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The gut microbiome as therapeutic target

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ABSTRACT

Obesity, type-2 diabetes and low-grade inflammation are becoming worldwide epidemics. In this regard, the literature provides a novel concept that we call “MicroObesity” (*Microbes and Obesity*), which is devoted to deciphering the specific role of dysbiosis and its impact on host metabolism and energy storage. In the present review, we discuss novel findings that may partly explain how the microbial community participates in the development of the fat mass development, insulin resistance and low-grade inflammation that characterise obesity. In recent years, numerous mechanisms have been proposed and several proteins identified. Amongst the key players involved in the control of fat mass development, Fasting induced adipose factor, AMP-activated protein kinase, G-protein coupled receptor 41 and G-protein coupled receptor 43 have been linked to gut microbiota. In addition, the discovery that low-grade inflammation might be directly linked to the gut microbiota through metabolic endotoxaemia (elevated plasma lipopolysaccharide levels) has led to the identification of novel mechanisms involved in the control of the gut barrier. Amongst these, the impacts of glucagon-like peptide-2, the endocannabinoid system and specific bacteria (e.g., *Bifidobacterium* spp.) have been investigated. Moreover, the advent of probiotic and prebiotic treatments appears to be a promising “pharmaco-nutritional” approach to reversing the host metabolic alterations linked to the dysbiosis observed in obesity.

Although novel powerful molecular system biology approaches have offered great insight into this “small world within”, more studies are needed to unravel how specific changes in the gut microbial community might affect or counteract the development of obesity and related disorders.

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Abbreviations: ACC, Acetyl-CoA Carboxylase; AMPK, AMP-Activated Protein Kinase; CB₁, Cannabinoid Receptor type 1; ChREBP, Carbohydrate Responsive Element Binding Protein; JNK, c-Jun N-terminal Kinase; eCB, Endocannabinoid; FAS, Fatty Acid Synthase; FIAF, Fasting-Induced Adipose Factor; GLP-1, Glucagon-Like Peptide-1; GLP-2, Glucagon-Like Peptide-2; GPR41, G-Protein Coupled Receptor 41; GPR43, G-Protein Coupled Receptor 43; IL-1, Interleukin-1; IL-6, Interleukin-6; iNOS, inducible Nitric Oxide Synthase; LPL, LipoProtein Lipase; LPS, Lipopolysaccharide; MCP-1, Monocyte Chemoattractant Protein-1; MAPK, Mitogen-Activated Protein Kinase; NAFLD, Non-Alcoholic Fatty Liver Disease; NF-κB, Nuclear Factor κB; SCFA, Short-Chain Fatty Acid; SREBP-1, Sterol Responsive Element Binding Protein; TNF-α, Tumor Necrosis Factor-α; TLR-4, Toll-Like Receptor-4.

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1. Introduction

Compelling evidence supports the concept that obesity is influenced by lifestyle factors and host susceptibility. The most frequent idea is a dysbalance between energy intake and energy expenditure. Although this hypothesis is widely accepted, the growing incidence of obesity over the last 30 years cannot solely be explained by genetic factors. Obesity is classically associated with a wide cluster of metabolic alterations, including glucose intolerance, type 2 diabetes, hypertension, dyslipidaemia, fibrinolysis disorders, cardiovascular diseases and non-alcoholic fatty liver disease (NAFLD)

(Eckel et al., 2005; Ogden et al., 2007). Most of these are related to glucose homeostasis and to the development of cardiovascular diseases, and they probably result from a combination of variable associations of genetic and environmental factors (Alberti et al., 2005; Kahn et al., 2006; Matarese et al., 2007). Along with this increasing worldwide epidemic, obesity is associated with a low-grade chronic inflammation that contributes to the development of insulin resistance, type 2 diabetes and cardiovascular diseases (Hotamisligil, 2008; Shoelson and Goldfine, 2009). The mechanisms underlying obesity, fat mass development and the development of inflammation are not fully defined. Over the past decade, the physiological processes regulating body weight and energy homeostasis, including peripheral and central food intake regulatory signals and their integration, have received intense investigation (Small & Bloom, 2004; Wynne et al., 2005; Levin, 2006; Chaudhri et al., 2008; Neary & Batterham, 2009). In the present review, we will discuss the importance in obesity of one specific environmental factor that evolves with us and our dietary habits from birth to death, namely the gut microbiota.

2. Gut microbiota: the small world within

Since Robert Koch and Ilya Mechnikov were awarded two Nobel prizes in physiology and medicine in the 1900's for their discoveries linking microbes and human health, several determinants of host-microbe interactions, both spatial (e.g., skin, mouth, and gut) and temporal (e.g., birth and senescence), have been partially unravelled. Nevertheless, knowing that the human microbiota consist of as many as 10 to 100 trillion microorganisms, representing at least 10-fold more cells than exist in the human body (Savage, 1977), it is easily conceivable that both humans and microbes have been influenced by their continuous intimate co-evolutionary history. In addition, the microbiome encodes a consortium of genes exceeding the human genome by a magnitude of 150. Altogether, these data support the astounding concept that we are not 100% human, but merely 10% human and 90% microbes (Fig. 1). Therefore, it is accepted that in this "small world within" the gut provides important biological and metabolic functions that cannot be performed by our human metabolism (Jia et al., 2008). Thus, this complex symbiosis and its development are probably dependent on interactions between host-microbe genetics and the environment (Ley et al., 2006a; Khachatryan et al., 2008). Although the exact composition of the gut microbiota is not known, advances in metagenomic technologies have recently begun to unravel our microbial partners (the human microbiome)

(Qin et al., 2010). It is estimated that each individual houses at least 160 such species from a consortium of 1000 to 1150 prevalent bacterial species (Qin et al., 2010). Amongst these bacteria, 90% of the bacterial phylotypes are members of two *phyla* (Bacteroidetes and Firmicutes), followed by Actinobacteria and Proteobacteria (Eckburg et al., 2005; Ley et al., 2005; Qin et al., 2010) (Fig. 1). Importantly, due to the difficulty of obtaining samples from the different regions of the intestine, most of the studies investigating the ecology and activity of microbiota within the intestinal tract have been carried out using faecal samples (Roberfroid et al., 2010). However, the gut microbiota community observed in the faecal samples is not exactly representative of that of the small intestine as a whole, thereby, this caveat merits to be mentioned and further supports the need of specific investigations.

3. Evidence for interrelations between the gut microbiota and energy homeostasis

Today, much attention is paid to the role of the gut microbiota and host energy homeostasis and metabolic functions. Hence, the gut microbiota have been recently proposed to be an environmental factor involved in the control of body weight and energy homeostasis (Backhed et al., 2004, 2005, 2007; Ley et al., 2005, 2006b; Turnbaugh et al., 2006). This "exteriorised organ" contributes to our homeostasis via multiple metabolic functions and diverse control mechanisms involved in the extraction of calories from ingested dietary substances, and it helps to store these calories in host adipose tissue for later use. To date, unequivocal evidence for the role of the gut microbiota in energy homeostasis comes from studies performed in mice lacking gut microbiota (Backhed et al., 2004, 2007; Fleissner et al., 2010; Rabot et al., 2010). For instance, mice raised in the absence of microorganisms (bacteria-free, axenic or germ-free) were 40% leaner (less body fat) than mice living with normal gut microbiota (conventionalised) (colonisation of the gut at birth or by inoculation), even though the latter ate about 30% less chow than the axenic mice (Backhed et al., 2004). More strikingly, conventionalisation of axenic mice with microbes harvested from the cecum of lean mice induced a dramatic increase of body weight (total body fat mass) within two weeks unless food intake was decreased (Backhed et al., 2004). This fat mass gain was even more pronounced when the gut microbial community was derived from genetically obese (*ob/ob*) mice (Turnbaugh et al., 2006). These results are likely due to differences in the microbiomes and derived metabolites of the lean and obese

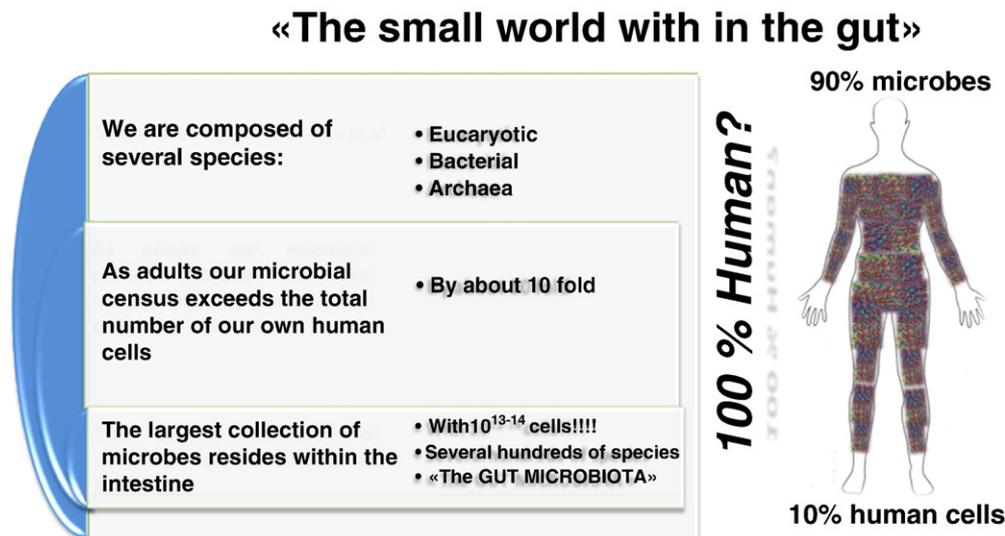


Fig. 1. The small word within. We are composed of multiple species: eukaryotic, bacterial and archaeal. With densities as high as 10^{11} cells/g in the colon, it has been estimated that our microbial census outnumbers our eukaryotic cells by a factor of ten, lending support to the astounding concept that we are not 100% human, but 90% microbes and 10% human.

mice. In this regard, Ley et al. have shown that obese mice exhibit a division-wide shift between the two major *phyla* (Firmicutes and Bacteroidetes), with a higher Firmicutes to Bacteroidetes ratio in the cecum biota of obese mice than in that of lean mice (Ley et al., 2005).

To fill out this interesting story linking the composition of the gut microbial community to energy homeostasis, a series of mechanisms has been proposed.

The first pathway suggested by investigators was that gut microbiota conventionalisation results in a doubling of the density of capillaries in the small intestinal villus epithelium, thereby helping to promote intestinal monosaccharide absorption (Stappenbeck et al., 2002).

The second set of mechanisms is related to energy extraction from undigested food components, which are directly fermented into short chain fatty acids (SCFAs) and eventually participate in hepatic de novo lipogenesis through the expression of several key enzymes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Both ACC and FAS are controlled by carbohydrate responsive element binding protein (ChREBP) and sterol responsive element binding protein (SREBP-1) (Denechaud et al., 2008). Accordingly, Backhed and colleagues have provided evidence that conventionalisation of axenic mice promotes hepatic ChREBP and SREBP-1 mRNA expressions (Backhed et al., 2004) (Fig. 2). Interestingly, the development of adipose tissue observed following gut colonisation with microbes was not directly explained by the modulation of adipose tissue differentiation or lipogenesis. A role for adipocyte lipoprotein lipase (LPL) activity was proposed. Consistent with this hypothesis, the authors have suggested that gut microbiota promote fat storage through a mechanism linking circulating triglycerides with suppression of the intestinal expression of an LPL inhibitor (FIAF, fasting-induced adipose factor) (Backhed et al., 2004). FIAF inhibits LPL activity, thereby reducing fatty acid release from circulating triacylglycerols. Hence, upon gut colonisation with microbiota, FIAF expression is reduced, leading to higher LPL activity and greater fat storage (Backhed et al., 2004) (Fig. 2). In accordance with this hypothesis,

these authors found that FIAF-deficient mice were resistant to gut microbiota-induced body weight gain (Backhed et al., 2004). Nevertheless, a recent study has suggested that the FIAF mechanism is not universally associated with gut microbiota-related fat mass development. For instance, it has been recently shown that axenic mice on high-fat diet showed increased intestinal mRNA expression of FIAF with no major changes in circulating FIAF compared with conventionalised mice (Fleissner et al., 2010).

A third pathway further explores the underlying mechanisms related to the resistance of axenic mice to high-fat diet induced obesity and associated metabolic disorders (Fig. 3) (Backhed et al., 2007). In this study, the activation of AMP-activated protein kinase (AMPK) explained the apparent resistance of axenic mice to the development of obesity in response to a high-fat diet (Backhed et al., 2007). More precisely, the gut microbiota were found to suppress AMPK-driven fatty acid oxidation in the liver and in skeletal muscle (Backhed et al., 2007) (Fig. 3).

A fourth pathway involving SCFAs has been proposed. Samuel et al. have demonstrated that G-protein coupled receptor 41 (GPR41) knockout mice colonised with a specific fermentative microbial community resist fat mass gain compared to their wild-type littermates (Samuel et al., 2008). SCFAs act as signalling molecules and are specific ligands for at least two G protein-coupled receptors, GPR41 and GPR43 (Le et al., 2003). In accordance with the potential role of these GPRs in fat mass development, a recent study has shown that G-protein coupled receptor 43 (GPR43) knockout mice are resistant to diet-induced obesity (Bjursell et al., 2011). Therefore, this set of experiments strongly supports the idea that specific metabolites coming from the gut (i.e., SCFAs) act in a variety of ways (e.g., as energy substrates and as metabolic regulators) (Fig. 3).

The original idea that the gut microbiota contribute to energy extraction from the diet through higher production of SCFAs has been challenged by multiple paradoxes. For instance, it is not clear whether the higher amount of SCFAs found in the faeces of obese animals or subjects directly contributes to the development of fat mass and body weight gain (Ley et al., 2005, 2006b; Turnbaugh et al., 2006). In

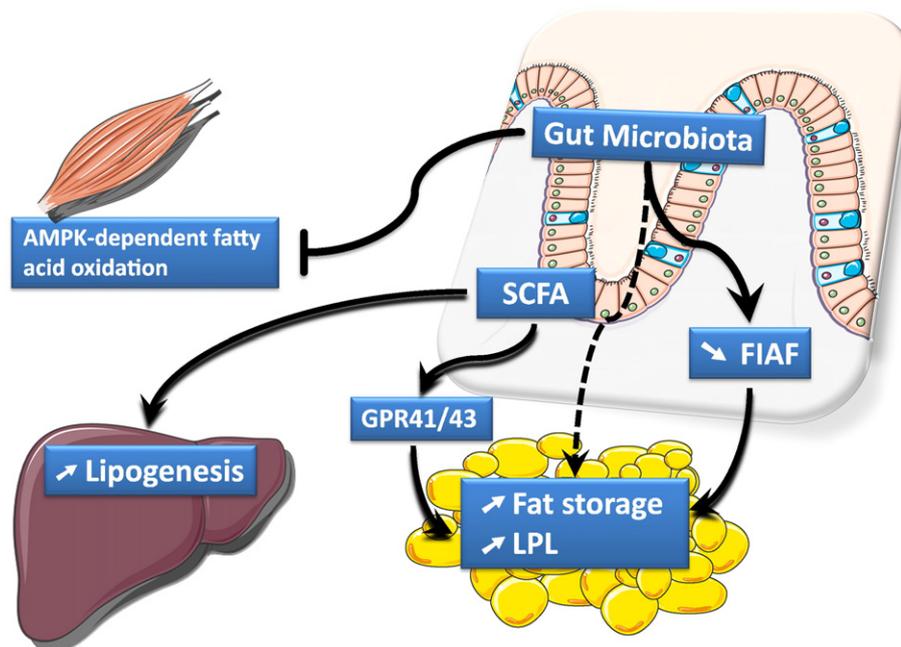


Fig. 2. Gut microbiota increase energy storage. Gut microbiota might be involved in energy storage through various mechanisms, mostly demonstrated in axenic mice. By increasing short chain fatty acid production and absorption, the gut microbiota provide lipogenic substrates to the host, thereby increasing hepatic lipogenesis and fat storage via several mechanisms. For instance, by suppressing Fasting-Induced Adipose Factor (FIAF) in the gut, the gut microbiota indirectly control the activity of the enzyme lipoprotein lipase (LPL). In addition, SCFA participates in fat storage by acting through GPR41 and GPR43. Finally, in response to a high-fat diet, the gut microbiota inhibit AMPK-dependent fatty acid oxidation; however, it should be noted that other direct or indirect mechanisms exist (dotted line).

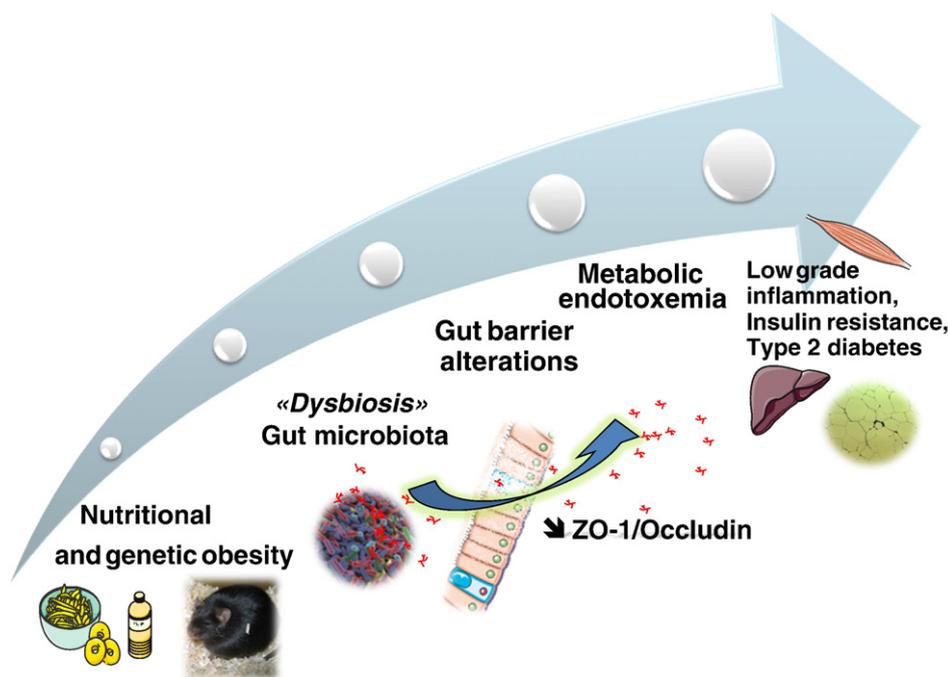


Fig. 3. The gut microbiota are involved in the onset of metabolic disorders associated with obesity: a model. Nutritional (high-fat diet) and genetic (*ob/ob* mice) obesity are associated with gut microbiota dysbiosis. This leads to the occurrence of gut permeability (altered distribution of the tight junction proteins ZO-1 and Occludin), promoting metabolic endotoxaemia and initiating the development of low-grade inflammation and insulin resistance in the liver, muscles and adipose tissue.

contrast to normal chow diet experiments, axenic mice fed a high-fat diet were resistant to western-type diet-induced obesity, whereas energy intake and faecal energy content were equivalent between the axenic and the conventionalised mice. Moreover, our results indicate that a diet enriched with specific non-digestible carbohydrates leads to greater intestinal SCFA production and thereby mitigates body weight gain, fat mass development and the severity of diabetes (Cani et al., 2004, 2005a, b, 2006a, b). These specific non-digestible carbohydrates embody the prebiotic concept: “The selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host” (Roberfroid et al., 2010). In addition, these prebiotic compounds promote the blooming of specific strains able to digest polysaccharides and provide extra energy to the host as they increase the total mass of bacteria in the colon (Kleessen et al., 2001; Kolida et al., 2007, 2006).

In the quest of potential therapeutic approaches based on gut microbiota modulation in humans, only few but promising studies have been published. It is well known that specific changes in the gut microbiota composition by using prebiotics strongly promote SCFA production (Roberfroid et al., 2010). Interestingly, this phenomenon has been associated with changes in ingestive behaviour by mechanisms linked to the modulation of gut peptide production and secretion (i.e., glucagon-like peptide-1 [GLP-1], peptide-YY and ghrelin). For instance, in healthy subjects, inulin-type fructans feeding (18 to 20 g/day) significantly increased gut microbiota fermentation (Piche et al., 2003; Cani et al., 2009a). In these studies, feeding of prebiotics was associated with increased plasma levels of GLP-1 (Piche et al., 2003; Cani et al., 2009a) and peptide-YY (Cani et al., 2009a). In two different studies, fermentation of prebiotics by gut microbiota has been related to reduced hunger and increased satiety, thereby decreasing total energy intake by about 10% (Cani et al., 2006a, 2009a). Two other studies, one by Archer et al. (2004) and one by Whelan et al. (2006), have confirmed that gut microbiota fermentation of non-digestible carbohydrates controlled food intake behaviour and impacted on energy intake. Importantly, in obese patients prebiotic-induced changes in the gut microbiota decreased

circulating ghrelin and increased peptide-YY (Parnell & Reimer, 2009). Nevertheless, some papers report that acute or low dose prebiotic (<8 g/day) treatment does not necessarily affect appetite (Peters et al., 2009; Hess et al., 2011). A recent study has shown that a single dose of prebiotics (i.e., inulin) significantly increased postprandial plasma levels of GLP-1 and decreases plasma levels of ghrelin (Tarini & Wolever, 2010). This finding contradicts the previous idea that persistent and prolonged gut microbiota modulation is required to establish an effect on gut endocrine function.

As regards the mechanisms involved in the endogenous secretion of gut peptides, it has been proposed that SCFAs come directly into play following gut microbiota fermentation. For instance, some authors proposed that acetate plays a role in this important regulation, as they found that the modulation of plasma SCFAs was related to changes in gut peptides involved in appetite regulation but also to decreases of inflammatory markers in insulin-resistant subjects (Freeland et al., 2010a; Freeland & Wolever, 2010). Therefore, the data support a role of a microbiome-dependent metabolic flux in the regulation of energy storage that does not solely favour fat storage.

The complexity of the gut microbiota is still under investigation in both lean and obese humans. Even though several observational studies have associated some specific phyla or genera with obesity (Ley, 2010) or anorexia (Armougom et al., 2009), conflicting results exist. Besides these discrepancies, therapeutic intervention or specific modulation of the gut microbiota composition by prebiotics is consistent, at least, with regard to the bloom of some genera, whose presence is often associated with beneficial health effects (e.g., *Bifidobacterium* spp.). For example, the relative contribution of the *Bifidobacterium* spp. merits further investigations in the field of obesity (Boesten & de Vos, 2008; Boesten et al., 2009; Turroni et al., 2009). Nevertheless, the prebiotic approach also seems interesting because it may help promote other beneficial bacteria. In addition, inulin-type fructans have been shown to increase *Faecalibacterium prausnitzii* in healthy volunteers (Ramirez-Farias et al., 2009). These bacteria have been shown to modulate inflammation and diabetes in obese individuals (Furet et al., 2010). Finally, the genus *Lactobacillus*

spp., belonging to the Firmicutes phylum, is subject to controversial findings and discussions within the literature (Raoult, 2008; Armougom et al., 2009; Delzenne & Reid, 2009; Ehrlich, 2009; Santacruz et al., 2009; Andreasen et al., 2010; Aronsson et al., 2010; Balamurugan et al., 2010; Kadooka et al., 2010; Luoto et al., 2011, 2010). This debate is related to the potential association of lactobacilli and obesity. To date, this debate remains unresolved, but it is likely that such an association exists with some specific species being protective against obesity whereas other species are in fact associated with weight gain. A very simple analogy could be proposed with the commensal strains of *Escherichia coli*, which could be viewed as potential pathogens, whereas another specific strain such as *E. coli* strain Nissle 1917 is known to positively impact on intestinal inflammation (Trebichavsky et al., 2010). These data raise the crucial questions whether the obesity-related effects are strain-specific and, more importantly, what mechanisms are behind these differential actions.

4. Gut microbiota, inflammation and insulin resistance

Although a large body of evidence supports the idea that extraction of energy from the diet by gut microbiota leads to the development of obesity and related metabolic disorders via multiple mechanisms, these theories have not unravelled the interplay between gut microbes, obesity-related metabolic disorders and the onset of low-grade inflammation. Numerous studies support the idea that this inflammation may derive from macrophage infiltration into organs (adipose tissue, muscles, and liver), which promotes the secretion of pro-inflammatory factors (Weisberg et al., 2003; Xu et al., 2003; Tordjman et al., 2008; Olefsky & Glass, 2010). Nonetheless, the exact role of macrophages and the source and type of triggering factors activating the immune system in this specific context remain a matter of debate (Odegaard & Chawla, 2008; Kosteli et al., 2010).

Given the plethora of inflammatory factors (e.g., interleukin-1 [IL-1], tumour necrosis factor- α [TNF- α], monocyte chemoattractant protein-1 [MCP-1], inducible nitric oxide synthase [iNOS], interleukin-6 [IL-6]) causally related to the development of impaired insulin action (or insulin resistance) and the multiple molecular interactions between immunity and insulin signalling, we have been seeking a potential integrating factor able to elucidate these mechanisms.

For instance, c-Jun N-terminal kinase (JNK), the transcription nuclear factor κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) control specific molecular pathways that play crucial roles in the development of inflammation and insulin resistance.

The pro-inflammatory effect of a high-fat diet has mainly been attributed to the inflammatory properties of dietary fatty acids (i.e., palmitic acid). Recently, it has been proposed that such fatty acids trigger an inflammatory response by acting via LPS receptor (Toll-like receptor-4 [TLR-4]) signalling in adipocytes and macrophages, which might contribute to the inflammation of adipose tissue in obesity (Shi et al., 2006; Suganami et al., 2007a, b). However, the direct link between fatty acids and TLR4 has been revisited and contested (Erridge & Samani, 2009). Strikingly, all these molecular steps play a pivotal role in the integration of the metabolic and immune responses upon infection through gram-negative bacteria-derived compounds, namely lipopolysaccharides (LPS) (Guha & Mackman, 2001). Thus, given that obesity and type 2 diabetes are closely associated with a low-grade inflammatory state and an intricate interplay of receptors involved in host-microbe interactions, we have investigated the role of a microbe-related factor in the aetiology of obesity and associated disorders.

Recently, we defined gut microbiota-derived LPS as the *primum movens* in the early development of inflammation and metabolic diseases (Cani et al., 2007a). More precisely, we demonstrated that excess dietary fat not only increases systemic exposure to potentially pro-inflammatory free fatty acids and their derivatives but also

facilitates the development of metabolic endotoxaemia (e.g., increased plasma LPS levels) (Cani & Delzenne, 2007; Cani et al., 2007a). Given that LPS can affect inflammation throughout the body and interfere with both metabolism and the function of the immune system, this novel hypothesis provides a new insight into the role of gut microbiota-derived products and metabolism. Accordingly, it is increasingly recognised that the innate immune system and metabolic pathways are functionally intertwined (Olefsky & Glass, 2010).

A series of experiments has shown that gut bacteria can initiate the inflammatory processes associated with obesity and insulin resistance by modulating plasma LPS levels (Fig. 4). The first experiment supporting a connection between the gut microbiota and a high-fat diet led to the discovery of a microbial dysbiosis between lean normal chow-fed mice and high-fat diet fed mice. In this study, a high-fat diet increased plasma LPS levels (metabolic endotoxaemia) two- to three-fold. Interestingly, the high-fat diet was also linked to specific changes in the gut microbial community, with a marked reduction in *Bifidobacterium* spp., *Bacteroides*-related bacteria and *Eubacterium rectale-Clostridium coccoides* (Cani et al., 2007a, c). The relevance of LPS signalling to the development of diet-induced low-grade inflammation was further explored by a study of mice lacking the co-receptor CD14 of the Toll-like receptor-4 (TLR-4): CD14^{-/-} mice. After four weeks of a high-fat diet, these mice exhibited more fat mass and higher body weight associated with a low-grade inflammatory state (liver, adipose tissues and muscles). Strikingly, in the absence of a functional LPS receptor, mice were resistant to diet-induced obesity and related disorders (including hepatic insulin resistance) (Cani et al., 2007a). We also demonstrated that chronic metabolic endotoxaemia produced by chronic subcutaneous infusion of LPS (mimicking metabolic endotoxaemia) significantly induces inflammation and insulin resistance. Regarding fat mass development, the chronic administration of LPS increased subcutaneous and visceral mass by about 30% and 40%, respectively. It should be noted that the relative increases in fat mass and body weight due to LPS administration or the high-fat/carbohydrate-free diet that we used in this protocol were lower than those observed with a western-type diet. Nevertheless, other studies favour this hypothesis because in the absence of the LPS receptor (CD14^{-/-} or TLR-4^{-/-} models), mice are resistant to diet-induced metabolic disorders (Cani et al., 2007a; Tsukumo et al., 2007; Davis et al., 2008; Roncon-Albuquerque et al., 2008). The role of gut microbiota-derived LPS as a factor triggering low-grade inflammation, type 2 diabetes and insulin resistance was subsequently investigated in both nutritionally and genetically obese mice through specific modulation of the composition of the gut microbiota (Cani et al., 2008; Membrez et al., 2008).

First, we found that changing the gut microbiota by antibiotic treatment protects against diet-induced fat mass development, glucose intolerance, insulin resistance, inflammation and oxidative stress. This set of studies strongly suggests that a high-fat diet might not be able to directly cause obesity.

Second, we found that genetically obese *ob/ob* mice exhibited an improved metabolic phenotype (i.e., insulin resistance and inflammation) upon gut microbiota manipulation, whereas their total body weight gain was unchanged (Cani et al., 2008). To gain better insight into the role of LPS in the pathogenesis of the inflammation and insulin resistance associated with obesity, we decided to interfere with LPS signalling using two specific models. In the first model, we administered chronic subcutaneous LPS quenchers (polymyxin B or endotoxin inhibitors) for 4 weeks in genetically obese *ob/ob* mice. The second model consisted of the generation of *ob/ob* mice lacking the LPS receptor CD14 (*ob/ob* CD14^{-/-}). The results obtained following the investigation of all these specific models indicated significant decreases in inflammation and macrophage infiltration markers together with improved glucose tolerance and insulin resistance (Cani et al., 2008). These experiments

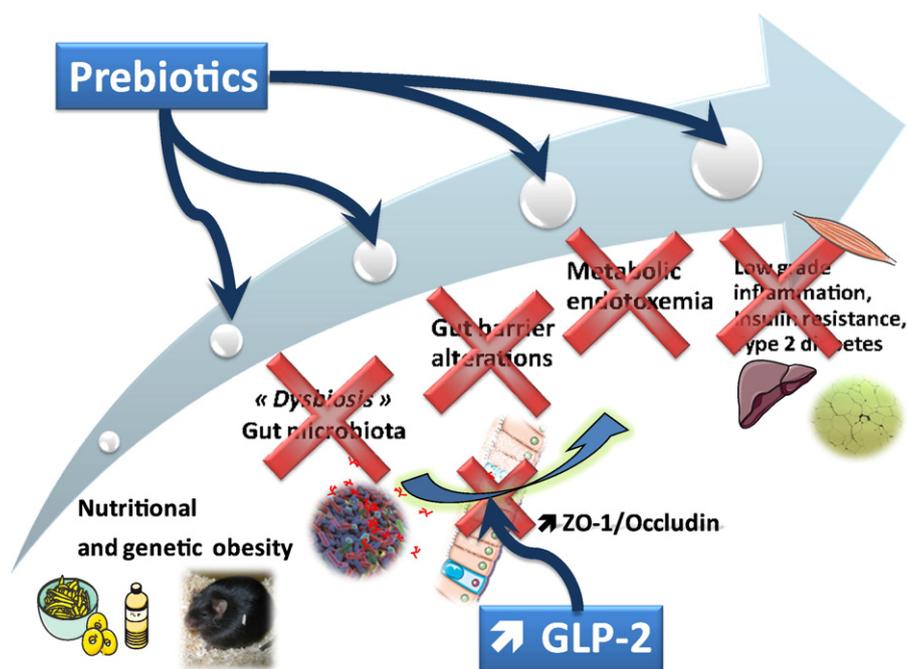


Fig. 4. Prebiotic-induced changes in the gut microbiota can abolish obesity-associated metabolic features. Prebiotics modulate the composition of the gut microbiota in a complex way in response to a high-fat diet or genetic obesity (i.e., increased *Bifidobacterium* spp.). Prebiotic treatment decreases gut permeability and metabolic endotoxaemia but improves insulin sensitivity, steatosis and low-grade inflammation. One of the mechanisms explaining this phenomenon is increased endogenous GLP-2 production, which restores gut barrier function.

clearly demonstrate the contribution of LPS derived from the gut microbiota to metabolic endotoxaemia. Consistent with this set of data, other studies have reported that plasma LPS is elevated in *ob/ob* and *db/db* mice (Brun et al., 2007). Furthermore, polymyxin B treatment, which specifically eliminates Gram negative bacteria and further quenches LPS, diminished hepatic steatosis (Pappo et al., 1991). Together with the earlier results, these findings strongly suggest that the gut microbiota contribute to the metabolic endotoxaemia related to both genetic and diet-induced obesity.

The relationship between a high-fat diet, obesity, type 2 diabetes and LPS was subsequently confirmed in human subjects. In the last three to four years, numerous studies have confirmed the concept of high-fat diet-induced metabolic endotoxaemia in healthy and obese humans. First, Erridge et al. examined baseline endotoxaemia concentrations in healthy human subjects and found that a high-fat meal induces a metabolic endotoxaemia that fluctuates rapidly to concentrations sufficient to induce some degree of inflammation (Erridge et al., 2007). We also found in a cohort of 211 subjects a link between energy intake (high-fat diet) and metabolic endotoxaemia (Amar et al., 2008). Furthermore, it has been shown that metabolic endotoxaemia increases adipose TNF- α and IL-6 concentrations and insulin resistance in healthy volunteers (Anderson et al., 2007). In addition, Creely et al. recently reinforced the hypothesis that metabolic endotoxaemia might act as a gut microbiota-related factor involved in the development of type 2 diabetes and obesity in humans (Creely et al., 2007). A recent study investigating the impact of a pancreatic and gastric lipase inhibitor has linked metabolic endotoxaemia to impaired glucose tolerance (Dixon et al., 2008). Moreover, it has been shown that changes in metabolic endotoxaemia in obese type 2 diabetic patients are inversely correlated with multiple plasmatic parameters (i.e., triglycerides, cholesterol, glucose, and insulin) (Al-Attas et al., 2009). Finally, the relationship between a high-fat diet and metabolic endotoxaemia has been confirmed in multiple independent studies (Ghoshal et al., 2009; Ghanim et al., 2009; Deopurkar et al., 2010; Laugerette et al., 2011). Altogether, these findings reinforce the role of fat intake (and absorption) in the development of metabolic endotoxaemia.

Although much data supports the role a mechanism dependent on LPS-TLR-4/CD14 complex activation, emerging evidence supports the concept that other TLRs might be involved in the development of the insulin resistance and low-grade inflammation associated with obesity. Recently, several independent studies investigating the role of TLR-2 have causally linked the development of diet-induced obesity and metabolic disorders to this pathogen-associated receptor (Davis et al., 2011; Ehses et al., 2010; Himes & Smith, 2010; Kuo et al., 2011). Interestingly, TLR-2 recognises a large number of lipid-containing molecules including bacterial lipopeptide (Lien et al., 1999). Moreover, the expression and induction of TLR-2 are directly under the control of LPS, but TLR-2 can also be induced by TNF- α and CD14 (Lin et al., 2000). These data have subsequently been confirmed, and it has been proposed that the up-regulation of TLR-2 in the presence of low but clinically relevant levels of microbial products is an important mechanism by which the immune system boosts its response to a recent infection (e.g., LPS) (Nilsen et al., 2004). Therefore, we propose that metabolic endotoxaemia triggers the activation of TLR-2, thereby amplifying LPS/TLR-4/CD14 complex signalling to stimulate the inflammatory response. In addition, different studies have proposed that saturated fatty acids promote low-grade inflammation and insulin resistance through a TLR-4 dependent mechanism (Shi et al., 2006; Suganami et al., 2007a, b). However, it has been suggested that the effect of saturated fatty acids on TLR-4 activation might be due to LPS contamination of the fatty acid preparations or of the bovine serum albumin used in these studies (Erridge & Samani, 2009). It can be argued with more certainty that fatty acids are indeed involved in the stimulation of the innate immune system, but probably in conjunction with initial stimulation by LPS of the TLR-4/CD14 complex and subsequent TLR-2 stimulation. Multiple observations support these hypotheses: i) altering gut microbiota with antibiotics protects mice from diet-induced obesity and metabolic disorders, even in the presence of functional TLR-4/2 receptors (Cani et al., 2008); ii) CD14 knock-out mice do not develop fat-induced insulin resistance and low-grade inflammation, even though the TLR-4 and TLR-2 receptors are fully expressed (Cani et al., 2007a; Roncon-

Albuquerque et al., 2008), however, it should be mentioned that CD14 is required for an appropriate functionality of both TLR-2 and TLR-4 (Buwitt-Beckmann et al., 2005; Heine & Ulmer, 2005); and iii) axenic mice fed a high-fat diet are resistant to the development of high-fat diet-induced inflammation and insulin resistance, even though they fully digest and absorb the ingested fat (Backhed et al., 2007; Rabot et al., 2010). Taken together, these experiments suggest that a signalling cascade initiated by an LPS/TLR-4/CD14-dependent mechanism in turn activates TLR-2 expression to support innate immune system inflammatory responses.

5. Gut microbiota and gut permeability: novel insights into “MicroObesity”

Amongst the causes potentially involved in the development of metabolic endotoxaemia, numerous studies support the idea that a host–bacterial mutualism leads to the control of gut barrier function (Brun et al., 2007; Cani et al., 2008, 2009b; De La Serre et al., 2010; Muccioli et al., 2010).

Metabolic endotoxaemia (or even higher plasma LPS levels) can result from multiple mechanisms, including increased production of endotoxins upon changes in the gut microbiota (Cani et al., 2007a, c). Under healthy physiological conditions, the gut epithelium acts as a powerful and continuous barrier that prevents bacterial translocation (i.e., LPS). However, various endogenous and/or exogenous situations are associated with alteration of this protective function. Amongst the recognised factors specifically leading to a leaky gut (and thereby promoting metabolic endotoxaemia), immobilisation stress (Mazzon & Cuzzocrea, 2008), alcohol consumption (Nanji et al., 1993; Nishida et al., 1994; Adachi et al., 1995; Enomoto et al., 1998; Rivera et al., 1998; Enomoto et al., 2001) and radiation (Paulos et al., 2007) have been proposed. In addition, we and others have recently proposed that changes in the distribution and localisation of Zonula Occludens-1 (ZO-1) and Occludin (two tight junction proteins) in intestinal tissue are associated with the increased gut permeability occurring in

obese and diabetic rodents (Fig. 4) (Brun et al., 2007; Cani et al., 2008, 2009b; De La Serre et al., 2010; Muccioli et al., 2010).

Various mechanisms have emerged to link the changes in the gut microbiome in obesity with changes in gut barrier function (Figs. 4 and 5). In a recent study, we demonstrated that altering the gut microbiota of genetically obese mice with prebiotics is associated with a significant improvement in gut permeability measured *in vivo*; this phenomenon is linked to increased tight junction mRNA expression and improved protein distribution (Fig. 4). In this study, we found that an improved gut barrier was strongly correlated with reduced portal plasma LPS levels and low-grade inflammatory tone (i.e., decreases in hepatic and circulating cytokines) (Cani et al., 2009b). Furthermore, we demonstrated that lowering systemic inflammation with prebiotics significantly correlates with marked decreases in oxidative and inflammatory stresses in liver tissue. Although these data strongly suggested that modulation of the gut microbiota in obese mice with prebiotics could act favourably on the intestinal barrier, the mechanisms by which prebiotics improve gut permeability in the particular context of obesity remained to be elucidated. Therefore, we investigated the role of a specific gut peptide involved in the control of epithelial cell proliferation and gut barrier integrity, namely glucagon-like peptide-2 (GLP-2) (Jeppesen et al., 2001; Thulesen et al., 2001; Martin et al., 2005; Chiba et al., 2007; Dube & Brubaker, 2007).

We investigated this particular peptide because in our previous work, we found that prebiotic-induced changes in the gut microbiota promote GLP-1 synthesis (proglucagon mRNA and GLP-1 peptide) in the proximal colon by a mechanism linked to the differentiation of precursor cells into enteroendocrine cells (Cani et al., 2004, 2005a, b, 2006b; Cani et al., 2007b; Delzenne et al., 2007). Given that both GLPs are produced and secreted by L-cells and that endogenous production of GLP-1 increases upon prebiotic-induced changes in the gut microbiota, we focused our research on GLP-2. We found that increased endogenous GLP-2 production was associated with improved mucosal barrier function via the restoration of tight junction protein expression and distribution (Fig. 4). To further investigate the role of GLP-2 in the protective effects of prebiotics, we blocked GLP-2

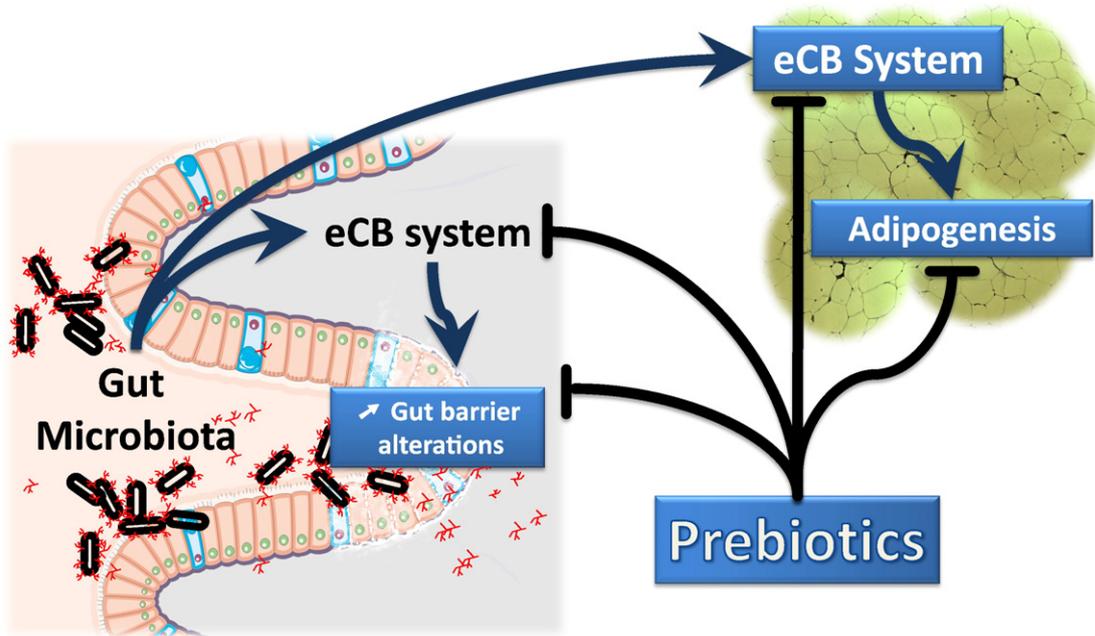


Fig. 5. Gut microbiota determine gut permeability and adipose tissue physiology through LPS–eCB system regulatory loops. The eCB system is hyper-activated in the intestines with consequent increases in gut permeability, plasma LPS levels and systemic inflammation. Crosstalk between the eCB system and gut microbiota participates in the regulation of adipogenesis directly by acting on adipose tissue and indirectly by increasing plasma LPS levels. Prebiotic-induced changes in the gut microbiota decrease both intestinal and adipose tissue eCB system responsiveness, thereby improving the gut barrier and normalising adipogenesis.

receptors concomitantly with prebiotic-associated changes in the gut microbiota. Interestingly, the GLP-2 antagonist completely blocked the major features of the prebiotic treatment. Hence, without a functional GLP-2 receptor, the prebiotic treatment failed to reduce metabolic endotoxaemia, hepatic inflammation and oxidative stress markers. Collectively, these data support the concept that specific changes in the gut microbiota improve gut permeability and inflammatory tone via a GLP-2-dependent mechanism (Cani et al., 2009b) (Fig. 4).

Besides these interesting changes in gut permeability upon prebiotic treatment, the measurements of gut permeability were carried out primarily in the jejunum and ileum, whereas the modulation of gut microbiota occurs in the distal part of the intestine, namely the colon. Amongst the potential mechanisms involved, we propose that changing the gut microbiota by prebiotics controls and increases endogenous production of the intestinotrophic proglucagon derived peptide GLP-2 not only in the colon but also in the jejunum (Cani et al., 2009b), and consequently improves gut barrier functions in the upper part of the gut via both auto- and paracrine regulatory loops (Cani et al., 2009b). In addition, it has been previously found that prebiotics increase villus height, crypt depth and increase the thickness of the mucosal layer in the jejunum and colon (Kleessen et al., 2003). In addition, we cannot rule out that prebiotic feeding impacts on the microbial community which resides within the first part of the gut, although this hypothesis remains to be confirmed.

One additional mechanism potentially involved in the impact of the gut microbiota on the development of obesity and related disorders is the endocannabinoid system (eCB). The investigation of this very specific biological system originated in the following observations:

- 1 the massive expansion of adipose tissues upon obesity is characterised by low-grade inflammation, possibly controlled by the gut microbiota (via LPS);
- 2 obesity is also characterised by greater eCB system responsiveness (i.e., altered expression of cannabinoid receptor 1 (CB₁ mRNA) and increased plasma eCB and increased adipose tissue eCB levels) (Engeli et al., 2005; Bluher et al., 2006; Matias et al., 2006; Cote et al., 2007; D'Eon et al., 2008; Starowicz et al., 2008; Di Marzo et al., 2009; Izzo et al., 2009; Muccioli et al., 2010);
- 3 LPS stimulates eCB synthesis (in vivo and in vitro) (Di Marzo et al., 1999; Maccarrone et al., 2001; Liu et al., 2003; Hoareau et al., 2009);
- 4 blocking the CB₁ receptor (genetically or pharmacologically) protects against obesity, steatosis and low-grade inflammation via unresolved mechanisms (Osei-Hyiaman et al., 2005; Gary-Bobo et al., 2007; DeLeve et al., 2008; Osei-Hyiaman et al., 2008).

Given this emerging evidence that the eCB system, inflammation and obesity are interconnected, we decided to investigate whether the gut microbiota and gut barrier function might be convergent molecular mechanisms. By using different models to study the interactions between the host and the gut microbial community, we found that specific changes in the gut microbiota (axenic versus conventional mice; dietary treatments that drastically or selectively change the composition of the gut microbiota; or genetic disruption of gut bacteria–host interactions) selectively decrease eCB system activity in the colon and in adipose tissue (Muccioli et al., 2010). In both diet-induced obesity and in genetically obese mice, the eCB system is overactivated in the intestine and in adipose tissue (Fig. 5). We found that the eCB system, and more specifically the CB₁ receptor, control gut barrier function. For instance, blocking the CB₁ receptor in obese mice reduces gut permeability via improved tight-junction protein (ZO-1 and Occludin) distribution and localisation (Fig. 5). In addition, CB₁ activation increases gut permeability markers in vivo and in vitro (Alhamoruni et al., 2010; Muccioli et al., 2010). Thus, this study was the first to demonstrate that CB₁ receptors control gut permeability through interactions with the gut microbiota (Muccioli

et al., 2010). In addition, we demonstrated the existence of eCB–gut microbiota crosstalk that participates in the regulation of adipogenesis (Muccioli et al., 2010) (Fig. 5).

Importantly, we found that changing the gut microbiota with prebiotics promotes the normalisation of eCB system responsiveness in both the gut and in adipose tissue. These effects are strongly associated with decreases in gut permeability, metabolic endotoxaemia and fat mass development (Fig. 5).

Nevertheless, it should be noted that even if strong correlations exist between the composition of the gut microbiota and elements controlling gut barrier function (e.g., GLP-2 and the endocannabinoid system), the direct involvement of specific gut microbes and/or of microbial metabolites remains to be elucidated.

6. Conclusions

The novel concept of “MicrObesity” has led to demonstrations of the impact of gut microbiota on host metabolism and energy storage. Each year, numerous findings emerge that help the scientific community to better understand this small world within hidden behind the shadow of our belly. Compelling evidence supports the concept that the microbial community participates in the development of the fat mass deposition, insulin resistance and low-grade inflammation that characterises obesity. The development of powerful analytical methods will provide novel data lending insight into the complexity of the gut microbiota. Nevertheless, this will also raise several novel questions regarding the mechanisms by which gut bacteria interact with the host. The answers to these key questions will be crucial for the future development of “à la carte” treatments for dysbiosis-linked pathologies. In this regard, even though this should be appropriately verified, prebiotics are promising tools that are already available.

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