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Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity

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Obesity is associated with metabolic alterations related to glucose homeostasis and cardiovascular risk factors. These metabolic alterations are associated with low-grade inflammation that contributes to the onset of these diseases. We and others have provided evidence that gut microbiota participates in whole-body metabolism by affecting energy balance, glucose metabolism and low-grade inflammation associated with obesity and related metabolic disorders. Recently, we defined gut microbiota-derived lipopolysaccharide (LPS) (and metabolic endotoxemia) as a factor involved in the onset and progression of inflammation and metabolic diseases. In this review, we discuss mechanisms involved in the development of metabolic endotoxemia such as the gut permeability. We also discuss our latest discoveries demonstrating a link between the gut microbiota, endocannabinoid system tone, leptin resistance, gut peptides (glucagon-like peptide-1 and -2) and metabolic features. Finally, we will introduce the role of the gut microbiota in specific dietary treatments (prebiotics and probiotics) and surgical interventions (gastric bypass).

Introduction

A growing body evidence suggests that obesity is influenced by both genetic and lifestyle factors. Although an imbalance between energy intake and energy expenditure could explain the growing incidence of obesity, the metabolic alterations associated with obesity are not solely explained by genetic factors. Obesity is classically associated with metabolic alterations related to glucose homeostasis (e.g., glucose intolerance, type 2 diabetes and insulin resistance) and cardiovascular risk factors (e.g., hypertension and dyslipidemia).[1] These metabolic alterations are associated with low-grade inflammation that contributes to the onset of these diseases.[2] Given the existing link between inflammation and metabolism in the context of the metabolic syndrome, identifying the origin of inflammation is of the utmost importance. Although numerous investigations have demonstrated a relationship between inflammation and macrophage infiltration into organs (e.g., adipose tissue, muscles and the liver), the exact role of macrophages and the source and type of triggering factors activating the immune system remain a matter of debate.[2-8] Hence, the major pathogenic mechanism linking inflammation with changes in liver and adipose tissue metabolism remain to be determined. Given the plethora of inflammatory markers causally associated with the development of impaired insulin action (or insulin resistance) and the numerous molecular interactions between the immunity and insulin signaling, we have researched potential integrating factors that might provide a mechanism. Our studies focused on the gut microbiota as a putative candidate.

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Gut Microbiota and Metabolic Endotoxemia

Among the environmental factors that are hypothesized to interfere with energy homeostasis, growing evidence demonstrates that the gut microbiota plays a critical role.[9-16]

The human microbiota consists of as many as 10 to 100 trillion microorganisms, a number that is at least 10-fold more than cells that make up the human body,[17] meaning that the cells that compose our body are 10% human and 90% microbes.[18] Therefore, it is now widely accepted that this consortium of cells provides important metabolic and biological functions that cannot be performed by our human metabolism.[19]

We and others have provided evidence that gut microbiota participates in whole-body metabolism by affecting energy balance,[11,13,14] glucose metabolism,[9,14,20-22] and low-grade inflammation[9,10,14,23-25] associated with obesity and related metabolic disorders.

Recently, we defined gut microbiota-derived lipopolysaccharide (LPS) as factors involved in the onset and progression of inflammation and metabolic diseases.[14] We found that in pathological situations (obesity and type 2 diabetes) specific microbes-associated molecular patterns (MAMPs) such as LPS play a major role in the onset the diseases associated with obesity.[9,10,14,22,25-27] LPS is a component of Gram-negative bacteria cell walls and are among the most potent and well-studied inducers of inflammation. We demonstrated that dietary fat facilitates the development of metabolic endotoxemia (e.g., increased plasma LPS levels).[14,28] This process can be initiated by physiological mechanisms, such

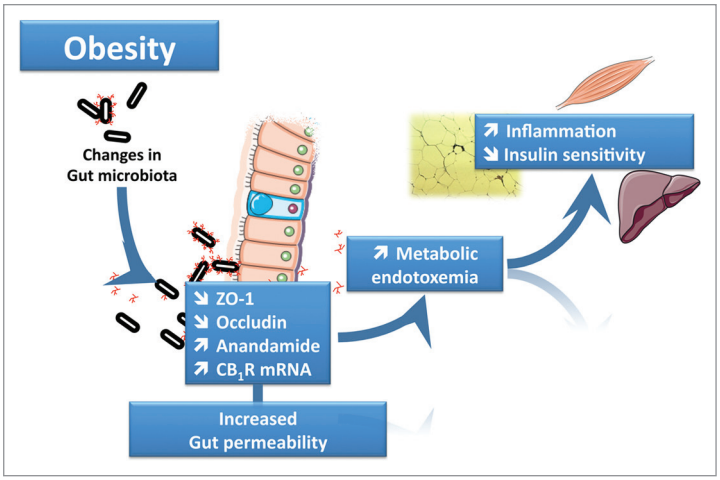

Figure 1. The gut microbiota controls gut barrier function and the onset of metabolic endotoxemia. Diet-induced obesity and genetic (*ob/ob* or *db/db* mice) obesity are associated with changes in gut microbiota composition. This leads to gut barrier function alteration through several mechanisms, including an altered distribution of the tight junction proteins ZO-1 and Occludin and an increased eCB system tone with a higher expression of anandamide and CB_1R. These phenomena promote metabolic endotoxemia and initiate the development of low-grade inflammation and insulin resistance in the liver, muscles and adipose tissue.

as the transport of LPS from the gut lumen toward target tissues by newly synthesized chylomicrons from epithelial intestinal cells in response to fat feeding.[29,30] We and others have confirmed these data in human subjects; therefore, this phenomenon could contribute to higher plasma LPS levels and low-grade inflammation, as is observed with a high-fat diet.[14,31-35] Given that genetic models of obesity fed with a normal chow diet still develop metabolic endotoxemia, we cannot exclude the involvement of factors other than fat absorption.[10,25,36,37] Therefore, we hypothesized that metabolic endotoxemia could be linked to the gut microbiota.

Gut Microbiota and High-Fat Diet

We first demonstrated that high-fat diet profoundly affects gut microbiota composition in 2007.[14,27] More precisely, we found, that diet-induced obesity strongly altered gut microbiota composition by reducing Bifidobacterium spp and Bacteroides-related bacteria, *Eubacterium rectale-Blautia coccoides* group content.[14,27] These data have been confirmed and extended by the discovery that fat feeding also decreases Lactobacillus spp and Roseburia spp.[38] Several other studies have characterized the gut microbiota composition of diet-induced obese mice with different metagenomic approaches.[39-43] Importantly, all of these studies are relatively consistent regarding gut microbiota modulation in mice fed a high-fat diet; Firmicutes become more abundant, and Bacteroidetes decrease in number. More specifically, Hildebrandt et al. showed decreased Bacteriodetes and increased Firmicutes and Proteobacteria in mice fed a high-fat diet.[41] Murphy et al. found an increased proportion of Firmicutes and a reduction in Bacteroidetes in similar dietary conditions.[42] More recently, Ravussin et al. confirmed that mice on a high-fat diet had increased levels of Firmicutes.[43] Although most of the results exploring the impact of high-fat diet on gut microbiota composition are convergent, no study has identified a specific link with metabolic endotoxemia onset.

Gut Microbiota and the Innate Immune System Link Metabolic Endotoxemia to Insulin Resistance

Toll-like receptors (TLRs) are a family of pattern-recognition receptors that play a critical role in innate immunity by integrating signals from microbiota-host interactions (e.g., proinflammatory signals). The innate immune system detects LPS via its interaction with specific proteins that complex with TLR4 (CD14/TLR4 complex).[44] Several experimental arguments support metabolic endotoxemia as a factor involved in the development of low-grade inflammation associated with insulin resistance and type 2 diabetes (**Fig. 1**). We found that a high-fat diet increased fat mass, body weight and low-grade inflammatory state (in the liver, adipose tissues and muscle) through a LPS-dependent mechanism. We also discovered that mice that lack functional LPS receptors (CD14 knockout mice) are resistant to diet-induced obesity and related disorders, including hepatic insulin resistance.[14] We demonstrated that the chronic subcutaneous infusion of LPS (mimicking metabolic endotoxemia) induces significant inflammation and insulin resistance to a similar extent as observed following high-fat diet feeding. The origin of gut microbiota in this phenotype has been further demonstrated in mice chronically treated with broad-spectrum antibiotics. In these conditions, mice fed a high-fat diet that received antibiotics exhibited reduced metabolic endotoxemia, inflammation, insulin resistance and fat mass development.[9] These effects were confirmed in genetically obese (*ob/ob*) mice.[9,45] More recently, it was shown that germ-free mice did not develop inflammation following a high-fat diet, further supporting the role of gut microbiota in low-grade inflammation onset.[23] Different studies have proposed that saturated fatty acids promote low-grade inflammation and insulin resistance through a TLR-4 dependent mechanism;[46-48] however, this effect is still a matter of debate.[49] It can be proposed with more certainty that fatty acids stimulate the innate immune system, but probably in conjunction with initial stimulation by LPS of the TLR-4/CD14 complex and subsequent TLR-2 stimulation. For instance, both axenic mice and antibiotic treated mice are resistant to the development of high-fat diet-induced inflammation and insulin resistance. Given that these mice fully digest and absorb the ingested fat, and display functional TLR-4/2 receptors[9,23,50] suggest that a signaling cascade initiated by an LPS/TLR-4/CD14-dependent mechanism in turn activates TLR-2 expression to support innate immune system inflammatory responses.

Because of these studies, it is now increasingly recognized that the innate immune system and metabolic pathways are functionally intertwined,[2] making them attractive obesity and diabetes targets. Recent data indicate that low-grade inflammation and insulin resistance are also controlled by TLR2.[51-53] It has been proposed that both TLR5,[16] and TLR2,[54] knock out mice

exhibited altered gut microbiota composition and these receptors play a central role in the development of obesity and associated disorders. These data further support the hypothesis that microorganisms and/or derived compounds play a crucial role in the onset of metabolic disorders associated with obesity. However, there is no clear evidence of a unique coupling system between gut microbial-host signals and the onset or progression of metabolic alterations associated with obesity.

Metabolic Endotoxemia and Gut Barrier Function

One of the mechanisms explaining the development of obesity-related metabolic endotoxemia is that gut microbiota links gut permeability to low-grade inflammation and insulin resistance.[9,14,25,37] More specifically, we have shown that a high-fat diet contributes to the disruption of the tight-junction proteins (Zonula Occludens-1 and Occludin) involved in the gut barrier function (**Fig. 1**). This effect is directly dependent on the gut microbiota because antibiotic treatment abolished diet-induced gut permeability.[9,10] In accordance with this hypothesis, we also found that the specific modulation of gut microbiota composition with non-digestible carbohydrates (e.g., prebiotics) improves gut barrier integrity, reduces metabolic endotoxemia, and lowers inflammation and glucose intolerance (**Fig. 1**).[10,25,27,37]

Among the putative mechanisms linking the gut microbiota with the development of obesity and related disorders, we have proposed a role for the endocannabinoid system (eCB). The eCB system is composed of endogenous lipids that activate specific G protein-coupled receptors termed cannabinoid receptors 1 and 2 (CB_1R and CB_2R). Among these lipids, *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are the most studied.[55] AEA and 2-AG are both widely present throughout the body, and their tissue levels are regulated by the balance between synthesis and inactivation.[56]

Importantly, our latest discoveries demonstrate a link between the gut microbiota, eCB system tone and metabolic features associated with obesity.[24,37] For instance, we have demonstrated that the gut microbiota controls the eCB tone in intestine and adipose tissue.[24,37] We also found that the gut microbiota regulates the CB_1R expression, AEA content and its degrading enzyme fatty acid amide hydrolase (FAAH), not only in the intestine but also in mouse adipose tissue (**Fig. 1**).[37] However, the mechanisms behind this constitutive crosstalk in obese and type 2 diabetes are largely unknown. Interestingly, several studies have suggested a relationship between LPS and the eCB system. Indeed, LPS controls eCB synthesis in macrophages, and the macrophage infiltration of adipose tissue and the liver during obesity is a key factor in metabolic disorder development.[5,57] We demonstrated that macrophage infiltration is dependent on LPS activation and its interaction with its co-receptor CD14, as well as gut microbiota composition.[9,14] Finally, we used pharmacological interventions to demonstrate that the eCB system contributes to gut barrier function (in vivo and in vitro) and metabolic endotoxemia via putative CB_1 receptor-dependent mechanisms. More precisely, the eCB system controls gut barrier function through the distribution and localization of tight junction proteins (ZO-1 and occludin), independently of food intake behavior. Furthermore, we identified the eCB system in the gut and adipose tissue as a prominent pathway for adipogenesis regulation.[37] Altogether, these data support interplay between these three partners, namely, the gut microbiota, the innate immune system and the endocannabinoid system, in the development of obesity and related disorders.

Communication between Gut Microbes and Adipose Tissue Metabolism

Adipose tissue is a key organ with a central role in metabolism regulation.[58] Although its role in energy storage has been recognized for centuries, its function as an endocrine organ affecting energy homeostasis, innate immunity and inflammation has only been known for a few decades.[58] Insight into the mechanisms underlying adipose tissue biology is important for a better understanding of altered metabolism in obesity and type 2 diabetes. As mentioned already, the gut microbiota can be considered as an "exteriorized organ" that contributes to host metabolism and homeostasis via different functions and mechanisms.[18] Growing evidence suggests that the gut microbiota contributes to host metabolism through communication with adipose tissue, which influences the development of metabolic alterations associated with obesity.

Several studies support such a communication axis. First, mice with gut microbiota (conventionally raised) have over 40% more fat mass than germ-free mice (without gut microbiota).[11] Second, the transplantation of cecal content (gut microbiota) isolated from obese mice to germ-free mice resulted in a greater increase in total body fat mass compared with colonization with gut microbiota isolated from lean donors.[13] A recent study demonstrated an increased lipolysis and decreased lipogenesis in the brown adipose tissue of germ-free vs. conventionally raised mice, suggesting that gut microbiota stimulates brown adipose tissue lipid metabolism.[59] *Ob/ob* mice fed prebiotics have a different gut microbiota composition and decreased adiposity index compared with obese mice fed a normal chow diet.[25] Conventionalization of germ-free mice has also highlighted a general increase in activity of the enzyme lipoprotein lipase (LPL), which catalyzes the release of fatty acids from circulating triglycerides and lipoproteins in muscle and adipose tissue. This mechanism may be related to suppression of the fasting-induced adipose factor (FIAF) in the gut. FIAF is an inhibitor of LPL activity, and its blunted activity in conventionalized mice may participate in the accumulation of fatty acids in adipose tissue.[11] Finally the demonstration that germ-free mice are resistant to high-fat diet-induced body weight gain and fat mass accumulation suggests that gut microbiota promotes fat storage.[50] Different mechanisms leading to the resistance of diet-induced obesity have been proposed and are extensively discussed in recent reviews in references 18 and 60.

We have previously suggested a link between gut microbiota composition, metabolic endotoxemia and adipose tissue development during high-fat diet feeding.[9,14,27] In addition, we showed that gut microbiota modulation by prebiotics may control adipogenesis by acting on lipogenic enzymes and markers of adipocyte

differentiation.[37] We proposed that selective changes in the gut microbiota of obese mice impact LPS levels, a process that may participate in adipogenesis regulation.[37]

Based on a recent study from our lab,[24] we suggest that the impact of gut microbiota modulation on adipose tissue metabolism can be mediated by the eCB system. Different studies suggest a role for eCBs in adipose tissue metabolism in both rodents and humans,[61-66] but the exact molecular mechanisms underlying this regulation are still under investigation. Recently, we discovered that modulating gut microbiota with prebiotics changes the eCB system tone, not only in the gut (as discussed earlier) but also in adipose tissue.[37] eCB activation stimulates adipogenesis, whereas LPS stimulates eCB system tone.[37,67] More recently, we observed that genetically obese and diabetic mice (*db/db*) exhibited a different gut microbiota composition compared with their lean littermates.[24] With high-throughput culture-independent technologies (pyrosequencing and MITchip analysis), we found that gut microbiota composition varied between the two groups. In addition, we have shown that *db/db* mice present an altered adipose eCB system tone, characterized by elevated AEA levels and increased expression of CB_1 receptor and synthesizing enzyme N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD), in comparison to their lean littermates (**Fig. 1**).[24] We also examined adipose tissue metabolism, represented here by apelin, a newly identified adipokine. This key peptide is involved in several physiological functions in both the central nervous system and peripheral tissues. Apelin plays a role in cardiovascular functions (e.g., heart contractility, fluid homeostasis and blood pressure) and seems to be a potential regulator of metabolism; it has been demonstrated that apelin affects glucose homeostasis by stimulating glucose uptake in skeletal muscle and adipose tissue.[68-70] Interestingly, we found that higher expression of apelin and its receptor (APJ) in the adipose tissue of obese and type 2 diabetic mice was significantly correlated with several taxa.[24] These genetic models are known to have inflammation, altered gut barrier function and endotoxemia.[36] We confirmed this by measuring inflammatory markers (cytokine IL-1β, chemokine MCP-1, macrophage markers F4/80, CD11c, CD68), which are all increased in obese vs. lean mice; there were positive correlations between these inflammatory markers and the apelinergic system.[24] To ascertain the mechanistic link between altered adipose tissue metabolism and inflammation (i.e., LPS and cytokines) or the eCB system, we stimulated adipose tissue explants from lean wild-type mice with an eCB system agonist, with or without concomitant LPS administration. We found that eCB activation downregulates expression of apelin and its receptor, and low-dose LPS significantly increases these two markers. Surprisingly, concomitant treatment of adipose tissue with eCB agonist and LPS increases apelin and APJ; in this scenario the eCB agonist is unable to neutralize LPS-mediated effects on the apelinergic system.[24] These results indicate that eCBs impact adipose tissue metabolism in a physiological state and that these effects can be modified in pathological situations (e.g., obesity with endotoxemia, inflammation and eCB activation), which strongly suggests that LPS and inflammation regulate adipose tissue metabolism.[24] Taken together, these data suggest a potential relationship between gut microbiota and adipose tissue that may be mediated by the eCB system.

Gut Peptides as Communication Agents between Gut Microbiota and Host Metabolism in a Context of Obesity

Gut microbiota composition is influenced by several factors, including host-dependent factors (e.g., genetic background, age, sex, immune system and gut motility), treatment (e.g., antibiotics and gastric bypass) and diet (e.g., nondigestible carbohydrates, fat, prebiotics or probiotics).[60] Prebiotics are selectively fermented dietary ingredients that cause specific changes in the composition and/or activity of the gut microbiota (e.g., bifidobacteria and lactobacilli) that confer benefit(s) to host health.[71,72] Hence, prebiotics are often used to modulate gut microbiota and to promote health. We have previously shown that prebiotics improve gut barrier function and reduce the metabolic inflammation and insulin resistance associated with obesity by increasing release of gut peptides, such as glucagon-like peptide-1 and -2 (GLP-1 and -2).[10,18,20-22,26,73,74] We found that prebiotic-induced changes in the gut microbiota promote GLP-1 and GLP-2 synthesis (proglucagon mRNA, GLP-1 and GLP-2 peptides) in the proximal colon (**Fig. 2**).[25,74] Recently, we demonstrated that prebiotics, such as oligofructose, modulate 102 different taxa in obese and type 2 diabetic mice.[25] These modulations were associated with lower fat mass accumulation, increased muscle mass and improved glucose and lipid metabolism.[25] In addition, we confirmed that these effects were associated with decreased gut permeability, metabolic endotoxemia and whole body inflammation.[25] In the same study, we identified a novel mechanism explaining higher endogenous GLP-1 and GLP-2 production; prebiotic treatment increases the number of enteroendocrine cells producing GLP-1 and GLP-2 (L-cells) in the jejunum and colon (**Fig. 2**).[25] Therefore, these findings suggest that targeting enteroendocrine function could be a novel therapeutic approach to treat the inflammatory phenotype associated with obesity and type 2 diabetes.

Leptin is produced by adipocytes in proportion to the amount of adipose tissue to inform whole the body of nutritional status.[75,76] Leptin is involved in food intake and energy homeostasis regulation and is also linked to glucose homeostasis regulation and numerous gastrointestinal functions such as induction of GLP-1 secretion[77] (**Fig. 2**). Leptin resistance is a hallmark of obesity[78] and we were the first to demonstrate that gut microbiota control leptin action.[25] More precisely, we found that altering gut microbiota composition with prebiotics improves leptin sensitivity in diet-induced obese and type 2 diabetic mice (**Fig. 2**).[25] We cannot exclude that improved leptin sensitivity could be responsible for increased plasma GLP-1. Therefore, we propose that targeted gut microbiota modulations could be a novel therapeutic target to reset leptin sensitivity during obesity.

In addition to data obtained in pre-clinical studies, several effects of prebiotics have been partially confirmed in humans. For instance, it has been shown that prebiotic consumption (5 to 20 g per day) changes the gut microbiota composition and increases plasma GLP-1 levels.[79-82] These effects have been

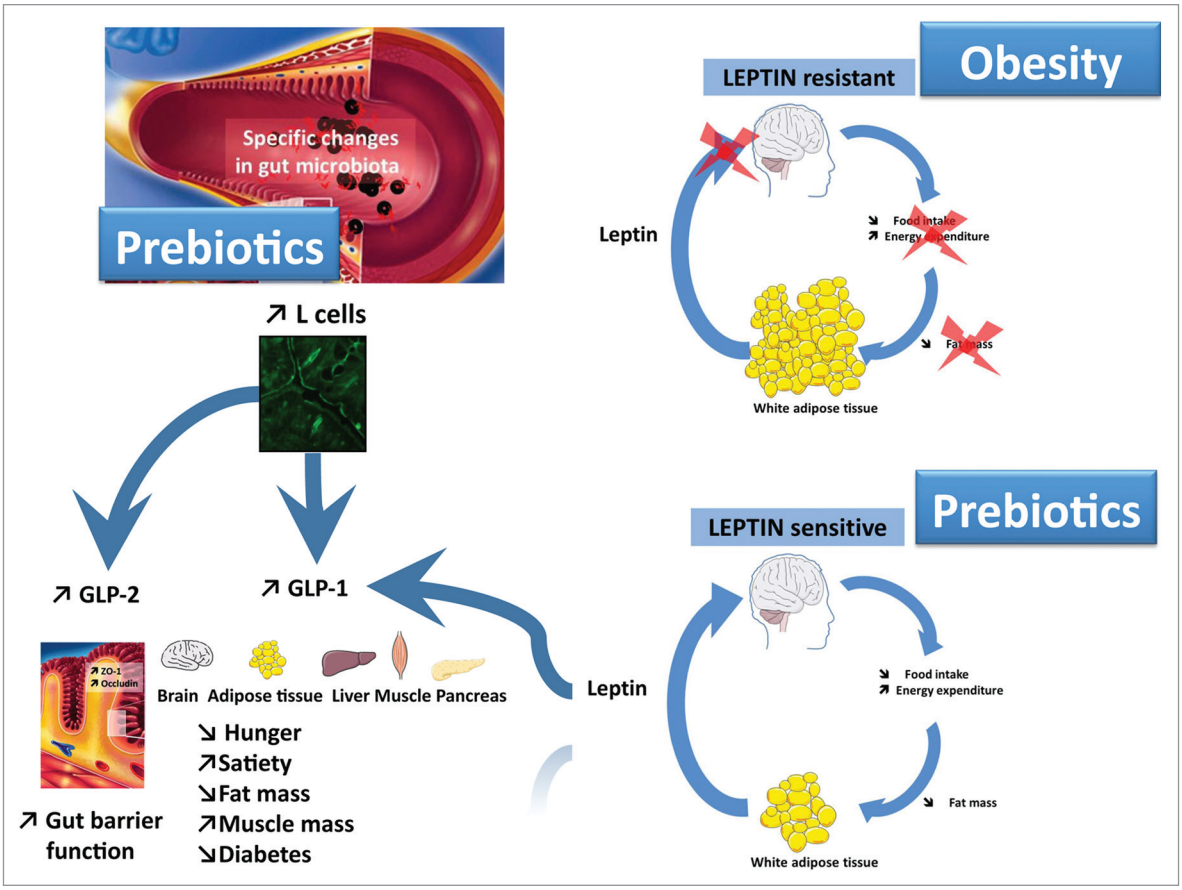

Figure 2. Prebiotic-induced changes in the gut microbiota affect enteroendocrine function and leptin sensitivity. Prebiotics profoundly affect gut microbiota composition in a complex way in response to a high-fat diet or genetic obesity (e.g., increased Bifidobacterium spp and *Akkermansia muciniphila*). Prebiotic treatment decreases gut permeability and metabolic endotoxemia and improves insulin sensitivity, steatosis and low-grade inflammation via several mechanisms, including the following: (1) an increased L-cell number and endogenous GLP-1 and GLP-2 production and (2) an increased leptin sensitivity, which controls energy homeostasis and GLP-1 production.

associated with several interesting effects, including the following: (1) lower postprandial glycemia,[81,83] (2) an increased satiety and a decreased hunger and energy intake[80-83] and (3) a reduced visceral fat mass.[80-83] Altogether, these results strongly suggest that gut peptides and their "regulator elements" contribute to communication between gut microbiota and the host, with benefits in terms of obesity pathology. Deciphering the mechanisms involved in this communication will allow the design of novel therapies for the treatment of obesity and associated metabolic disorders.

Gut Microbiota and Non-Alcoholic Fatty Liver Disease

The relationship between changes in gut microbiota and the development and progression of liver diseases has been known for over 50 y. Endotoxemia and gut-derived toxins are suggested to have causative roles in the onset and progression of liver inflammation and damage in chronic liver diseases.[84,85] Non-alcoholic fatty liver disease (NAFLD) is the most typical chronic liver complication observed in obesity and metabolic syndrome. This hepatic component of metabolic syndrome involves a complex spectrum of pathological changes, including steatosis, nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis.[86]

Similar to the mechanisms underlying endotoxemia and inflammation described in the previous sections, diet-induced intestinal bacterial overgrowth, gut leakiness and increased endotoxin absorption have all been associated with NAFLD/NASH in both rodents and human patients.[10,36,87,88] Changes in tight junction protein expression and distribution are suggested as critical factors in the impairment of gut barrier function and subsequent alterations in gut permeability observed in NAFLD patients.[89] As a consequence, hepatic exposure to gut-derived endotoxins is thought to increase TLR activation, especially on Kupffer cell membranes, and to activate nuclear transcription factors resulting in the release of numerous proinflammatory cytokines that ultimately lead to hepatic injury and fibrosis.[90-92]

Both high-fat and high-fructose diets induce important changes in gut microbiota and trigger inflammatory reactions associated with the development of systemic and hepatic insulin resistance and NAFLD/NASH.[14,27,93,94] Recently, several studies have attempted to show that proinflammatory pathway activation in Kupffer cells and hepatic resident macrophages is involved in the development of diet-induced hepatic insulin resistance.[57,95-97]

Although the inhibition of Kupffer cell functioning prevents adiposity, adipose tissue inflammation and exerted antidiabetic and antiobesity effects in response to a short exposure to HFD,[57] the role of Kupffer cells in chronic HFD-induced insulin resistance remains unclear.[95,96]

However, treatment with antibiotics or loss of TLR-4 significantly attenuate the development of hepatic steatosis in fructose-fed mice.[98] It was recently demonstrated that TLRs other than TLR4 may be involved in fructose-induced NAFLD onset.[99]

The hypothesis is that diet-induced NAFLD may be mediated by a MyD88-dependent pathway,[94] whereas the development of other forms of steatohepatitis (e.g., alcohol-related) seems to involve the MyD88-independent pathway.[100]

Additional confirmation of the close relationship between gut and liver comes from work in mice with non-alcoholic liver steatosis, in which probiotics ameliorate NAFLD/NASH and metabolic syndrome. Li et al. demonstrated that probiotic treatment improves insulin resistance, hepatic histology and total fatty acid content in mice with NASH.[101] Furthermore, the use of VSL#3, a mix of probiotic strains, decreases levels of TNFα and reduces hepatic inflammatory signaling in liver steatosis.[102,103] Similar data were reproduced in humans, suggesting that prebiotic administration could ameliorate oxidative and inflammatory liver damage associated with NAFLD.[102]

However, the lack of randomized clinical trials prevented a recent Cochrane Library review to assess the clinical effects of probiotic therapy in NAFLD/NASH patients. Therefore, additional studies are required to further prove the beneficial effects of probiotic therapy for NASH.[104]

Gut Microbiota and Gastric Bypass Surgery

Currently, strategies involving diet and increased physical activity as well as the use of anti-obesity medications offer only limited efficacy in terms of significant weight loss and long-term effectiveness. In fact, body weight loss is usually rapidly regained once dieting or drug therapy is discontinued.[105]

Presently, the most effective weight loss strategy is bariatric surgery.[106] Several reports and clinical studies analyses reported that bariatric surgery significantly reduces body weight and fat mass in obese patients over time; it also ameliorates or even cures type 2 diabetes and metabolic syndrome and has profound effects on the cardiovascular system.[107-110] Among the different bypass surgeries, including biliopancreatic diversion, ileal transposition and duodenal-jejunal bypass, the Roux-en-Y gastric bypass (RYGB) surgery is the most effective treatment for morbid obesity.[111]

The modification of food intake behavior, altered gut hormone secretion,[112] gastric emptying, bile acid metabolism and changes in intestinal gluconeogenesis[113] have all been postulated as possible explanations for improvements in body composition and nutritional and inflammatory metabolism associated with RYGB. However, mechanisms for weight loss and metabolic changes in RYGB are not fully understood.

RYGB induces eating behavior changes, with decreased appetite and meal frequency[114] and, paradoxically, increased energy expenditure.[115] Gut hormone signaling is also modified by the surgery. Postprandial levels of PYY and GLP-1 rise soon after surgery and remain elevated for many months.[107,116] GLP-2 levels and mucosal crypt cell proliferation are also increased after surgery.[117] Higher GLP-2 production may account for gut absorptive surface area restoration, which would limit malabsorption after RYGB.[117] Interestingly, these metabolic and hormonal changes can be detected before weight loss occurs. Furthermore, diabetes remission is estimated to be approximately 80–85% just days after surgery.[106] For these reasons, RYGB is now considered a potential treatment strategy for diabetes, even in the absence of obesity. In addition to the immediate and long-term adaptations associated with gastric bypass surgery, recent studies reported that microbial ecology also changes in obese subjects after RGYB.[118] In fact, post-surgery changes in gut microbiota have been suggested to play a role in the improvement of several features that characterize the pathological statuses of human obesity and type 2 diabetes.[119,120] Zhang et al. used pyrosequencing technology to show that gastric bypass surgery significantly decreased Firmicutes and increased Gammaproteobacteria, a member of the Enterobacteriaceae family, in three obese patients.

Additionally, the proportion of Bacteroides and Prevotella (a subgroup of Bacteroidetes) were shown to rapidly increase in the distal gut microbiota of 30 morbidly obese subjects after surgery.[120] *Escherichia coli* species were inversely associated with body fat mass and metabolic changes in the same patients 3 months after surgery. Interestingly, other specific bacterial groups (e.g., *Faecalibacterium prausnitzii*) were better correlated with changes in inflammatory markers and inflammation than with changes in calorie intake.[120]

However, in the same study, Lactobacillus/Leuconostoc/Pediococcus and Bifidobacterium levels were decreased after surgery.[120] This is in contrast with previous studies that reported positive correlations between Bifidobacterium levels and improvements in metabolic and inflammatory states.[121-123] However, another study in morbidly obese patients undergoing RYGB revealed that probiotic treatment with Lactobacillus significantly increased the percentage of weight loss compared with control patients up to three months after surgery.[124] Li et al. reported higher concentrations of Proteobacteria, specifically *Enterobacter hormaechei*, after RYGB in a non-obese rat model. Gut microbiota analysis of fecal samples from RYGB rats showed lower concentrations of *Firmicutes* and *Bacteroidetes* in comparison with sham-operated rats.[125] Although all of these results support a new role of gut microbiota in adaptations associated with RYGB, a definitive explanation for the beneficial improvements observed following bypass surgery has not been fully elucidated.[118]

Future studies are needed to explore the molecular and physiological mechanisms observed post-RYGB, with particular attention given to the relationships between changes in eating behavior, gut hormone levels and the gut microbial community.

In addition to the specific dietary treatments (prebiotics and probiotics) and surgical interventions (gastric bypass) showing the interaction between the gut microbiota and host metabolism, recent preliminary experiments have shown the interest of fecal transplantation in humans. Vrieze et al. have shown that

fecal transplantation from lean donors to 18 obese patients significantly improved peripheral insulin sensitivity compared with those who received an autologous transplant. Although the final characterization of the gut microbiota composition was not known, and more information are required to strengthen the results, this original approach challenge the hypothesis that fecal transplantation might be view as a "new way to treat the metabolic syndrome" (Vrieze A, et al. EASD 2010; Abstract 90).

Conclusion

Compelling evidence supports the concept that the gut microbiota participates in the development of fat mass, insulin resistance and low-grade inflammation associated with obesity. Over the last five years, numerous emerging concepts have helped elucidate this "small world within"[126] in the context of the "MicrObesity."[18]

The gut microbiota seems to play a crucial role in numerous conditions associated with obesity and type 2 diabetes, from metabolic endotoxemia to gut barrier function and liver diseases. It also seems to be involved in specific dietary treatments (prebiotics and probiotics) and surgical interventions (gastric bypass).

Nevertheless, the numerous mechanisms by which gut bacteria interact with the host, including the direct involvement of specific gut microbes and/or of microbial metabolites, remains to be elucidated.

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