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Gut microbiome, endocrine control of gut barrier function and metabolic diseases

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Abstract

Overweight and obesity are associated with several cardiometabolic risk factors, including insulin resistance, type 2 diabetes, low-grade inflammation and liver diseases. The gut microbiota is a potential contributing factor regulating energy balance. However, although the scientific community acknowledges that the gut microbiota composition and its activity (e.g., production of metabolites and immune-related compounds) are different between healthy subjects and subjects with overweight/obesity, the causality remains insufficiently demonstrated. The development of low-grade inflammation and related metabolic disorders has been connected with metabolic endotoxaemia and increased gut permeability. However, the mechanisms acting on the regulation of the gut barrier and eventually cardiometabolic disorders are not fully elucidated.

In this review, we debate several characteristics of the gut microbiota, gut barrier function and metabolic outcomes. We examine the role of specific dietary compounds or nutrients (e.g., prebiotics, probiotics, polyphenols, sweeteners, and a fructose-rich diet) as well as different metabolites produced by the microbiota in host metabolism, and we discuss how they control several endocrine functions and eventually have either beneficial or deleterious effects on host health.

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Introduction

Obesity is linked with many cardiometabolic risk factors, such as insulin resistance, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD). Although lowering body weight is effective for alleviating many of these metabolic abnormalities, prevention remains the greatest challenge. Among the different factors contributing to the regulation of energy balance, the microorganisms that reside in the human gut (called the gut microbiota) have received increasing attention. Initially, the gut microbiota was studied because of its association with classical infectious diseases, such as gut infections (*Escherichia coli*, *Shigella*), acute colitis, Crohn's disease, and inflammatory bowel disease (IBD) (Frank, et al. 2007; Lupp, et al. 2007; Macpherson and Harris 2004; Voth and Ballard 2005), but in the last two decades, it has been investigated also because of functions beyond those of pathogens (Bäckhed, et al. 2004; Cani and Delzenne 2009). This incredible awareness of the potential of the gut microbiota is translated by more than five thousand publications in 2019 alone, many of which are dedicated to the study of the gut microbiota and cardiometabolic disorders associated with overweight and obesity. However, caution must be taken with regard to the causality raised in the literature (Cani 2017, 2018; Lynch and Pedersen 2016). In this review, we specifically discuss different aspects of the link between the gut microbiota, gut barrier function and metabolic outcomes. We discuss the role of different metabolites produced by the microbiota in host metabolism and how specific nutrients may promote either beneficial or deleterious effects on host health.

Role of the microbiota in the onset of metabolic diseases

In 2004, pioneering work from Jeffrey Gordon and his team showed that mice lacking a microbiota (i.e., germ-free mice) were characterized by specific energy metabolism and even resistance to diet-induced obesity (Backhed, et al. 2007; Backhed, et al. 2004). In the same period, we identified a causal association between the gut microbiota and the development of low-grade inflammation and insulin resistance associated with obesity and lipid-rich diets (i.e., a high-fat diet, HFD) (Cani, et al. 2007). We found that some constituents of gram-negative bacteria, such as lipopolysaccharide (LPS), were the key factors triggering the onset of low-grade inflammation and insulin resistance (Cani et al. 2007). By using different animal models

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(i.e., genetic, diet-induced obesity and diabetes models), we discovered that these animals had an increased level of circulating LPS, a condition termed metabolic endotoxaemia (Cani et al. 2007). This finding has since been confirmed in several human studies (Amar, et al. 2008; Gomes, et al. 2017; Gummesson, et al. 2011; Horton, et al. 2014; Jayashree, et al. 2014; Lassenius, et al. 2011; Laugerette, et al. 2011; Monte, et al. 2011; Pussinen, et al. 2011; Radilla-Vazquez, et al. 2016) (Figure 1). Since this discovery, other pathogen-associated molecular patterns (PAMPs) (e.g., flagellin and peptidoglycans) have also been shown to play a fundamental role in the regulation of similar metabolic pathways (Chassaing, et al. 2014; Denou, et al. 2015; Vijay-Kumar, et al. 2010). Over the years, thriving literature has demonstrated that alterations in gut microbiota composition and function are widely associated with the development of metabolic diseases, especially obesity and type 2 diabetes (T2D), in humans (Cotillard, et al. 2013; Karlsson, et al. 2013; Le Chatelier, et al. 2013; Pedersen, et al. 2016). In this context, faecal microbiota transplantation (FMT) has recently emerged as a good option to assess the causal relationship between the gut microbiota and the onset of metabolic diseases (Kootte, et al. 2017; Le Roy, et al. 2013; Vrieze, et al. 2012). In addition, an increasing number of studies are seeking to identify specific microbial signatures in the gut and liver that could predict the onset and/or severity of metabolic disorders, such as liver diseases (Boursier, et al. 2016; Michail, et al. 2015; Sookoian, et al. 2020; Wang, et al. 2016). In addition to changes in the gut microbiota, it was discovered that the barrier function of the gut also played a key role (for a review: (Cani 2018; Cani, et al. 2019)). Because gut microbes are located close to intestinal epithelial cells, gut barrier function must be highly efficient to prevent the enteric microbiota and potent immunostimulatory molecules from entering the circulation. However, the gut barrier must also be permissible to allow uptake of essential nutrients and fluids. This delicate balance is part of a multifaceted system controlled through intricate mechanisms. Over the last decade, the role of gut barrier function has been investigated, and numerous studies have discovered that maintaining an adequate gut barrier requires finely tuned mechanisms that are dependent on the microbial composition. More precisely, the gut barrier is composed of several physical and chemical components. A single layer of epithelial cells that display densely packed microvilli (brush border) and are joined at their apical side by tight junction proteins (TJPs, e.g., zonula occludens 1 (ZO-1), occludin, and claudins) act as the transcellular barrier. This monolayer is renewed constantly (every 4–5 days

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due to Intectin and Cyclin D1) and is covered by a protective mucus layer that is impregnated with several immune factors (e.g., antimicrobial factors) produced by the host. Together, the mucus layer and the different antimicrobial factors (e.g., C-type lectin, primarily regenerating islet-derived 3-gamma, Reg3γ, several defensins, lysozyme C and phospholipases) contribute to maintaining gut microbes at a certain distance from intestinal epithelial cells (Bevins and Salzman 2011; Hooper and Macpherson 2010; Pott and Hornef 2012) (Figure 1). Maintaining the integrity of the gut barrier is critical and avoids structural and functional disorganization of the intestine that can lead to several disorders. IBD is the hallmark example of compromised gut barrier function (Stange and Schroeder 2019; Wehkamp, et al. 2008). Altered mucus function and chemical defence by defensins are characteristic of ulcerative colitis and Crohn's disease, respectively (Johansson, et al. 2014; Salzman, et al. 2010). It is important to note that the antibiotic action of specific defensins is reinforced by their proteolytic fragmentation into shorter peptides and thereby constitutes an interesting way to modulate gut barrier function (Ehmann, et al. 2019). Defects in this line of defence have also been correlated with metabolic diseases. By using metaproteomic resources, Zhang et al. showed that several AMPs are depleted in the faeces of T2D subjects in comparison to those in prediabetic and healthy subjects (Zhong, et al. 2019). In addition to the innate immune system, the adaptive immune system is another important contributor; for example, immunoglobulin A (IgA) is able to inhibit bacterial penetration into the host mucus and mucosal tissue (Macpherson, et al. 2012).

Therefore, gut barrier function is a very complex and multifaceted mechanism (Figure 1), and alterations in this line of defence are the first signal that allows the penetration of bacteria and thereby contributes to a local inflammatory response (e.g., IBD) and/or metabolic disorders (e.g., T2D). (reviewed in (Konig, et al. 2016; Paone and Cani 2020; Stange and Schroeder 2019; Wells, et al. 2017)).

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Effect of specific nutrients on the microbiota composition and their impact on health*: "beneficial"* versus *"deleterious"* effects

Maintaining normal intestinal barrier function is an essential aspect of human health. The growing significance of gut barrier integrity and concomitant translocation of bacteria and bacterial components raises the question of how we can improve gut barrier function. This aspect is of particular interest since alteration of intestinal homeostasis (gut microbiota composition and gut barrier function) directly or indirectly (via microbial-produced metabolites) disturbs the production and secretion of gut endocrine hormones, thereby triggering metabolic diseases (Figure 2). The most obvious strategy to maintain gut barrier integrity is to maintain a healthy nutritional status, as it has been shown that certain dietary patterns are associated with improved health (for example, a Mediterranean versus Western diet), whereas high-fat, high-sugar diets or diets depleted of certain nutrients, such as zinc, glutamine and tryptophan, could compromise gut barrier integrity (see below the section "*deleterious effects*").

Dietary patterns are dominant factors in shaping the gut microbiota. Therefore, understanding the key mechanisms involving the different components of our diet is a challenge that needs to be met. Effectively manipulating the microbiota can reduce low-grade intestinal inflammation and improve gut barrier integrity, thereby reducing plasma glucose and serum lipid levels, ultimately resulting in weight loss and decreased insulin resistance (Cani, et al. 2009a; Dewulf, et al. 2013; Dray, et al. 2007; Parnell and Reimer 2009). This constitutes a promising and feasible approach, and several dietetic concepts, including prebiotics as well as probiotics, are currently being researched (Reid, et al. 2019; Sanders, et al. 2019). Traditionally, the quality and quantity of fatty acids and dietary fibres are denoted as crucial modulators of the gut microbiota composition (Caesar, et al. 2015; Devkota, et al. 2012; Just, et al. 2018; Lam, et al. 2015; Makki, et al. 2018); however, it is now clear that other potential actors, including many different metabolites, are involved (Holmes, et al. 2011; Wikoff, et al. 2009; Zierer, et al. 2018). In the next section of this review, we will focus on specific nutrients or food additives that are currently used in the Western diet. We focus first on the "beneficial" factors and then the "deleterious" factors.

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Beneficial effects

Gut permeability and prebiotics

Since the beginning of the 2000s, the expansion of original scientific work has tended to show that the modulation of the gut microbiota population by prebiotics has a major impact on human health (Roberfroid, et al. 2010). This is particularly the case for fermentable dietary fibres, such as inulin-type fructans that include inulin and oligofructose (Gibson, et al. 2017), which are known to improve glucose homeostasis (Cani, et al. 2006; Roberfroid et al. 2010). Evidence suggests a potential link between the intake of prebiotics and the modulation of gut permeability. The increased use of prebiotics to improve insulin sensitivity and/or reduce food intake in metabolic disorders is well described in animal models and in humans (reviewed in (Delzenne, et al. 2011)).

Animal models: Deciphering the mode of action of prebiotics on insulin sensitivity or energy homeostasis is still a competitive research topic. In 2004, we discovered that the colonic fermentation of a specific prebiotic called oligofructose (i.e., an inulin-type fructan) had the capacity to modulate endogenous production of appetite-controlling gut hormones (Cani, et al. 2004). Indeed, both oligofructose and inulin have the capacity to reduce dietary intake in rodents by a mechanism that involves anorexigenic and orexigenic gut hormones, i.e., an increase in glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) concentrations in the intestine and a decrease in the orexigenic hormone ghrelin concentration (Cani et al. 2004; Delzenne, et al. 2005). Prebiotics are generally known for their health benefits, but some recent papers bring nuances to the debate. Singh et al. showed that mice fed a compositionally defined diet (CDD) enriched with inulin developed hepatocellular carcinoma (HCC) through a mechanism dependent on the gut microbiota (Singh, et al. 2018). These results are in line with another study showing that inulin, when incorporated into a CDD, exacerbated colitis in mice exposed to dextran sulfate sodium (DSS) (Miles, et al. 2017). Although this effect has been observed in very specific animal models, these findings prove the importance of the dietary context in the effects of prebiotics in general. Regarding their mechanisms of action, many questions remain, but it is well known that due to their

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utilization by specific bacteria (i.e., fermentation), prebiotic fibres are transformed into different bacterial metabolites, such as short-chain fatty acids (SCFAs) (Koh, et al. 2016). These SCFAs act on a specific family of G-coupled protein receptors (GPCRs) called GPR41 and GPR43 (Le Poul, et al. 2003) and trigger the secretion of gut peptides involved in the regulation of appetite, energy homeostasis and glucose metabolism (Brooks, et al. 2017). However, this mechanism is still debated since Zou et al. showed that SCFAs and GPR43 are dispensable in the beneficial effects of inulin on HFD-induced low-grade inflammation and metabolic syndrome (Zou, et al. 2018). They demonstrated that IL-22, which is already known to protect against several intestinal infections (Zheng, et al. 2008; Zhong et al. 2019), is involved in fortification of the intestine through increased epithelial cell proliferation, thus contributing to protection against HFD-induced disorders.

In 2006, we demonstrated that the anti-diabetic action of oligofructose required a GLP-1 signalling pathway. Indeed, blocking the GLP-1 receptor by using pharmacological agents or using GLP-1 receptor knockout mice abolished the anti-diabetic effect of oligofructose (Cani et al. 2006). Treatment of obese and diabetic mice with oligofructose is associated with changes in the gut microbiota that rescue intestinal permeability. This physiological effect is associated with a restoration of the distribution and localization of the TJPs ZO-1 and occludin, thereby reinforcing the gut barrier, decreasing the LPS concentration in the portal vein and eventually reducing hepatic steatosis and systemic inflammation (Cani, et al. 2009b; Everard, et al. 2011). Importantly, blocking the GLP-2 receptor, a gut peptide increased by prebiotics and regulating epithelial cell proliferation and the gut barrier, abolished the effects of prebiotics on the gut barrier. This last result shows that prebiotics have the capacity to reduce low-grade inflammation and contribute to reducing insulin resistance. In addition, mice lacking the receptor GPR43 do not respond to prebiotic fibres, thereby showing the link between SCFAs, specific gut peptides and metabolism (Brooks et al. 2017). However, recent data also suggest that not all fermentable dietary fibres exert their effects on either the gut barrier or glucose metabolism by similar mechanisms and likely not only via SCFAs and gut peptides (Van Hul, et al. 2020). The reduction in HFD-induced obesity, fat mass accumulation and glucose intolerance with oligofructose and soluble corn fibre involves a distinct remodelling of the gut microbiota and subsequent different SCFA profiles.

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Therefore, although some mechanisms of action have been identified, it has also been established that gut microbes exert other functions via, for example, the release of other biological factors (such as neurotransmitters, bioactive lipids, gases) that also have an impact on gut physiology and contribute to the cross-talk observed between gut microbes and the host (review in (Cani and Knauf 2016; Rastelli, et al. 2018; Rastelli, et al. 2019)).

Humans: Although numerous data have been obtained in animals, human data also support that changing the gut microbiota by using fermentable fibres modifies gut peptide production (i.e., GLP-1, PYY, and Ghrelin) (Cani et al. 2009a; Parnell and Reimer 2009). Other studies have also confirmed the key role played by SCFAs in the beneficial effects, suggesting that they may be due to increased plasma levels of the enteroendocrine hormones PYY and GLP-1 (Chambers, et al. 2015; Freeland and Wolever 2010). In addition to their effects on gut peptides, inulin-type fructans exert beneficial effects on glucose metabolism in humans, including an improvement in fasting glycaemia, hyperinsulinaemia, HOMA-IR and HbA1c (Rao, et al. 2019; Zhang, et al. 2020). Several recent studies also found that a mix of prebiotics (i.e., inulin/oligofructose) correlated with bacterial-related metabolites (phosphatidylcholine, lactate and others) and to a specific gut microbiota composition that could explain the beneficial effect on glucose metabolism (Dewulf et al. 2013; Hiel, et al. 2019; Rodriguez, et al. 2020).

The results obtained regarding satiety and food intake are more discussed in humans. In 2018, Korczak and Slavin showed that fructan fibres used at a dose less than 10 g/day did not modify satiety or food intake (Korczak and Slavin 2018). The authors claimed that these fibres could not be used as the sole satiating agent since their impact on food intake is observed only at very high doses (>16 g/day) and when used for a long period (12-16 weeks).

Overall, the impacts of prebiotics on food consumption, glucose metabolism and body weight in humans is less significant than those observed in rodents.

Gut permeability and polyphenols

In addition to dietary fibres, plant-derived polyphenols represent other important substrates for the gut microbiota. Like dietary fibres, they are catabolized by and may

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influence the intestinal microbial ecosystem. In recent decades, polyphenols and their metabolites have gained attention for their promising beneficial health effects, which are generally attributed to their antimicrobial, antioxidant and anti-inflammatory properties (Anhe, et al. 2019). Recent studies also show the role of plant polyphenols in the regulation of the intestinal barrier. An overview of the main evidence from *in vitro* and *in vivo* studies supporting the role of polyphenols in modulating gut barrier permeability was the subject of a recent publication and will not be repeated here (Bernardi, et al. 2020). At present, it remains largely unknown how polyphenols exert their beneficial effects. They may be mediated by the microbial production of bioactive polyphenol-derived metabolites and/or by the modulation of the gut microbial community itself. However, several hypotheses have been proposed regarding how polyphenols influence the gut barrier. For example, polyphenols may improve barrier function by regulating oxidative stress through the downregulation of reactive oxygen species (ROS). Another mechanism by which polyphenols could exert their activity is by targeting different members of the NF-κB pathway or by antagonizing its activation. This pathway is responsible for the transcriptional induction of pro-inflammatory cytokines, chemokines and additional inflammatory mediators in different types of innate immune cells. By interfering with this signalling, polyphenols prevent the disassembly of TJPs and restore barrier integrity. Polyphenols also reinforce gut barrier function and morphology through the maintenance of the epithelial mucus layer in different mouse models of defective gut epithelium (Pierre, et al. 2013; Rodríguez-Daza, et al. 2020). However, many other pathways have also been suggested as potential polyphenol targets, many of which cross-talk with each other (reviewed in (Yang, et al. 2017), making this a complex issue to disentangle and highlighting the diversity within the polyphenol family. Indeed, in a comparative study using polyphenols derived from different sources (grapes or cinnamon), we demonstrated that both polyphenol extracts produced similar metabolic outcomes and that both improved gut barrier integrity via different underlying mechanisms (Van Hul, et al. 2017).

When discussing the health-promoting effects of polyphenols, it is important to consider their phenolic composition, bioavailability, distribution, metabolism and elimination. All of these parameters vary importantly from one study to another and explain, among others, strong interindividual variations (Boccellino and D'Angelo 2020; Teng and Chen 2019). The composition of polyphenol-rich solutions (e.g., sugar, fibre,

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and impurities) is not fully known in preclinical and clinical studies, and it remains difficult to determine the exact contribution of polyphenols rather than other components to the observed effects. Therefore, some efforts need to be made to standardize the chemical forms of dietary polyphenols used in preclinical and clinical studies to fully assess the effect of polyphenols on gut barrier function and health.

Deleterious effects

Impact of high-fat diets on the gut barrier

Chronic excess dietary fat not only increases systemic exposure to potentially pro-inflammatory free fatty acids but also disrupts gut barrier function by several mechanisms. First, a HFD can directly increase gut barrier permeability because both saturated and unsaturated fatty acids can impair the expression and distribution of TJPs. Moreover, lipid-rich diets facilitate the physiological absorption of LPS via the formation of chylomicrons (Ghoshal, et al. 2009). This phenomenon will trigger an immune response and enhance the secretion of pro-inflammatory factors (e.g., cytokines), which will then disrupt TJPs. This activity will in turn increase the permeability and lead to the leakage of even more LPS, resulting in a vicious circle (Cani, et al. 2008; Fujiyama, et al. 2007; Hamilton, et al. 2015; Kawano, et al. 2016; Luck, et al. 2015; Yoshida, et al. 2001; Zou et al. 2018). It has also been proposed that saturated fatty acids directly bind to toll-like receptor 4 (TLR-4) in the intestine via the adaptor protein fetuin A, thereby suggesting that saturated fatty acids may directly increase the production of inflammatory markers (Pal, et al. 2012) and contribute to alteration of the gut barrier. In contrast, mucus integrity has been positively associated with fatty acid synthase (FAS), a rate-limiting enzyme of fat-producing lipogenesis (Wei, et al. 2012). Defective intestinal lipogenesis in the absence of FAS is detrimental to the palmitoylation of Muc2, a key component of the mucus layer. These findings highlight the importance of lipid sources (dietary fat vs. de novo synthetized fat) in gut barrier function.

The HFD have become the gold standard for the study of the relationship between gut function and obesity (Buettner, et al. 2007). It is now well accepted that a HFD leads to alterations in gut microbiota composition and gut barrier function. However, caution should be exercised with regard to the composition of diets. The amounts of fibres,

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one of the main substrates used by the gut microbiota, differ among chow diets and are often greater than those found in HFDs (Pellizzon and Ricci 2018). Pioneering studies were performed using fibre-rich chow diets as a control and demonstrated for the first time that mice fed a HFD displayed increased gut permeability not only because of an alteration of TJPs but also because of an alteration of the intestinal mucus layer, further participating in gut barrier dysfunction and endotoxaemia (Cani et al. 2007; Everard, et al. 2013; Gulhane, et al. 2016). Since then, many works have been carried out with compositionally defined diets (CDDs) as a control, which mirror the composition of HFDs (excluding fat) (Chassaing, et al. 2015b; Dalby, et al. 2017; Jensen, et al. 2016). While exposure to the CDD (vs chow diet) induced significant alterations in the gut microbiota composition and in the intestine morphology, this effect is not sufficient to induce obesity and glucose intolerance that are characteristic of HFD feeding, thereby suggesting that gut microbiota alterations do not necessarily determine the onset of obesity (Dalby et al. 2017).

In addition to the mechanisms described above, dietary fat may also induce gut barrier dysfunction via alteration of the luminal bile acid profile. For example, dietary fat increases the concentration of deoxycholic acid (DCA), which is very hydrophobic and capable of disrupting cell membranes, whereas it reduces the concentration of the more hydrophilic and membrane-stabilizing ursodeoxycholic acid (UDCA) (Stenman, et al. 2012). In addition, BA has been shown to modulate gut permeability by affecting tight junction structure (Raimondi, et al. 2008; Stenman et al. 2012; Suzuki and Hara 2010). All these mechanisms support the notion that the HFD induces health disorders through an alteration of the gut environment.

The effects of sweeteners

In addition to the higher fat and sugar content, the Western-type diet is made up of ultra-processed food containing a large number of food additives (Carocho, et al. 2014). Among them, sweeteners are widely present to make food more palatable and stable. Despite their approbation by regulatory agencies, artificial sweeteners are not all safe, and some of them present adverse health effects. Saccharin, sucralose and aspartame induced glucose intolerance more than glucose did (with saccharin having the most pronounced effect) through an alteration in the composition and function of the gut microbiota (Suez, et al. 2014). Mice exposed to saccharine exhibited glucose

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intolerance associated with increased *Bacteroides* and decreased *Clostridiales* abundance. Metagenomic analysis revealed an enrichment in pathways targeting lipopolysaccharide biosynthesis, thereby revealing the potential mechanism by which sweeteners enhance susceptibility to T2D (Suez et al. 2014). In the same study, the authors demonstrated a positive correlation between the consumption of sweeteners and metabolic parameters such as HBA1c and glucose in humans. In another study that linked sweeteners with the gut microbiota, the authors reported that several "beneficial" phyla, such as *Bifidobacterium*, *Lactobacillus* and *Bacteroides*, were depleted in animals exposed to a combination of sweeteners and maltodextrin (Abou-Donia, et al. 2008). Given that sweeteners are able to bind sweet-taste receptors that are essential for the release of incretins, a large number of studies have aimed to investigate the potential of sweeteners as agents to trigger the secretion of gut endocrine hormones. Although sweeteners (mainly sucralose) have been reported to stimulate the release of GLP-1 in several enteroendocrine cell lines (Geraedts, et al. 2011; Jang, et al. 2007; Kidd, et al. 2008; Margolskee, et al. 2007), oral exposure in mice and humans failed to show the same effects (Brown, et al. 2009; Ford, et al. 2011; Fujita, et al. 2009; Ma, et al. 2009; Steinert, et al. 2011). Recently, two studies aimed to investigate the role of sweeteners in gut barrier integrity. The authors demonstrated that artificial sweeteners disrupted gut barrier function by increasing intestinal permeability and apoptosis *in vitro* (Santos, et al. 2018; Shil, et al. 2020). Shil et al. proposed that sweet-taste receptors were involved in this phenomenon since mice deleted for one of these receptors (T1R3) exhibited attenuated gut hyperpermeability (Shil et al. 2020). Given the presence of conflicting results regarding the effects of artificial sweeteners, additional studies on the topic have to be conducted to truly appreciate the safety of these additives.

The effects of emulsifiers

Another class of common additives is emulsifiers, which help to stabilize emulsions of liquids (such as mixes of oil and water). Emulsifiers are widely used, but some findings raise questions about their safety. In particular, carboxymethylcellulose (CMC) and polysorbate 80 (P80), two major emulsifiers, have been intensively studied for their capacity to trigger metabolic disorders. It has been demonstrated that CMC targets the microbial environment by promoting bacterial overgrowth in mice and that P80

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enhances the translocation of *E. coli* in intestinal-derived M-cells (Roberts, et al. 2010; Swidsinski, et al. 2009). A few years later, Chassaing et al. showed that CMC and P80 exposure predisposed mice to low-grade inflammation and metabolic syndrome and enhanced susceptibility to colitis in *Il10*$^{-/-}$ mice by a mechanism involving alteration of the mucus layer (Chassaing, et al. 2015a). Indeed, they discovered that the use of specific emulsifiers changes the penetrability of the mucus by different bacteria, thereby increasing the close vicinity of microbial cells with intestinal epithelial cells. In this pioneering work, they demonstrated that the gut microbiota plays a major role since transferring the microbiota from emulsifier-treated mice to germ-free mice reproduced the alteration of the gut barrier with an altered mucus layer, increased disruption of TJPs and induced metabolic endotoxaemia (Chassaing et al. 2015a). Similarly, in the absence of the microbiota, the mice were protected against emulsifier-induced gut barrier dysfunction, low-grade inflammation and eventually the onset of metabolic disorders. These findings are strengthened by ex vivo studies showing that CMC and p80 exposure in a human gut microbiota simulator (M-SHIME) enhanced the pro-inflammatory potential by increasing the levels of bioactive flagellin through a mechanism involving the gut microbiota (Chassaing, et al. 2017). Moreover, germ-free mice receiving this altered gut microbiota recapitulated all the metabolic disorders listed above, thereby confirming the involvement of the gut microbiota in mediating the deleterious effects of emulsifiers on health. In addition to the potential implication of the gut microbiota, a recent study proposed that emulsifiers might alter food intake by modulating the expression of neuropeptides (i.e., increase in appetite-stimulating AgRP and decrease in appetite-suppressing α-MSH), thereby suggesting that emulsifiers may endanger health by modulating the gut-to-brain axis (Holder, et al. 2019).

The effect of fructose-rich diets

The consumption of fructose soared across the world after the introduction of high-fructose corn syrup in the food industry in 1960. The overconsumption of fructose is strongly correlated with metabolic disorders, such as obesity, T2D, hepatic steatosis and cardiovascular diseases (Malik, et al. 2010; Ouyang, et al. 2008; Stanhope and Havel 2009). At the intestinal level, fructose alters gut barrier integrity by several mechanisms. First, chronic fructose intake is associated with a drop in the levels of

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TJPs in the duodenum, especially occludin and Claudin-1 (Jegatheesan, et al. 2016; Ochoa, et al. 2015; Ritze, et al. 2014; Volynets, et al. 2017; Zhou, et al. 2014). Because they observed an increase in serum endotoxin levels (i.e., metabolic endotoxaemia) and transcriptional activation of bacterial toll-like receptors, the authors proposed that fructose in combination with fat altered the gut barrier, leading to the translocation of bacterial products and thereby promoting hepatic inflammation (Mazzotti, et al. 2016; Ochoa et al. 2015; Spruss and Bergheim 2009). As observed with a high-fat diet, fructose intake alters mucus integrity by reducing mucus thickness (Rahman, et al. 2016; Volynets et al. 2017). Interestingly, Rahman et al showed that F11r$^{-/-}$ mice exhibited all the features of NASH (hepatic steatosis with lobular inflammation and ballooning), in contrast to similarly fed WT mice, suggesting that disrupted gut barrier function is instrumental for the progression of hepatic steatosis to NASH (Rahman et al. 2016).

Because enteroendocrine cells express the fructose transporter GLUT5 (Parker, et al. 2009; Reimann, et al. 2008), it is assumed that they adapt to the production of gut hormones as a function of fructose concentrations. However, the effect of fructose on gut hormones is still under investigation. In humans, fructose is able to stimulate CCK, PYY and neutrotensin (NTS) in a manner similar to that of glucose and GLP-1 to a lesser extent. However, fructose has no effect on GIP kinetics in both humans and rodents, suggesting that fructose acts on GIP and GLP-1 in different ways (Kuhre, et al. 2014).

Role of specific microbial metabolites in triggering metabolic diseases

We have shown that modulation of the microbiota itself is sufficient to affect host metabolism. Via its contribution to the production of bioactive metabolites, such as organic compounds (e.g., nitric oxide (NO), carbon oxide, indole, ammonia, and hydrogen sulfide), branched-chain amino acids (BCAAs) and SCFAs and their derivatives, the gut microbiota is also able to use these channels to influence peripheral metabolism (Koh et al. 2016; Pedersen et al. 2016; Tomasova, et al. 2016). As discussed in the chapter focused on prebiotics, most of the studies focused on the regulation of glucose tolerance and insulin signalling by SCFAs through a mechanism involving the endogenous production of GLP-1 and PYY.

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However, the gut microbiota produces a multitude of metabolites, many of which enter the bloodstream and may have an impact on specific metabolic pathways as well. These include metabolites that are derived from the gut-driving metabolism of amino acids, and of these, trimethylamine (TMA) is the best documented. TMA is produced by the gut microbiota from dietary choline and carnitine and is converted into trimethylamineoxide (TMAO) by flavin-containing monooxygenase 3 (FMO3) in the liver. TMAO is strongly associated with the development of cardiovascular diseases and insulin resistance in humans (Bennett, et al. 2013; Shih, et al. 2015; Tang, et al. 2013; Wang, et al. 2011). Moreover, insulin-resistant mice lacking hepatic insulin receptor (LIRKO) exhibited an upregulation of *Fmo3* and subsequent increased levels of TMAO (Miao, et al. 2015). Consistently, knockdown of *Fmo3* in LIRKO mice prevented hyperglycaemia and atherosclerosis by suppressing FOXO1 protein expression and activity (Miao et al. 2015).

Another microbially produced amino acid-derived metabolite involved in the development of insulin resistance is imidazole propionate, which results from the histidine degradation pathway. Imidazole propionate is increased in individuals with type 2 diabetes (T2D) and impairs glucose tolerance and insulin signalling in HFD-fed mice by a mechanism involving inhibition of insulin receptor substrate (IRS) through activation of the p38y/p62/mTORC1 pathway (Koh, et al. 2018).

Recently, the role of indolepropionic acid (IPA), a microbiota-produced deamination product of the amino acid tryptophan, has also been described. IPA is a bioactive compound that binds to pregnane X receptor (PXR) and aryl hydrocarbon receptor (Ahr) to exert effects on gut barrier integrity and glucose homeostasis (Agus, et al. 2018; Hubbard, et al. 2015; Venkatesh, et al. 2014; Zelante, et al. 2013). IPA has been described as inversely correlated with type 2 diabetes in humans (Tuomainen, et al. 2018). In animals, data are controversial because IPA improved glucose metabolism and metabolic endotoxaemia in Sprague-Dawley rats (Abildgaard, et al. 2018; Konopelski, et al. 2019; Zhao, et al. 2019), but its supplementation in mice fed a Western diet had no effect on glucose and lipid metabolism (Lee, et al. 2020).

Taken together, these data on the different microbial metabolites illustrate their importance and pleiotropic roles in the functional capacity of the gut microbiota.

General conclusions and perspectives

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Gut microbiota research has undoubtedly broadened our view on how metabolic pathways are regulated in an organism. However, despite the substantial technical progress made in the field, we still lack a real gold-standard analysis method. Most of the strategies rely on complementary approaches (i.e., taxonomic profiling, gene counts, and functional metagenomics), often in combination with metabolomics (i.e., analysis of the different metabolites produced by the microbiota). There is, however, a necessity for further technical advances, and many interrogations are still debated (Cani 2018). For example, while most of the studies use relative abundancies to evaluate microbial composition, the quantification of the absolute number of bacteria (i.e., cell counts or microbial load) may be an important aspect to take into account when exploring taxonomic changes. Jeroen Raes and his team have revealed new perspectives by showing that the relative quantification versus absolute quantification of gut bacteria could completely change the conclusions related to the association between specific bacteria and either health or diseases (Vandeputte, et al. 2017). Their study strongly argues that most of the previous works using only relative proportions of microbes are possibly not capturing the entirety of a health situation. One striking example highlighted by the team is that the abundance of *Bacteroides* is connected with colitis (Crohn's disease) only after using relative abundance and not when using quantitative microbiota analysis (Vandeputte et al. 2017). Furthermore, these data emphasize the limitations of using relative abundance analysis since they can lead to specious interpretations.

Additionally, given the number of potential combinations of bacteria/metabolites and host genes/susceptibilities, it is difficult to generalize all the findings discussed in this review. For example*, Prevotella copri*, which produces succinate, has been associated both positively and negatively with insulin resistance in two dietary approaches (Kovatcheva-Datchary, et al. 2015; Pedersen et al. 2016). Therefore, the effects of specific microbial signatures and subsequently produced metabolites can differ drastically depending on the type of diet used (HFD or fibre-rich chow diet). The scientific community agrees on the fact that we have to progressively move towards personalized medicine, and it is likely that nutritional approaches will also need to be tailored to individual needs.

Finally, while there is little doubt about the existence of a causal link between gut microbes and energy homeostasis, further studies are still warranted, for example, to characterize the numerous interactions between microbes and different nutrients.

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Only then will it one day be possible to target the gut microbiota to address obesity and related disorders.

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Conflict of interests

P.D.C. and C.K. are co-founders of Enterosys S.A. (Labège, France). P.D.C. is a co-founder of A-Mansia Biotech S.A. (Belgium) and owner of several patents concerning the use of microbiota and health.

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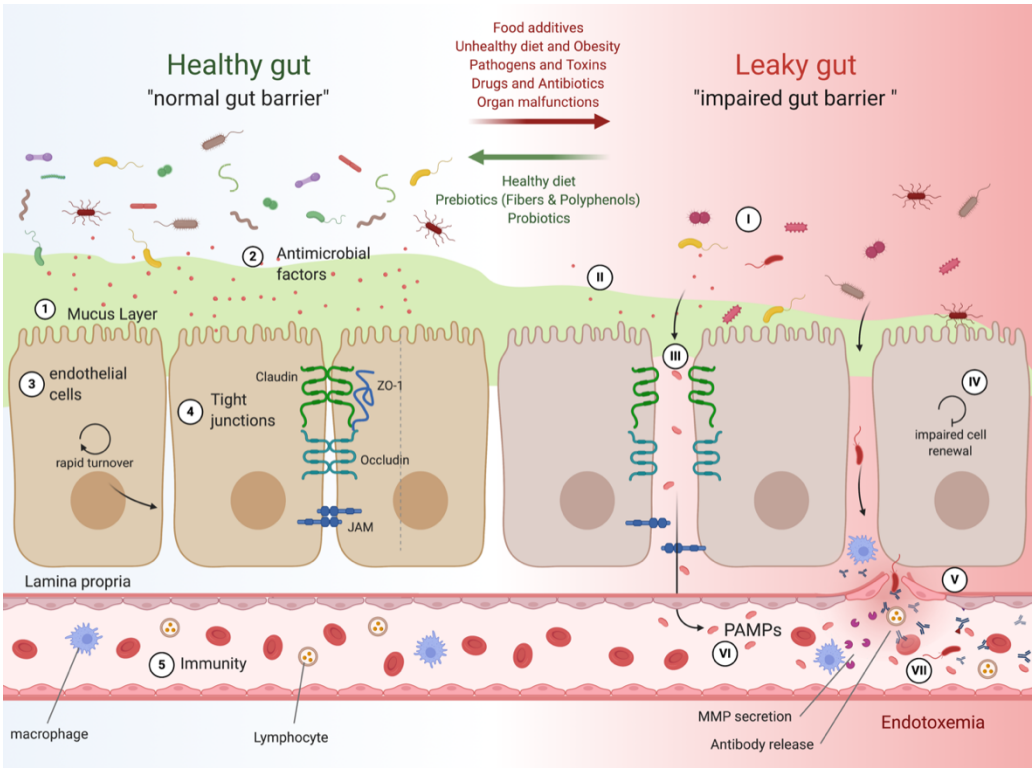

Figure 1: The gut barrier is composed of several physical and chemical components

The mucus layer (1) acts as a physical barrier to keep the local microbiota at a distance. It is impregnated with factors that provide additional antibacterial activity (2). Epithelial cells (3), joined together by tight junction proteins (4), are the primary gatekeepers and are renewed every 4-5 days. Immune cells (5) serve as the body's border patrol and limit the inner body's exposure to allergens, pollutants, viruses, bacteria, and parasites.

Disruption of the gut barrier (right) is associated with alterations in microbiota composition (I), reduction in mucus layer thickness and consistency (II), disruption of TJPs (III), impaired cell renewal (IV), increased permeability (V), translocation of PAMPS and pathogens (endotoxaemia) (VI) and inflammation (VII).

(ZO-1 = Zonula occludens-1; JAM = junctional adhesion molecule; PAMPs = pathogen-associated molecular patterns, MMP = matrix metalloproteinase)

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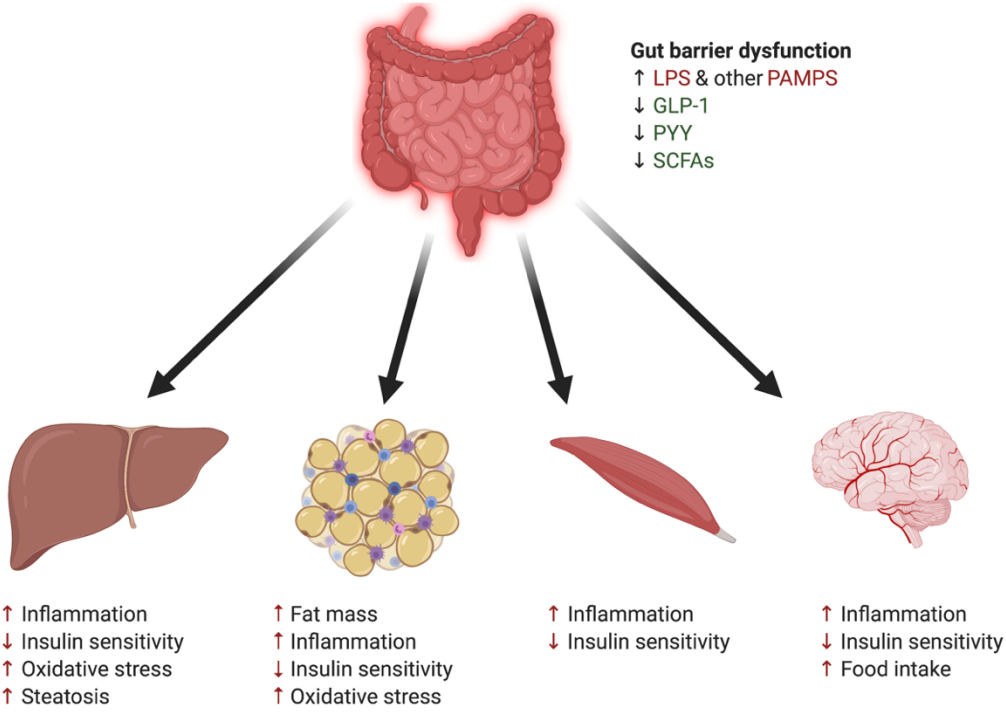

Figure 2: Gut barrier dysfunction has systemic consequences

Disruption of the gut barrier leads to metabolic endotoxaemia and impaired production of circulating gut hormones. This phenomenon translates to metabolic disorders in various organs.