaginal delivery, microbes from the mother and the environment rapidly colonize the intestinal tract of the infant. Multiple other factors, such as type of feeding and hygiene measures, have also been shown to play a

part in our eventual gut microbial composition.^{6,7} The role of the host genetics in determining the composition of the gut microbiota remains poorly understood.⁸ However, the recent identification of quantitative trait loci in mice has shed some light on the mechanisms of host genetic control in shaping individual microbiome diversity in mammals.⁹

Recent evidence suggests that the human gut microbiota are unique to each person, highly variable between individuals, and remarkably stable after the first year of life.^{10,11} Despite this uniqueness, among the microbes present within the human intestinal tract, members from 2 phylotypes, *Bacteroidetes* and *Firmicutes*, account for more than 90%.¹² Importantly, there appears to be major differences in surface adherence (ie, mucosal) and luminal microbial species along the length of the gastrointestinal tract.¹³

Gut Microbiota and Its Role in Energy Homeostasis and the Development of Obesity

The metabolic activities of the gut microbiota have the end results of extracting calories from ingested dietary substances, helping to store those calories in host adipose tissue for later use, and providing energy and nutrients for microbial growth and proliferation. Bäckhed et al¹ demonstrated that conventionally raised mice have a 40% higher body fat content and 47% higher gonadal fat content than germ-free (GF) mice, despite lower food intake. When the cecal microbiota of the conventionally raised mice were transferred into the intestines of the GF mice in a process known as conventionalization (CONV), a 57% increase in total body fat content, a 61% increase in epididymal fat weight, and development of insulin resistance occurred within 2 weeks despite unchanged food consumption or energy expenditure. These findings support the hypothesis that the gut microbiota affects the amount of energy extracted from the diet. To understand potential mechanisms involved, these investigators performed a series of experiments and determined that, among other factors, the suppression of a circulating lipoprotein lipase inhibitor, Fasting-induced adipocyte factor (Fiaf), was essential for the microbiota-induced deposition of triglycerides in adipocytes. Because the metabolic rate of GF mice was lower than that of their conventional counterparts, the authors speculated that the greater delivery of calories led to inefficient metabolism by futile cycles (and thus higher metabolic rates) in conventional mice. In contrast, Fleissner and colleagues¹⁴ failed to confirm the importance of Fiaf in gut microbiota-mediated effects on fat storage. In this study, GF mice on both a high-fat diet (HFD) and a Western diet (WD) showed an increase in intestinal mRNA expression of Fiaf, but no

major changes in circulating Fiaf compared with CONV mice. In addition, analysis of the fecal microbiota composition revealed that in CONV mice, HFD and WD resulted in an increase in the proportion of *Firmicutes* at the expense of the *Bacteroidetes*.

Turnbaugh et al¹⁵ also performed studies in GF mice confirming that the obese microbiome has an increased capacity to harvest energy from the diet. Importantly, they demonstrated that colonization of GF mice with “obese microbiota” resulted in a significantly greater increase in total body fat than colonization with “lean microbiota.” More recently, this group showed that obesity is associated with phylum-level changes (eg, fewer *Bacteroidetes*) in the microbiota, reduced bacterial diversity, and altered representation of bacterial genes affecting metabolic pathways.¹⁶

In an attempt to identify more specific changes in the gut microbiota that may be responsible for these metabolic effects, Ley et al¹⁷ studied genetically obese, leptin receptor-deficient (*ob/ob*) mice and found a 50% reduction in the abundance of *Bacteroidetes* and a proportional increase in *Firmicutes*. They subsequently showed similar findings in obese compared with lean humans, a finding that could be reversed following a successful low-fat or low-carbohydrate diet.¹⁸

A recent investigation into the gut microbiota changes following Roux-en-Y gastric bypass (RYGB) for extreme obesity also provides insight into the role of the gut microbiota in energy harvest. Zhang et al¹⁹ detected significantly higher numbers of H₂-using methanogenic *Archaea* in obese individuals than in normal-weight or RYGB individuals. *Firmicutes* were dominant in normal-weight and obese individuals but significantly decreased in RYGB individuals, along with a proportional increase of *Gammaproteobacteria*. It was hypothesized that interspecies H₂ transfer between bacterial and archaeal species was an important mechanism for increasing energy uptake by the human large intestine in obese individuals. The potential role of the gut microbiota on weight loss after RYGB was also recently suggested by the demonstration of enhanced weight loss following administration of a probiotic postoperatively.²⁰ Furet et al²¹ further demonstrated that RYGB results in rapid adaptation of the microbiota. In this study, the *Faecalibacterium prausnitzii* species was found to be directly linked to the reduction in the low-grade inflammatory state in obesity and diabetes independently of caloric intake. Both the improvements and decompensation of NAFLD after RYGB may be, in part, related to changes in microbiota as well.²²

Further supporting a role of the gut microbiota in energy homeostasis are the findings of a recent study investigating the impact of the administration of a probiotic organism on obesity and in improving obesity-related disorders. Female C57BL/6 mice were fed either a normal- or high-fat diet and administered *Lactobacillus*

plantarum strain No. 14 (LP14) or vehicle intragastrically daily for 11 weeks. LP14 administration significantly attenuated high-fat diet–induced changes in the mean adipocyte size and tended to reduce the white adipose tissue weight and serum total cholesterol and leptin concentrations as compared with the vehicle-administered mice.²³ Despite these intriguing findings, controversy persists regarding the contribution of the microbiota on the development of obesity in humans²⁴ because of conflicting findings from studies that have failed to confirm differences in the abundance of *Bacteroidetes* and *Firmicutes* between lean and obese humans.^{25,26} Furthermore, recent data from animals^{14,27,28} suggest that diet, a high-fat diet specifically, independent from the obesity itself, is responsible for changes in the gut microbiota. Clearly, the relationship between the gut microbial composition and its energy-harvesting capacity is complex, and much more work remains to be done to identify the role of the gut microbiota in relation to other external factors such as diet in the development of obesity.

The Influence of Diet on the Gut Microbiota, Intestinal Permeability, and Inflammation

Recent investigations indicate that an individual's diet may strongly influence changes in the microbiota, which, in turn, affect intestinal permeability and result in a state of chronic low-grade inflammation. These changes may historically have represented a competitive and advantageous adaptation for the development of the host organism.²⁹ The effect of dietary interventions, including high-fat and high-fructose diets, on the gut microbiota, intestinal permeability, and inflammation is reviewed in this section.

High-Fat Diet

A number of animal studies have investigated the influence of high-fat diets on the composition of gut microbiota and the effects on intestinal permeability, inflammation, and development of obesity-related metabolic complications. The information gathered from these investigations reveals that high-fat diets induce predictable changes in the microbiota that, in turn, result in changes in intestinal permeability, leading to the metabolic endotoxemia, which has been postulated to be essential in the development of the metabolic syndrome.

Cani et al⁴ demonstrated a causal relation between high-fat (HF) diets (49.5% fat g/100 g of total dry diet corresponding to 72% of the total energy content), increased lipopolysaccharide (LPS)–containing microbiota, increased serum LPS concentrations, and resultant features of the metabolic syndrome (eg, weight gain,

NAFLD, and insulin resistance) in mice. These investigators subsequently investigated HF-fed diabetic mice treated with the prebiotic, oligofructose (OFS), for 4 weeks in the presence or absence of the glucagon-like peptide 1 (GLP-1) receptor antagonist, exendin 9-39 (Ex-9). Dietary supplementation with OFS improved glucose tolerance, fasting blood glucose, glucose-stimulated insulin secretion, insulin-sensitive hepatic glucose production, and reduced body weight gain. Ex-9 prevented the beneficial effects seen with OFS, thereby supporting a role for GLP-1 in the beneficial effects of OFS.³⁰ Next, they demonstrated that HF feeding in mice significantly reduced levels of *Bifidobacteria* and increased endotoxemia compared to mice fed a regular diet. Subsequent OFS treatment restored quantities of *Bifidobacteria*, reduced levels of endotoxemia, and lowered proinflammatory cytokines. Furthermore, in HF/OFS mice, levels of *Bifidobacterium* spp in the cecum significantly and positively correlated with improved glucose tolerance and glucose-induced insulin secretion.³¹ In addition to simply altering the microbiota, HF feeding strongly increased intestinal permeability and reduced the expression of genes coding for tight junction proteins, suggesting a possible underlying mechanism responsible for this effect on permeability and the resultant increase in endotoxemia. The above findings suggesting a role for the gut microbiota in metabolic endotoxemia and inflammation are further supported by the recent demonstration of an attenuation in metabolic endotoxemia/inflammation/fatty liver by use of antibiotic treatment and by use of the CD14 knockout mouse lacking the LPS receptor.³²

Because of the difficulties involved in performing similar yet adequately controlled investigations in free-living humans, a mouse model representative of the human gut microbiome has recently been developed by transplanting adult human fecal microbial communities into GF C57BL/6J mice. This animal model with humanized gut microbiota was demonstrated to undergo a rapid shift in the makeup of the microbiota a single day after switching from a low-fat, plant polysaccharide–rich diet to a high-fat (HF), high-sugar “Western” diet. This dietary change induced adiposity and resulted in altered microbiome gene expression. Furthermore, this propensity toward obesity was shown to be transmissible via microbiota transplantation. These findings confirm that diet plays an important role in the composition of the gut microbiota and that these changes may occur rapidly after dietary changes. The use of this “humanized” mouse model may be very useful in future investigations on the role of diet and other interventions on the gut microbiota and its function.³³

Several other investigators have now confirmed changes in the microbiota composition and the development of an inflammatory response in mice ingesting an HF, Western diet. Ding et al³⁴ showed that ingestion of an

HF diet resulted in increased body weight and adiposity and induced ileal tumor necrosis factor (TNF)- α expression in CONV mice but not in GF mice. The increase in inflammation was found to precede the development of obesity and significantly correlated with diet-induced weight gain, adiposity, and plasma insulin and glucose levels. Finally, they demonstrated that the inflammatory response to an HF diet was transmittable through fecal slurries from CONV mice to GF mice. Rabot et al³⁵ found that GF mice fed an HF diet consumed fewer calories, excreted more fecal lipids, and weighed significantly less than CONV mice. Furthermore, the metabolic (eg, insulin resistance and dyslipidemia) and proinflammatory (eg, plasma TNF- α concentrations) consequences of an HF diet were attenuated in GF animals, suggesting that insulin sensitivity and cholesterol metabolism are metabolic targets influenced by the gut microbiota.

In an experiment using Sprague-Dawley rats, de La Serre et al³⁶ investigated whether changes in gut epithelial function and microbiota in mice are associated with diet, obesity, or both. The obesity-prone phenotype (DIO-P) rats exhibited an increase in toll-like receptor-4 (TLR4) activation with associated ileal inflammation and a decrease in intestinal alkaline phosphatase, a luminal enzyme that detoxifies LPS, whereas the obesity-resistant phenotype (DIO-R) rats did not. Measurement of bacterial 16S rRNA showed a decrease in total bacterial density and an increase in the relative proportion of *Bacteroidales* and *Clostridiales* orders in rats fed HF diets regardless of phenotype. An increase in *Enterobacteriales* was seen only in DIO-P rats. The results imply that consumption of an HF diet induces changes in the gut microbiota; however, it is the obese phenotype that develops obesity-induced inflammation. In contrast, Hildebrandt et al²⁷ found large alterations in the gut microbiota of RELM- β knockout mice associated with an HF diet, independent of obesity. Principal microbial alterations included a decrease in *Bacteroidetes* and an increase in both *Firmicutes* and *Proteobacteria*.

Further supporting the role of diet in the composition of the gut microbiota is a study from Zhang et al³⁷ that assessed the relative contributions of host genetics and diet in shaping the gut microbiota. They found that diet changes explained 57% of the total structural variation in gut microbiota, whereas genetic mutation accounted for no more than 12%. All the groups with impaired glucose tolerance had significantly different gut microbiota relative to healthy wild-type/normal chow-fed animals. *Bifidobacterium* spp were nearly absent in all animals on an HF diet, regardless of genotype. Sulfate-reducing, endotoxin-producing bacteria of the family *Desulfovibrionaceae* were enhanced in all animals with impaired glucose tolerance.

In summary, the studies described above indicate that (1) aside from its caloric contributions, an HF diet causes alterations in the gut epithelial integrity and the

microbiota, resulting in a propensity toward an increased low-grade systemic inflammation that contributes to the development of obesity; (2) a complex interaction between the original microbiota and diet-induced changes in the microbiota contributes to the development of metabolic endotoxemia, insulin resistance, and metabolic syndrome; and (3) alterations in the microbiota and resultant obesity-prone environment caused by an HF diet are transmissible.

High-Fructose Diets

A high-fructose diet in mice has been demonstrated to induce hepatic and extrahepatic insulin resistance, obesity, and hypertension. Because fructose (eg, high-fructose corn syrup) has now become a major constituent of our modern diet, the role of fructose in the obesity epidemic is under much study.³⁸ Although a number of mechanisms have been proposed, several studies have implicated the gut microbiota and its effects on intestinal permeability in high-fructose diet-induced obesity and obesity-related metabolic disturbances. Bergheim et al³⁹ compared C57BL/J6 mice with free access to solutions containing 30% glucose, 30% fructose, 30% sucrose, water sweetened with artificial sweetener (AS), or plain water. Whereas total caloric intake and weight gain were highest in mice exposed to glucose, hepatic lipid accumulation was significantly higher in mice consuming fructose compared to all other groups. Interestingly, fructose-fed mice had significantly higher levels of endotoxin in portal blood as well as increased hepatic lipid peroxidation and TNF- α expression. Treatment with the antibiotics, polymyxin B and neomycin, attenuated the endotoxemia and hepatic lipid accumulation in fructose-fed mice. Another study compared TLR4-mutant (C3H/HeJ) mice and wild-type (C3H/HouJ) mice with fructose-induced NAFLD. In fructose-fed TLR4 mutant mice, hepatic triglyceride accumulation was significantly reduced by approximately 40% in comparison to fructose-fed wild-type mice, and plasma alanine aminotransferase levels were at the level of water-fed controls. In addition, hepatic lipid peroxidation, MyD88, and TNF- α levels were significantly decreased in fructose-fed TLR4-mutant mice. The authors concluded that fructose-induced NAFLD is associated with intestinal bacterial overgrowth, increased intestinal permeability, and endotoxin-dependent activation of hepatic Kupffer cells.⁴⁰

Microbiota, Endotoxemia, Hepatic Steatosis/Inflammation/Injury

We have thus far reviewed potential interactions of the microbiota, diet, and gut permeability with low-grade endotoxemia and inflammation. We have shown that both high-fat and high-fructose diets can induce hepatic

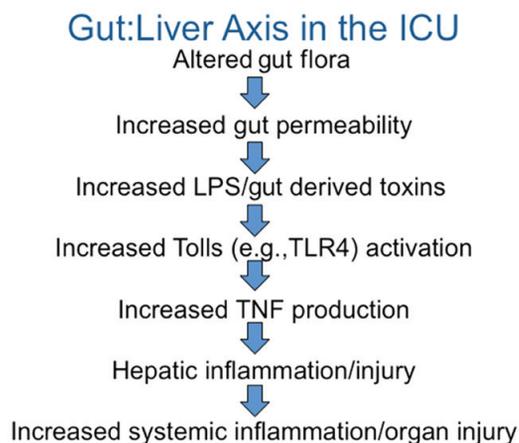


Figure 1. Alterations in gut flora/permeability can lead to activation of hepatic Tolls, subsequent hepatic inflammation/injury, and ultimately systemic inflammation and organ injury. ICU, intensive care unit; LPS, lipopolysaccharide; TLR4, toll-like receptor-4; TNF, tumor necrosis factor.

steatosis. We now review how gut-derived endotoxin can act as a “second hit” or insult to convert hepatic steatosis to steatohepatitis and cause both local hepatic and systemic inflammation (Figure 1). Importantly, hepatic inflammation can predispose to other organ dysfunction/injury, including lung, kidney, and brain. This dysregulated inflammatory response has major implications for the organ failure that occurs in many intensive care unit (ICU) patients. Moreover, patients in the ICU are likely to have hepatic steatosis for multiple reasons, including previously mentioned dietary factors, alcohol abuse, or parenteral nutrition; thus, they have a fatty liver that is susceptible to a “second hit.” We now focus on the interactions of the microbiome, gut permeability, endotoxin, and proinflammatory cytokines as one pathway for driving steatosis to steatohepatitis and systemic inflammation.

It has been recognized for more than a half century that the gut flora and gut-derived toxins play a critical role in the development of both liver disease and certain complications such as portal systemic (PSE) encephalopathy.⁴¹⁻⁴⁴ Indeed, more than 50 years ago, it was shown that germ-free rodents or rodents treated with antibiotics to “sterilize the gut” were resistant to both nutrition and toxin-induced liver injury. Elegant studies by Broitman and coworkers⁴³ showed that rats fed a choline-deficient diet developed cirrhosis that could be prevented by oral neomycin. However, when endotoxin was added to the water supply, neomycin could no longer prevent the development of liver injury and fibrosis. This study clearly documented the critical role of endotoxin in the development/progression of liver inflammation/injury/fibrosis. Subsequently, it was shown that dietary factors such as fat, fructose, and alcohol can alter gut flora and intestinal permeability. Alterations in permeability allow gut-derived toxins to

cross the intestinal barrier and activate Kupffer cells, which are “primed” to overproduce inflammatory cytokines, which, in turn, can cause liver inflammation/injury and systemic inflammation (Figure 1). Mechanisms for intestinal permeability changes are multiple and include alterations in microbiota, acetaldehyde generation from alcohol, nitric oxide production, alterations in individual nutrients such as zinc deficiency, and others.⁴⁵ Gut-derived toxins that cross the gut barrier include not only endotoxins or LPS but also other gut-derived products such as peptidoglycan.

As introduced in the previous section, antibiotics, prebiotics, and probiotics have all been shown to prevent the development of both experimental alcohol- or diet-induced steatosis/steatohepatitis.^{39,46-49} Increased plasma/hepatic concentrations of proinflammatory cytokines such as TNF were noted in rodent models of alcohol- or diet-induced steatosis/steatohepatitis, and mice given anti-TNF antibodies or mice lacking TNFR1 were protected against the development of experimental diet- or alcohol-induced steatosis/steatohepatitis.⁴⁹⁻⁵² Moreover, diets inducing hepatic steatosis were shown to sensitize to the hepatotoxicity induced by gut-derived endotoxin and TNF, and specific components of the TLR4 pathway responsible for steatosis-related liver injury are currently being defined. Indeed, TLR4 activation by endotoxin results in recruitment of the adaptor molecules MyD88 and Toll/interleukin-1 receptor (TIR) domain-containing adapter-inducing interferon- β (TRIF), which each activate separate downstream signaling cascades. Recent data suggest that the MyD88-independent pathway (TRIF) is more important in the development of alcohol-related steatohepatitis, whereas nonalcoholic steatohepatitis appears to signal through the MyD88-dependent pathway.⁵³ Thus, our knowledge is rapidly expanding concerning how a fatty liver produces an exaggerated inflammatory response when challenged with gut-derived toxins, and new potential therapeutic targets are being discovered.

Concomitant studies in patients with steatohepatitis due to either obesity or alcohol also showed increased gut permeability and endotoxemia. More than 20 years ago, we first reported that patients with alcoholic steatohepatitis had increased basal- and endotoxin-stimulated monocyte TNF production, and subsequent studies showed that plasma and monocyte proinflammatory cytokines correlated with the clinical course of alcoholic steatohepatitis and survival.^{54,55} Similar but less prominent increases in TNF and chemokines, such as interleukin (IL)-8, were observed in nonalcoholic steatohepatitis, as well as significant decreases in plasma concentrations of the critical anti-inflammatory adipokine, adiponectin.^{56,57} Thus, human data appear generally to parallel animal studies and suggest that patients with fatty liver have low-grade systemic inflammation producing a greater inflammatory/cytokine response to gut-derived endotoxin stimulus.

Conclusion

Conventional thoughts regarding caloric intake, energy expenditure, and the development of obesity and obesity-related complications are being challenged by recent revelations regarding the role of the gut microbiota. Not only does this symbiotic relationship result in vast differences in nutrient acquisition and energy homeostasis, but it appears that diet composition can rapidly induce important changes in the microbiota, which, in turn, result in further metabolic consequences for the host organism. Although high-fat and high-fructose diets result in caloric excess, their deleterious health consequences appear also to be partially due to their effect on the microbiota, gut permeability, and resultant metabolic endotoxemia. There are multiple reasons for ICU patients to have both metabolic endotoxemia and fatty liver. A second insult such as bacteremia or hypotension can induce further inflammation and potential organ injury. Multiple pathways that are amenable to therapeutic interventions (including nutrition therapy) are being identified and investigated for anti-inflammatory potential.

References

- Bäckhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A*. 2004;101(44):15718-15723.
- Xu J, Gordon JI. Inaugural article: honor thy symbionts. *Proc Natl Acad Sci U S A*. 2003;100(18):10452-10459.
- Cani PD, Delzenne NM. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. *Curr Opin Pharmacol*. 2009;9(6):737-743.
- Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007;56(7):1761-1772.
- DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. *Mayo Clin Proc*. 2008;83(4):460-469.
- Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*. 2006;118(2):511-521.
- Manco M, Putignani L, Bottazzo GF. Gut microbiota, lipopolysaccharides and innate immunity in the pathogenesis of obesity and cardiovascular risk. *Endocr Rev*. 2010;31(6):817-844.
- Benson AK, Kelly SA, Legge R, et al. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci U S A*. 2010;107(44):18933-18938.
- Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007;5(7):e177.
- Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science*. 2009;326(5960):1694-1697.
- Bäckhed F. Intestinal microbiota during infancy and its implications for obesity. *J Pediatr Gastroenterol Nutr*. 2009;48(3):249-256.
- Hugenholtz P, Goebel BM, Pace NR. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol*. 1998;180(18):4765-4774.
- Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science*. 2005;308(5728):1635-1638.
- Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M. Absence of intestinal microbiota does not protect mice from diet-induced obesity. *Br J Nutr*. 2010;104(6):919-929.
- Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444(7122):1027-1031.
- Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;457(7228):480-484.
- Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A*. 2005;102(31):11070-11075.
- Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006;444(7122):1022-1023.
- Zhang H, DiBaise JK, Zuccolo A, et al. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A*. 2009;106(7):2365-2370.
- Woodard GA, Encarnacion B, Downey JR, et al. Probiotics improve outcomes after Roux-en-Y gastric bypass surgery: a prospective randomized trial. *J Gastrointest Surg*. 2009;13(7):1198-1204.
- Furet JP, Kong LC, Tap J, et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes*. 2010;59(12):3049-3057.
- Drenick EJ, Fisler J, Johnson D. Hepatic steatosis after intestinal bypass: prevention and reversal by metronidazole, irrespective of protein-calorie malnutrition. *Gastroenterology*. 1982;82(3):535-548.
- Takemura N, Okubo T, Sonoyama K. *Lactobacillus plantarum* strain No. 14 reduces adipocyte size in mice fed high-fat diet. *Exp Biol Med (Maywood)*. 2010;235(7):849-856.
- Laugerette F, Vors C, Peretti N, Michalski MC. Complex links between dietary lipids, endogenous endotoxins and metabolic inflammation. *Biochimie*. 2011;93(1):39-45.
- Duncan SH, Lobley GE, Holtrop G, et al. Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes (Lond)*. 2008;32(11):1720-1724.
- Schwartz A, Taras D, Schäfer K, et al. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)*. 2010;18(1):190-195.
- Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology*. 2009;137(5):1716-1724.
- Murphy EF, Cotter PD, Healy S, et al. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut*. 2010;59(12):1635-1642.
- Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*. 2008;3(4):213-223.
- Cani PD, Knauf C, Iglesias MA, Drucker DJ, Delzenne NM, Burcelin R. Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide I receptor. *Diabetes*. 2006;55:1484-1490.
- Cani PD, Neyrinck AM, Fava F, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia*. 2007;50(11):2374-2383.
- Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008;57(6):1470-1481.
- Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med*. 2009;1(6):6ra14.

34. Ding S, Chi MM, Scull BP, et al. High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PLoS One*. 2010;5(8):e12191.
35. Rabot S, Membrez M, Bruneau A, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J*. 2010;24(12):4948-4959.
36. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol Gastrointest Liver Physiol*. 2010;299(2):G440-G448.
37. Zhang C, Zhang M, Wang S, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J*. 2010;4(2):232-241.
38. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev*. 2010;90(1):23-46.
39. Bergheim I, Weber S, Vos M, et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. *J Hepatol*. 2008;48(6):983-992.
40. Spruss A, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. *Hepatology*. 2009;50(4):1094-1104.
41. Nolan JP. The role of intestinal endotoxin in liver injury: a long and evolving history. *Hepatology*. 2010;52(5):1829-1835.
42. Leach BE, Forbes JC. Sulfonamide drugs as protective agents against carbon tetrachloride poisoning. *Pro Soc Exp Bio Med*. 1941;48:361-363.
43. Broitman SA, Gottlieb LS, Zamcheck N. Influence of neomycin and ingested endotoxin in the pathogenesis of choline deficiency cirrhosis in the adult rat. *J Exp Med*. 1964;119:633-642.
44. McClain CJ, Zieve L. Portal systemic encephalopathy: recognition and variations. In: Davidson CS, ed. *Problems in Liver Diseases*. New York: Stratton Intercontinental Medical Book Corp; 1979:162-172.
45. Purohit V, Bode JC, Bode C, et al. Alcohol, intestinal bacterial growth, intestinal permeability to endotoxin, and medical consequences: summary of a symposium. *Alcohol*. 2008;42(5):349-361.
46. Keshavarzian A, Choudhary S, Holmes EW, et al. Preventing gut leakiness by oats supplementation ameliorates alcohol-induced liver damage in rats. *J Pharmacol Exp Ther*. 2001;299(2):442-448.
47. Nanji AA, Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). *Proc Soc Exp Biol Med*. 1994;205(3):243-247.
48. Gratz SW, Mykkanen H, El-Nezami HS. Probiotics and gut health: a special focus on liver diseases. *World J Gastroenterol*. 2010;16(4):403-410.
49. Li Z, Yang S, Lin H, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology*. 2003;37:343-350.
50. Honchel R, Ray MB, Marsano L, et al. Tumor necrosis factor in alcohol enhanced endotoxin liver injury. *Alcohol Clin Exp Res*. 1992;16(4):665-669.
51. Iimuro Y, Gallucci RM, Luster MI, Kono H, Thurman RG. Antibodies to tumor necrosis factor alpha attenuate hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat. *Hepatology*. 1997;26(6):1530-1537.
52. Yin M, Wheeler MD, Kono H, et al. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology*. 1999;117(4):942-952.
53. Szabo G, Bala S. Alcoholic liver disease and the gut-liver axis. *World J Gastroenterol*. 2010;16(11):1321-1329.
54. McClain CJ, Cohen DA. Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. *Hepatology*. 1989;9(3):349-351.
55. Khoruts A, Stahnke L, McClain CJ, Logan G, Allen JI. Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. *Hepatology*. 1991;13(2):267-276.
56. Chawla R, Watson W, Eastin C, Lee E, Schmidt J, McClain CJ. S-adenosylmethionine (adomet) deficiency and tumor necrosis factor (TNF) in lipopolysaccharide (LPS) induced hepatic injury. *Am J Physiol*. 1998;275:G125-G129.
57. Louthan MV, Barve S, McClain CJ, Joshi-Barve S. Decreased serum adiponectin: an early event in pediatric nonalcoholic fatty liver disease. *J Pediatr*. 2005;147(6):835-838.