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Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using prebiotics

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REVIEW ARTICLE

Abstract

Crosstalk between organs is crucial for controlling numerous homeostatic systems (e.g. energy balance, glucose metabolism and immunity). Several pathological conditions, such as obesity and type 2 diabetes, are characterised by a loss of or excessive inter-organ communication that contributes to the development of disease. Recently, we and others have identified several mechanisms linking the gut microbiota with the development of obesity and associated disorders (e.g. insulin resistance, type 2 diabetes, hepatic steatosis). Among these, we described the concept of metabolic endotoxaemia (increase in plasma lipopolysaccharide levels) as one of the triggering factors leading to the development of metabolic inflammation and insulin resistance. Growing evidence suggests that gut microbes contribute to the onset of low-grade inflammation characterising these metabolic disorders via mechanisms associated with gut barrier dysfunctions. We have demonstrated that enteroendocrine cells (producing glucagon-like peptide-1, peptide YY and glucagon-like peptide-2) and the endocannabinoid system control gut permeability and metabolic endotoxaemia. Recently, we hypothesised that specific metabolic dysregulations occurring at the level of numerous organs (e.g. gut, adipose tissue, muscles, liver and brain) rely from gut microbiota modifications. In this review, we discuss the mechanisms linking gut permeability, adipose tissue metabolism, and glucose homeostasis, and recent findings that show interactions between the gut microbiota, the endocannabinoid system and the apelinergic system. These specific systems are discussed in the context of the gut-to-peripheral organ axis (intestine, adipose tissue and brain) and impacts on metabolic regulation. In the present review, we also briefly describe the impact of a variety of non-digestible nutrients (i.e. inulin-type fructans, arabinoxylans, chitin glucans and polyphenols). Their effects on the composition of the gut microbiota and activity are discussed in the context of obesity and type 2 diabetes.

Keywords: endocannabinoid system, apelin, metabolic endotoxaemia, obesity, type 2 diabetes

1. Introduction

Crosstalk between organs is crucial for controlling energy homeostasis. In obesity and type 2 diabetes, inter-organ communication is significantly disturbed, which contributes to changes in food intake regulation, energy expenditure, adipose tissue development and insulin resistance. Of all the complex regulatory systems, both the gut-to-brain and gut-to-adipose tissue axes have been proposed as crucial paths that control these metabolic features.

Obesity is defined as a massive expansion of the adipose tissue. Obesity is typically associated with a wide cluster of metabolic alterations, including glucose homeostasis disorders (e.g. glucose intolerance, insulin resistance and type 2 diabetes), cardiovascular diseases or risk factors (e.g. hypertension, dyslipidaemia, and fibrinolysis disorders) and non-alcoholic fatty liver disease (Eckel *et al.*, 2005; Ogden *et al.*, 2007). The majority of these alterations likely result from a combination of variable associations between genetic and environmental factors. Low-grade chronic inflammation

appears to be a common feature that may contribute to the development of insulin resistance, type 2 diabetes and cardiovascular diseases (Hotamisligil, 2008; Shoelson and Goldfine, 2009). However, the mechanisms underlying obesity, fat mass development and the development of inflammation are not fully defined.

The gut microbiota may be a key exteriorised organ that can contribute to the onset of several metabolic dysregulations, leading to inflammation in intestinal and peripheral tissues (e.g. adipose tissue, muscles, liver and brain) and altered glucose and energy homeostasis (Cani and Delzenne, 2009; Cani *et al.*, 2012; Delzenne *et al.*, 2011). During food intake, the intestine senses various messages from different origins, including nutrients and hormones. In response, the intestine generates different types of messages (hormones, afferent nerves) that inform the peripheral organs (e.g. liver and adipose tissue) and the brain (i.e. hypothalamus) as to the nutritional status. In turn, these organs generate signals to modify energy storage or expenditure, and other key messages are generated by the brain such as efferent signals via the autonomic nervous system. In pathological situations associated with obesity, metabolic disorders are characterised by an insulin resistant state that could be due to the following: (1) a default in nutrient sensing in the intestine; (2) an abnormal signal from the gut sent to the peripheral organs and brain; (3) an aberrant signal generated by the brain to the periphery; and (4) a direct insulin resistant state in the peripheral organs. However, the intestinal and hypothalamic molecular components involved in gut-to-organ deregulation in type 2 diabetes remain unknown.

In the present review, we discuss recent findings that demonstrate a major mechanism linking the gut microbiota to low grade inflammation. In the second part of the review, we will discuss the potential relationships between recently identified physiological systems (i.e. the endocannabinoid and apelinergic systems) that link intestinal function and the peripheral organs (intestine, adipose tissue and brain) with metabolic regulation. Finally, we describe the effects of specific non-digestible carbohydrates (NDC) and their impacts on gut microbiota and host metabolism in the context of obesity and type 2 diabetes.

2. The role of metabolic endotoxaemia and its association with obesity and type 2 diabetes: evidence for altered gut barrier function

We recently defined gut microbiota-derived lipopolysaccharide (LPS) as a factor involved in the early development of inflammation and metabolic diseases (Cani *et al.*, 2007a). In pathological conditions such as obesity and type 2 diabetes, the gut microbiota can 'dialogue' with the host and contribute to the development of metabolic disorders (Cani *et al.*, 2006b, 2007a,b, 2008, 2009b). More precisely, intake of excess dietary fat increases systemic exposure to potentially pro-inflammatory free fatty acids and their derivatives and increases plasma LPS levels, which is defined as metabolic endotoxaemia (Cani *et al.*, 2007a,b). Because LPS can affect inflammation throughout the body and interfere with metabolism and function of the immune system, this major breakthrough provides new insight into the role of gut microbiota-derived products and metabolism. Of the mechanisms explaining the development of metabolic endotoxaemia in obesity, the gut microbiota was observed to link gut permeability with low-grade inflammation and insulin resistance (Figure 1) (Cani *et al.*, 2007a, 2008; Everard *et al.*, 2011; Muccioli *et al.*, 2010). For example, we investigated the role of the specific gut peptide glucagon-like peptide-2 (GLP-2), which is produced by L-cells and is involved in the control of epithelial cell proliferation and gut barrier integrity (Dube and Brubaker, 2007). Higher endogenous GLP-2 production was associated with improved mucosal barrier function via the restoration of tight junction protein expression and distribution (Figure 1) (Cani *et al.*, 2009b). Using complementary approaches involving specific modulation of the gut microbiota (antibiotics, prebiotics) and pharmacological inhibition or activation of the GLP-2 receptor, we discovered that gut microbiota and the gut peptide GLP-2 participate in the modulation of gut barrier function and the consequent systemic and hepatic inflammatory phenotype associated with obesity and type 2 diabetes (Cani *et al.*, 2009b). The increase in endogenous GLP-2 has been associated with an increased number of L-cells (Figure 1) (Everard *et al.*, 2011). These findings indicate that targeting enteroendocrine function may be a novel therapeutic approach for treating the inflammatory phenotype associated with obesity and type 2 diabetes. Although the enteroendocrine function of L-cells is an important mechanism in regulating gut barrier function, molecular links between the gut microbiota and enteroendocrine function of the gut remain unknown.

3. Links between gut microbiota and adipose tissue metabolism during obesity and type 2 diabetes

Growing evidence suggests that the gut microbiota contribute to host metabolism through an axis of communication with the adipose tissue, which influences the development of metabolic alterations associated with obesity. Germ-free mice have 40% less fat mass than normal conventionalised mice (Backhed *et al.*, 2004). Colonisation of germ-free mice with the gut microbiota from obese donors resulted in an increase in total body fat mass (Turnbaugh *et al.*, 2006). We previously demonstrated that mice treated with antibiotics were resistant to diet-induced fat mass development and insulin resistance (Cani *et al.*, 2008). In addition, several studies have demonstrated that germ-free mice are protected against glucose intolerance

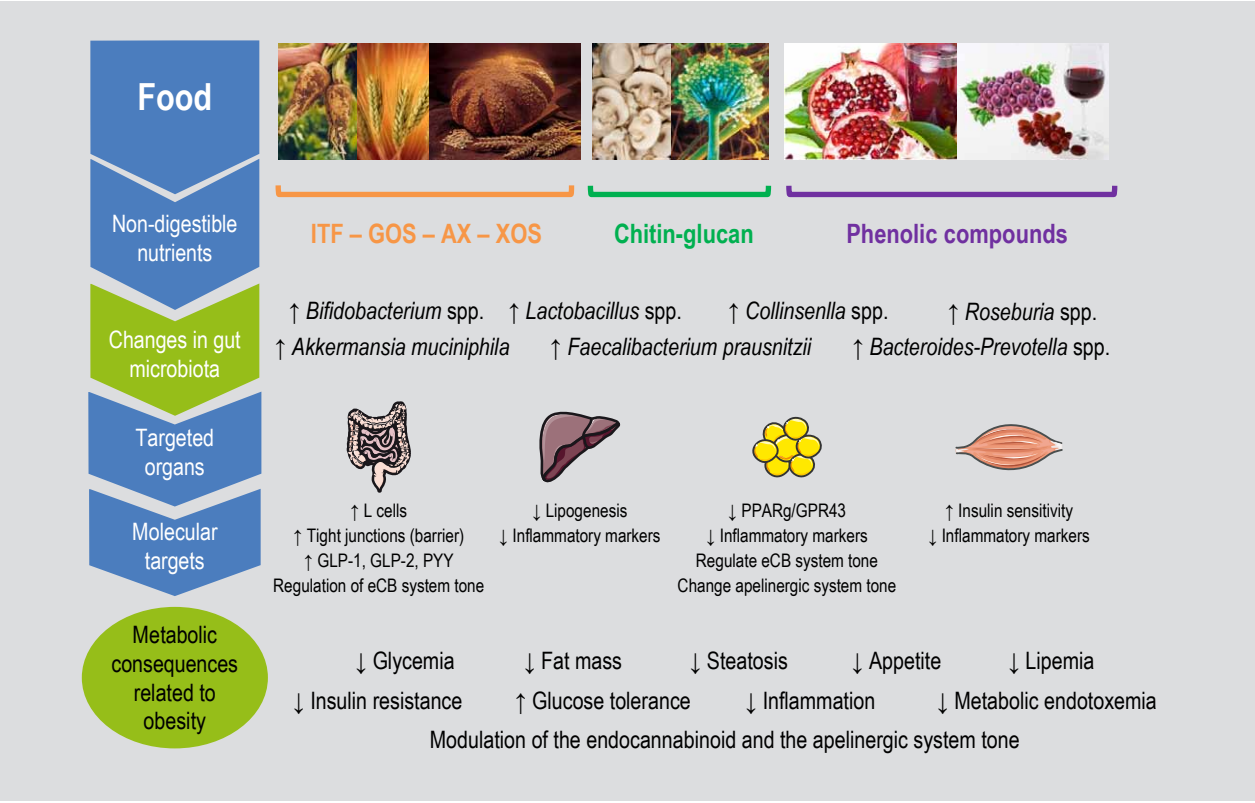

Figure 1. Effect of non-digestible nutrients with prebiotic properties on host pathophysiology related to obesity. In intervention studies in animals and humans, non-digestible nutrients with prebiotic properties, such as inulin-type fructans, galacto-oligosaccharides, arabinoxylan and arabinoxylan oligosaccharides derived from wheat, fungal chitin-glucan and several phenolic compounds present in pomegranate or grapes, have been shown to change the gut microbiota composition by favouring bacteria that confer health benefits to the host. Prebiotics reinforce the gut barrier and promote gut hormones that control appetite, glucose homeostasis and systemic inflammation. The prebiotic approach also counteracts hepatic steatosis (lipogenesis), hepatic insulin resistance, and adiposity by modifying gene expression at the tissue level. LPS = lipopolysaccharide, APJ = apelin receptor, eCB = endocannabinoid.

and insulin resistance induced by a high-fat diet (Backhed *et al.*, 2007; Ding *et al.*, 2010; Rabot *et al.*, 2010). A recent report showed that the gut microbiota is able to stimulate brown adipose tissue lipid metabolism (Mestdagh *et al.*, 2011). We demonstrated that nutritionally and genetically obese rodents fed prebiotics had a different gut microbiota composition and a different adiposity index compared to obese mice fed a normal chow diet (Cani *et al.*, 2005b, 2006b, 2007b; Dewulf *et al.*, 2011; Neyrinck *et al.*, 2012a,b). Over the last five years, numerous studies have contributed to demonstrate the link between the gut microbiota composition and obesity. In the present review, we will specifically focus on two physiological systems recently identified that we have linked with the gut microbiota (i.e. the endocannabinoid and apelinergic systems). It is worth noting that to provide an overview of the state of the art on the composition of the gut microbiota in the context of obesity and type 2 diabetes we recommend these recent reviews (Angelakis *et al.*, 2012; Clarke *et al.*, 2012; Flint, 2012; Ley, 2010; Musso *et al.*, 2010; Shen *et al.*, 2012; Tilg and Kaser, 2011; Tremaroli and Backhed, 2012).

The endocannabinoid system

We recently proposed that the endocannabinoid (eCB) system could be one of the mediators of communication between the gut and adipose tissue (Muccioli *et al.*, 2010). The eCB system consists of bioactive lipids that bind to cannabinoid receptors and elicit cell signalling. Of these lipids, the most well-characterised are AEA (anandamide or *N*-arachidonoylethanolamine) and 2-AG (2-arachidonoylglycerol) (Lambert and Muccioli, 2007). eCBs activate G-coupled receptors, namely CB1 and CB2, that present a distinct expression pattern with rare overlap in a given cell type (Pertwee, 2010; Engeli, 2012). Several other lipids are considered eCBs such as the *N*-acylethanolamine PEA (palmitoylethanolamine) or OEA (oleoylethanolamine) that activate the PPARα, GPR55 and GPR119 receptors (Hoareau *et al.*, 2009; Muccioli, 2010; Ryberg *et al.*, 2007). The tight control of eCB levels depends on the balance between synthesis and degradation. eCBs are synthesised by specific enzymes such as NAPE-PLD (*N*-acyl-phosphatidylethanolamine

phospholipase D), that synthesise AEA, PEA and OEA, or DAGL (diacylglycerol lipase) to synthesise 2-AG. eCBs are rapidly degraded by specific enzymes. AEA, PEA and OEA are primarily degraded by FAAH (fatty acid amide hydrolase), and 2-AG is degraded by MGL (monoglycerol lipase). Dysregulation of this tight control may result in pathological conditions, such as obesity or type 2 diabetes. eCBs are widely expressed in tissues that control the energy balance (i.e. pancreas, muscle, gut, adipose tissue, liver, and hypothalamus) and have a broad range of physiological effects, including regulating feeding behaviours and metabolism (Matias and Di Marzo, 2007). One of the roles of the eCB system in normal conditions is to facilitate energy intake and storage, which can promote obesity in pathological situations (Pagotto *et al.*, 2006). The eCB system has been shown to be over-activated during obesity in rodents and humans, with an increased expression of synthesising enzymes and receptors and increased plasma and adipose tissue eCB levels (Engeli *et al.*, 2005; Bluher *et al.*, 2006). However, there is no clear evidence that this concept may be generalised to the numerous lipids that belong to the eCB system (e.g. NAE and acylglycerols). The effects of eCBs on metabolism are thought to be mediated primarily by CB1 and CB2 receptors. Several studies have shown that stimulation of the eCB system increases food intake and that CB1 knockout mice or treatment with a CB1 antagonist such as SR141716 in rodents and humans reduces food intake and decreases body weight (Cota *et al.*, 2003; Ravinet *et al.*, 2004; Tam *et al.*, 2010; Van Gaal *et al.*, 2005). Mice treated with a CB1 antagonist and CB1 knockout mice have a pronounced reduction in adipose tissue mass and reduced plasma leptin, highlighting a central role for adipose tissue.

The eCB system links gut barrier function to the adipose tissue and the gut microbiota

We previously demonstrated that the eCB system is implicated in gut barrier alterations and increased gut permeability observed in obesity (Muccioli *et al.*, 2010). Colonic CB1 mRNA levels were modified in animal models of gut microbiota modulation (germ-free mice, antibiotic treatment, MyD88 knockout mice, high-fat diet-treated mice and prebiotic-treated mice), suggesting that the gut microbiota selectively modulates CB1 colonic mRNA (Muccioli *et al.*, 2010). Consistent with these data, the AEA content and FAAH expression were affected in the colon of these animal models (Muccioli *et al.*, 2010). Because gut microbiota contribute to low-grade inflammation associated with obesity by increasing gut permeability and metabolic endotoxaemia (Cani *et al.*, 2007a, 2008, 2009b), we hypothesised that the eCB system could link the gut microbiota to peripheral inflammation and metabolism (Muccioli *et al.*, 2010). Although not directly associated with energy homeostasis or gut barrier function, it has been previously shown that the administration of a specific strain of bacteria, *Lactobacillus acidophilus* NCFM, increases CB2 expression in the colon in mice, whereas four other bacteria strains (well known as probiotics) belonging to the *Lactobacillus* and *Bifidobacterium* genera (*L. salivarius* Ls-33, *L. paracasei* Lpc-37, *B. lactis* Bi-07 and *B. lactis* Bi-04) and two *Escherichia coli* strains have no effect on CB2 (Rousseaux *et al.*, 2007).

Intestinal eCBs have already been implicated in several metabolic pathways, including the gut-to-brain axis. The gastrointestinal tract is a key player in the control of food intake by sending messages to the central nervous system via gut hormones, eCBs and other lipid mediators (Cluny *et al.*, 2012). Several data support the hypothesis of a gut-to-adipose tissue axis where the eCBs may play a role. For instance, leptin-deficient *ob/ob* mice treated with a CB1 antagonist exhibited reduced metabolic endotoxaemia and improved gut barrier function by mechanisms independent of food intake (Muccioli *et al.*, 2010). Most of the effects associated with CB1 antagonists have not been observed in pair-fed animals (Gary-Bobo *et al.*, 2007; Muccioli *et al.*, 2010), suggesting that CB1 have a direct impact on gut barrier function. In accordance with this hypothesis, we demonstrated that expression of tight junction proteins (ZO-1 and occludin) and transepithelial electric resistance are controlled by CB1-dependent mechanisms, but not CB2 in a cellular model of the intestinal epithelial layer using Caco-2 cells (Muccioli *et al.*, 2010). These results were recently confirmed (Alhamoruni *et al.*, 2012).

In addition to recent data showing the impact of the eCB system on gut barrier regulation, numerous reports have demonstrated that the eCB system tone is dysregulated in adipose tissue during obesity (Bluher *et al.*, 2006). The altered eCB system tone observed in the gut and adipose tissue may be directly associated with specific changes in the composition of the gut microbiota (Muccioli *et al.*, 2010; Geurts *et al.*, 2011). For instance, the genera *Odoribacter, Prevotella,* and *Rikenella* were identified exclusively in obese and diabetic *db/db* mice, whereas *Enterorhabdus* was identified exclusively in lean mice (Geurts *et al.*, 2011). We also found that the genera *Prevotella, Tannerella, Barnesiella* and *Alistipes* were significantly increased in the genetic diabetic mice (Geurts *et al.*, 2011). Using two different models of genetic obesity (*ob/ob* and *db/db* mice), we consistently detected increased NAPE-PLD mRNA levels, AEA content and decreased FAAH levels in the adipose tissue of obese and diabetic mice compared to their lean littermates (Muccioli *et al.*, 2010; Geurts *et al.*, 2011). Altering the gut microbiota of *ob/ob* mice with prebiotics reduces the AEA content and CB1 mRNA expression in adipose tissue and improves adipose tissue metabolism, which is similar to the phenotype observed following CB1 receptor blockage (Muccioli *et al.*, 2010).

Whether the gut microbiota and metabolic endotoxaemia directly control these parameters is unclear. However, obesity and associated inflammation alter adipose tissue metabolism by impairing adipogenesis (Gustafson *et al.*, 2009) and support the increased eCB system tone (Bluher *et al.*, 2006). Several studies have proposed that LPS receptor activation could decrease the processes of adipogenesis (adipocyte differentiation and lipogenesis) (Poulain-Godefroy *et al.*, 2008; Muccioli *et al.*, 2010) and activate eCB production (Figure 2) (Maccarrone *et al.*, 2001; Liu *et al.*, 2003; Hoareau *et al.*, 2009). Understanding the underlying mechanisms responsible for altered adipogenesis is of utmost important in avoiding associated metabolic alterations. We recently suggested that modulation of the gut microbiota or blocking CB1 using an antagonist impacts the gut barrier integrity and reduces plasma LPS, thereby reducing low-grade inflammation (Muccioli *et al.*, 2010). This process may reduce the eCB system tone and improve adipogenesis during obesity (Figure 2). Because metabolic endotoxaemia is under the control of gut CB1 through the regulation of gut permeability (Muccioli *et al.*, 2010), LPS could be proposed as an additional factor in controlling adipogenesis and eCB system regulation, confirming a putative gut microbiota-to-adipose tissue regulatory loop (Figure 2). All of these data clearly suggest that LPS and eCB act as mediators in this regulatory loop.

The apelinergic system

Adipocytes produce and secrete adipokines. Disruption of their global actions can influence the emergence of type 2 diabetes. Of the adipokines produced and secreted by adipocytes (Galic *et al.*, 2010), apelin is considered as a promising target for treating metabolic disorders (Castan-Laurell *et al.*, 2012). Apelin has been proposed as a novel key peptide involved in the regulation of several physiological functions. The apelinergic system, which comprises apelin and the apelin receptor (APJ), is widely expressed in mammals and exert functional effects in both the central nervous system and the periphery (Sorli *et al.*, 2006). Apelin plays a key role in the cardiovascular system by acting on heart contractility, blood pressure, fluid homeostasis, vessel formation, and cell proliferation (Maenhaut and Van, 2011). In the peripheral organs, increased secretion and expression of apelin in different mouse models of obesity and obese subjects is strongly associated with insulin regulation (Dray *et al.*, 2008; Erdem *et al.*, 2008; Soriguer *et al.*, 2009). We demonstrated that apelin produced by the peripheral organs is considered beneficial with anti-diabetic properties and represents a promising target for managing insulin resistance (Dray *et al.*, 2008, 2010). Acute intravenous injection of apelin in normal mice has shown a powerful glucose-lowering effect by stimulating glucose utilisation in skeletal muscle via eNOS, AMPK and Akt-

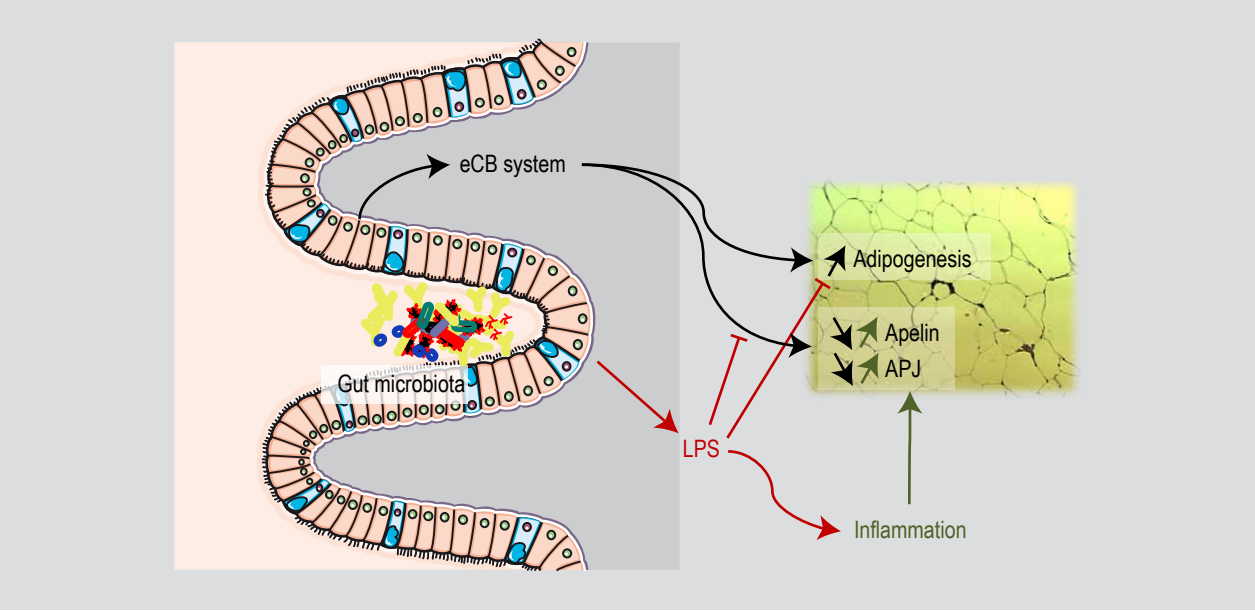

Figure 2. Crosstalk between the gut microbiota, the endocannabinoid system, the apelinergic system and impacts of metabolic endotoxaemia on adipose tissue development. In physiological situations, the endocannabinoid (eCB) system tone reduces the levels of apelin and apelin receptor (APJ) mRNA in adipose tissues, whereas the inflammatory tone increases these markers. Endocannabinoids increase the adipogenesis processes. In obesity, both the eCB system and the inflammatory tone are increased and associated with metabolic endotoxaemia (i.e. circulating lipopolysaccharide (LPS)). In this pathological condition, LPS completely abolished the effects of endocannabinoids on adipogenesis and the apelinergic system, suggesting that both eCBs and LPS are implicated in adipose tissue metabolism.

dependent pathways. Apelin restored glucose tolerance and increased glucose utilisation in skeletal muscle and adipose tissue in obese and insulin-resistant mice fed a high-fat diet (Dray *et al.*, 2008). Two weeks of chronic apelin treatment in insulin-resistant mice was shown to improve insulin sensitivity. The role of apelin in glucose homeostasis has been confirmed in apelin knockout mice that have decreased insulin sensitivity (Yue *et al.*, 2010).

Apelin is one of several rare adipokines present in the hypothalamic neurons. Apelin strongly colocalises with proopiomelanocortin (POMC), and its receptor is expressed in POMC neurons (Reaux *et al.*, 2002). This suggests that apelin may stimulate α-melanocyte-stimulating hormone release in an autocrine pathway, reinforcing the potential anorexigenic effect of central apelin (Reaux-Le Goazigo *et al.*, 2011; Lv *et al.*, 2012). We recently studied glucose metabolism in response to acute and chronic intracerebroventricular (icv) injection of apelin in normal and obese/diabetic mice. We demonstrated that acute icv injection of apelin improves glucose homeostasis via NO dependent mechanisms. Acute and chronic administration of high doses of apelin in the brain at concentrations similar to those observed in obese/diabetic mice (Duparc *et al.*, 2011a) provokes hyperinsulinaemia, hyperglycaemia, glucose intolerance, and insulin-resistance in fasted normal mice. Similar effects on glycaemia and glucose tolerance as a result of high dose apelin have been shown in high-fat fed mice. These data provide compelling evidence that central apelin participates in the regulation of glucose homeostasis and suggest a novel physiopathological mechanism involved in the transition from a normal to a diabetic state (Duparc *et al.*, 2011a). However, the mechanisms linking apelin production in the central nervous system and peripheral tissues in obesity and type 2 diabetes remain unknown.

We previously linked the gut microbiota with low-grade inflammatory tone. Several studies have suggested that inflammation and the apelinergic system are closely related. For example, the pro-inflammatory cytokine TNF-α induces apelin expression in massively obese humans and adipocytes isolated from different mouse models of obesity, indicating a direct link between inflammation and apelin in adipose tissue (Daviaud *et al.*, 2006). Inflammation promotes the hepatic apelinergic system (Melgar-Lesmes *et al.*, 2011). During intestinal inflammation, pro-inflammatory cytokines induce enteric apelin expression (Han *et al.*, 2008). We recently demonstrated that inflammatory markers were positively and significantly correlated with the apelinergic system in the adipose tissue of diabetic *db/db* mice (Geurts *et al.*, 2011). These data highlight an important relationship between adipose tissue and the apelinergic system. It has been previously proposed that the endocannabinoid system regulates specific adipokines (i.e. adiponectin and omentin) production of adipose tissue in obesity (Bensaid *et al.*, 2003; Ge *et al.*, 2013). The mechanisms involved in the regulation of the apelinergic system, have not been completely elucidated (Castan-Laurell *et al.*, 2011).

Thus, to unravel the mechanisms underlying apelin and APJ regulation, we investigated the impact of the eCB system, LPS and gut microbiota on the apelinergic system (Geurts *et al.*, 2011).

Linking the endocannabinoid system, the apelinergic system and metabolic endotoxaemia

We demonstrated that apelin and APJ expression were down-regulated by eCB in physiological conditions (Figure 2). The acute stimulation of eCB system with CB receptor agonist HU210 decreased apelin and APJ mRNA expression in adipose tissue explants (Geurts *et al.*, 2011). *In vivo* inhibition of AEA degradation using a potent FAAH inhibitor (URB597) decreased apelin and APJ mRNA in adipose tissue, supporting the potential implication of the eCB system in apelinergic tone (Figure 2) (Geurts *et al.*, 2011). To corroborate the link between adipose tissue metabolism and inflammation, we treated adipose tissue explants with LPS and found that LPS increases apelin and APJ mRNA expression (Figure 2). In a situation mimicking obese conditions (i.e. increased eCB system tone and inflammation), LPS completely abrogated CB agonist-induced downregulation of the apelinergic system, suggesting that both eCBs and LPS are implicated in adipose tissue metabolism (Figure 2) (Geurts *et al.*, 2011).

Leptin resistant *db/db* mice were recently shown to have a different gut microbiota composition compared to their lean littermates (Geurts *et al.*, 2011). By using pyrosequencing analyses and phylogenetic microarrays, we identified a significantly higher abundance of *Firmicutes*, *Proteobacteria* and *Fibrobacteres* phyla in *db/db* mice compared to lean mice. Ten genera were significantly affected by the genotype (Geurts *et al.*, 2011). To support the link between gut microbiota and apelin, we discovered more than 20 positive and negative correlations between several gut microbes and apelin and APJ mRNA, which support the idea that gut microbiota have direct impacts on adipose tissue metabolism and the apelinergic system in particular (Geurts *et al.*, 2011). Because LPS derived from gut microbiota-derived bacteria triggered low-grade inflammation and apelin and APJ expression, we postulate that these changes contribute to the phenotype of *db/db* mice, highlighting a new mechanism for apelin regulation in diabetes (Geurts *et al.*, 2011). We were the first to show a link between eCBs and the apelinergic system. These systems were shown to be closely involved in adipose tissue metabolism alterations (Geurts *et al.*, 2011). *db/db* mice were shown to have a higher eCB system tone, increased inflammation and an increased apelinergic system (apelin and receptor APJ) (Geurts *et al.*, 2011). Strong relationships between eCBs and the apelinergic system were demonstrated using multiple

correlation analyses. Specific gut microbes were correlated with adipose markers, suggesting that these microbes play a role in adipose tissue metabolism (Geurts *et al.*, 2011). To support an interaction between eCBs, gut microbiota and adipose tissue, we investigated the role of LPS derived from gut microbiota and found that LPS counteracted the effects of eCBs on adipose tissue metabolism (Figure 2) (Geurts *et al.*, 2011). Additional investigation is required to understand the precise impacts of gut microbiota and several specific taxa or species on host metabolism.

In contrast to the results observed in adipose tissue, the effects of apelin on brain or intestinal inflammation are unclear. Obese and diabetic mice characterised by an altered gut microbiota (Geurts *et al.*, 2011) and intestinal inflammation (Brun *et al.*, 2007; Duparc *et al.*, 2011b) were recently shown to exhibit altered enteric glucose detection and NO release in the hypothalamus, leading to peripheral insulin resistance (Duparc *et al.*, 2011a,b). In *db/db* mice, there was a positive correlation between intestinal apelin and APJ mRNA expression, inflammatory markers, endoplasmic reticulum stress and gut barrier disruption markers (P.D. Cani and C. Knauf, personal communication). A relationship between these findings and the gut microbiota, the apelinergic system and gut barrier function remain to be demonstrated.

4. Changes in the gut microbiota in the context of obesity and type 2 diabetes: lessons from intervention studies with prebiotics

Although discovery of the mechanisms involved in energy homeostasis, adipose tissue metabolism and gut barrier function are of utmost importance, most of the studies that initially contributed to these discoveries were performed using specific tools that are capable of changing the composition of the gut microbiota, such as prebiotics. Prebiotics are defined as '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). Some NDCs are recognised as prebiotics after they were preferentially fermented by specific types of bacteria generally considered beneficial for the host (Roberfroid *et al.*, 2010). *Bifidobacterium* spp. represent an important and complex group of bacteria with interesting health effects (Boesten and De Vos, 2008; Boesten *et al.*, 2009; Turroni *et al.*, 2009). We previously demonstrated that diet-induced obesity (high fat/low carbohydrate diet) in mice markedly affects the gut microbial community and that levels of *Bifidobacterium* spp. were significantly reduced (Cani *et al.*, 2007a,b). In accordance with these observations, several studies performed in humans have reported a low number of *Bifidobacterium* spp. correlated with the development of obesity and/or diabetes (Kalliomaki *et al.*, 2008; Wu *et al.*, 2010). Modulation of the gut microbiota with nutrients is of interest to reverse host metabolic alterations linked to gut microbiota dysbiosis. Based on animal and human studies, the intake of highly fermentable NDC could increase satiety, improve glucose tolerance, lower hepatic and serum lipids, and control hypertension (Cani *et al.*, 2006a, 2009a; Delzenne and Cani, 2005; Slavin, 2005).

The majority of scientific data (both experimental and human) on the effects of prebiotics have been obtained using food ingredients and supplements from two chemical groups, the inulin-type fructans and the galacto-oligosaccharides. These groups have repeatedly demonstrated the capacity to selectively stimulate the growth of bifidobacteria and, in some cases, lactobacilli, leading to significant changes in the composition of gut microbiota in various pathophysiological contexts (Roberfroid, 2007; Macfarlane *et al.*, 2008). In inulin-type fructans-treated mice, *Bifidobacterium* spp. was significantly and positively correlated with improved glucose homeostasis and normalised obesity-related inflammation (decreased metabolic endotoxaemia and tissue or serum proinflammatory cytokines) (Cani *et al.*, 2007b). Most of the physiopathological characteristics (glucose intolerance, metabolic endotoxaemia, systemic inflammation and gut permeability) induced by a high-fat diet were counteracted when the gut microbiota was intentionally modified by supplementation with gluco-oligosaccharides for 3 months (Serino *et al.*, 2012).

Several mechanisms have been proposed to link the events that occur in the gut following carbohydrate fermentation and control of metabolic disorders. Changes in the synthesis of gastrointestinal peptides that control food intake (glucagon-like peptide-1 (GLP-1), peptide YY (PYY), ghrelin, amylin, pancreatic polypeptide) can occur (Cani *et al.*, 2006b; Cani *et al.*, 2009a; Cani *et al.*, 2009b). However, the impact of gut microbiota on appetite regulation may not fully explain the phenotype because food restrictions or pair-feeding experiments were not able to replicate all of these metabolic features (Cani *et al.*, 2005a; Muccioli *et al.*, 2010). This suggests that regulation of fat storage is under the control of several mechanisms associated with the composition and activity of gut microbiota. Important mechanisms involving energy sparing resulting in part from the fermentation of NDC into short chain fatty acids have been identified. The release of those bacterial metabolites allow them to act as regulators of adiposity via G-protein-coupled receptors 41 and 43 (GPR41 and GPR43) and/or peroxisome proliferator-activated receptor-γ (PPAR γ)-dependent mechanisms (Dewulf *et al.*, 2011; Samuel *et al.*, 2008).

Are *Bifidobacterium* spp. doing the job alone?

Bifidobacteria may not explain the complex impact of prebiotic treatment on gut microbiota composition. Using pyrosequencing and phylogenetic microarray analysis, we identified more than 100 taxa that differed between the prebiotic treatment and control diet; 8 of the taxa were increased and 8 were decreased by more than 10-fold (Everard *et al.*, 2011). This allowed to identify bacteria that are promoted using a prebiotic approach, such as *Akkermansia muciniphila,* the abundance of which has been inversely correlated with body weight (Collado *et al.*, 2008; Everard *et al.*, 2011; Karlsson *et al.*, 2012; Santacruz *et al.*, 2010) and type 1 diabetes (Hansen *et al.*, 2012) in mice and humans. Other studies have also shown that prebiotic fibre decreased the *Firmicutes* to *Bacteroidetes* ratio in obese rats (Parnell and Reimer, 2012).

In an intervention study using prebiotics versus a placebo in obese women, complex modulation of the gut microbial ecology occurred after prebiotic treatment even though an increase in bifidobacteria remains the most common signature of prebiotic treatment with inulin-type fructans (Dewulf *et al.*, 2013). At the phylum level, treatment with inulin-type fructans increased *Firmicutes* and *Actinobacteria* and decreased *Bacteroidetes*. Increased levels of *Firmicutes* following prebiotic treatment were due to increases in bacilli and *Clostridium* clusters IV and XVI. Changes in bacilli were negatively associated with changes in metabolic endotoxaemia, whereas changes in *Clostridium* cluster IV were negatively correlated with changes in fat mass and fasting glucose homeostasis. Prebiotic-induced changes in *Faecalibacterium prausnitzii* were negatively correlated with changes in metabolic endotoxaemia. Levels of *Propionibacterium* and *Bacteroides vulgatus* both decreased after prebiotic treatment and were positively correlated with plasma levels of phosphatidylcholine and lactate. Increased levels of *Collinsella*, a genus belonging to *Actinobacteria* that can significantly increase with prebiotics, were correlated with higher urinary levels of hippurate. *Collinsella aerofaciens* has been associated with a low risk of colon cancer (Moore and Moore, 1995).

Numerous human studies have demonstrated the bifidogenic effect of galacto-oligosaccharides (for review see Macfarlane *et al.*, 2008). Multiplex sequencing of 16S rDNA tags revealed that consumption of galacto-oligosaccharides for 12 weeks induces significant compositional alterations in the faecal microbiota, mainly by increasing the abundance of organisms within *Actinobacteria*. Specifically, several distinct lineages of *Bifidobacterium* spp. were enriched (Davis *et al.*, 2011). Although increases in *Firmicutes* were also observed, these changes were detectable in only a few individuals. The enrichment of bifidobacteria was generally at the expense of one group of bacteria, the *Bacteroides*.

Impact of other non-digestible carbohydrates on gut microbiota and metabolism

Several NDCs, such as glucans, galactans, arabinoxylans (AX), resistant starch (dextrin), hemicellulose, pectins and their oligosaccharides, are fermented by the gut microbiota (Neyrinck and Delzenne, 2010; Slavin *et al.*, 2009). Most of them are present in cereal grains. AX are complex carbohydrates found in the cell walls of the starch endosperm, the aleurone layer and the pericarp tissues of cereals and represent 50% of the dietary fibres in wheat bran (Broekaert *et al.*, 2011; Grootaert *et al.*, 2009; Neyrinck and Delzenne, 2010). Supplementing a concentrate of water-extractable high molecular weight AX in the diet decreased high-fat-induced adiposity, body weight gain, serum and hepatic cholesterol accumulation and insulin resistance (Neyrinck *et al.*, 2011). AX added to a high-fat diet led to an increase in bifidobacteria, particularly *Bifidobacterium animalis subsp. lactis*. AX also restored the caecal content of gram negative *Bacteroides-Prevotella* spp. and gram positive *Roseburia* spp. bacterial populations to control levels, which were decreased following a high-fat diet. Correlation analysis revealed that *Roseburia* spp. and *Bacteroides-Prevotella* levels were inversely correlated with the host metabolic parameters. Hydrolysis of the highly polymerised AX from bran through wheat-associated endoxylanases or specific intestinal bacteria possessing arabinoxylan-degrading enzymes leads to the formation of arabinoxylan oligosaccharides (AXOS). AXOS are characterised by their average degree of polymerisation and arabinose substitution. In mice, we demonstrated that AXOS supplementation induced caecal and colon enlargement associated with an important bifidogenic effect (Neyrinck *et al.*, 2012b). The number of lactobacilli were decreased with AXOS supplementation compared to a high-fat diet alone or control diet, while the number of *Bacteroides-Prevotella* spp. was not significantly modified by AXOS treatment. AXOS increased the level of circulating satietogenic peptides, such as PYY and GLP-1, and counteracted high-fat-induced body weight gain and fat mass development (Figure 1). High-fat-induced hyperinsulinaemia and the homeostasis model assessment of insulin resistance index (HOMA-IR) were decreased with AXOS intake. In addition, AXOS reduced high-fat-induced metabolic endotoxaemia and macrophage infiltration in the adipose tissue, whereas tight junction proteins altered with high-fat intake were upregulated by AXOS treatment, suggesting that a lower inflammatory tone was associated with the improvement of gut barrier function (Neyrinck *et al.*, 2012b).

In humans, the levels of bifidobacteria significantly increased after AXOS treatment, but this effect was not restricted to this specific genus (Cloetens *et al.*, 2010; Walton *et al.*, 2012). An increase in total bacteria occurred following consumption of AXOS bread. Consumption of

AXOS bread increases faecal butyrate and reduces isovalerate, suggesting a beneficial shift in fermentation end products (Walton *et al.*, 2012). Another study showed a specific increase in bifidobacteria but not in *Lactobacillus* spp., *Bacteroides*, *Clostridium coccoides*, *Roseburia intestinalis-Eubacterium rectale* group, *F. prausnitzii* and *Clostridium* clusters I and II after consumption of 4.8 g/day of AXOS in ready-to-eat cereal for 3 weeks in 5 healthy men and women (Maki *et al.*, 2012).

Links between the effects of wheat NDCs on bacterial metabolism and obesity-related phenotypes (e.g. body weight, hunger and satiety scores) and/or type 2 diabetes (e.g. glycaemia, insulinaemia) in human interventions should be assessed to evaluate the relevance of AXOS on host energy metabolism. Health benefits related to weight control using wheat dextrin or glycaemic control using AX fractions have been reported in healthy subjects and diabetic populations. However, whether changes of the gut microbiota composition observed in healthy subjects (Maki *et al.*, 2012) directly contribute to the beneficial effects of AX observed in diabetic population remains to be investigated (for review see Neyrinck and Delzenne, 2010).

Fungi and edible mushrooms are natural sources of NDCs. For example, chitin combined with beta-glucan is the main component of the fungal cell exoskeleton. Both chitin-glucan and the deacetylated form of chitin, chitosan, share characteristics with dietary fibre, including resistance to mammalian digestive enzymes. In mice, supplementation of a high-fat diet with fungal chitin-glucan (10% w/w) extracted from *Aspergillus niger* induced enhanced caecal fermentation with prominent changes in the gut microbiota. Specifically, chitin-glucan restored the number of bacteria from *Clostridium* cluster XIVa, including the number of *Roseburia* spp., which were decreased due to high-fat intake (Neyrinck *et al.*, 2012a). The chitin-glucan treatment significantly decreased high-fat-induced body weight gain, fat mass development, fasting hyperglycaemia, glucose intolerance, hepatic triglyceride accumulation and hypercholesterolaemia independent of caloric intake. All of these parameters were negatively correlated with these specific bacteria (Neyrinck *et al.*, 2012a). In contrast to prebiotic inulin-type fructans that specifically target the bifidobacteria species, the effects of chitin-glucan on obesity appear to be independent of GLP-1 production. Although the chitin-glucan extract did not significantly affect high density lipoprotein cholesterol, triglycerides, glucose, insulin, or F2-isoprostane levels in humans, administration of 4.5 g/day for 6 weeks significantly reduced the level of oxidised low-density lipoproteins compared to the placebo (Bays *et al.*, 2013). In rodents, the improvement in this marker of atherosclerosis is related to modulation of the gut microbiota. These effects cannot be generalised to all edible mushroom extracts, because it was recently shown that an *Agaricus blazei* Murill extract protected against diet-induced obesity in rats without affecting the composition of the gut microbiota, although the pancreatic lipase activity and lipid absorption were inhibited (Vincent *et al.*, 2013).

Resistant starches (RS) are also fermentable non-digestible carbohydrates (Bird *et al.*, 2010; Robertson, 2012). Although they are not regarded widely as a prebiotics, most forms of RS induce changes in gut microbiota composition (Flint, 2012). In rodents, data suggest that chronic RS feeding upregulates proglucagon expression (i.e. GLP-1 precursor) in the colon with concomitant increases in neuropeptide expression in the hypothalamus (Shen *et al.*, 2009; Zhou *et al.*, 2008). These effects result in weight loss and improvements in glycaemic control. However, to date there is no evidence for this in humans (for review see Robertson, 2012).

Other classes of non-digestible dietary substances that are generally not considered prebiotics should be evaluated because they possess chemical structures that are different from carbohydrates. For example, some phenolic compounds abundant in fruit, vegetables, chocolate, nuts and beverages (tea, coffee, wine and soy milk) may be poorly absorbed in the upper part of the gut and can reach the colon to be fermented by bacteria. Polyphenols present in pomegranates (ellagitannins and anthocyanins) have important antioxidant and anti-inflammatory bioactivities that have been demonstrated *in vitro* (Basu and Penugonda, 2009). Promising results obtained using pomegranate products against cardiovascular disease and diabetes have been reported in human clinical trials (for review, see Johanningsmeier and Harris, 2011). However, phenolic components of common foods can readily contribute to modulation of the gut bacteria (Parkar *et al.*, 2008; Selma *et al.*, 2009; Tzounis *et al.*, 2008). One study examined the metabolism of pomegranate by-products by gut bacteria using culture fermentation systems reflective of the distal region of the human large intestine. Exposure to the pomegranate extract enhanced the total growth of *Bifidobacterium* spp. and *Lactobacillus* spp. without influencing the *C. coccoides-E. rectale* and the *Clostridium histolyticum* groups (Bialonska *et al.*, 2010). The authors suggest that pomegranate oligomers composed of gallic acid, ellagic acid and glucose units may account for the enhanced growth of specific bacteria.

We tested the prebiotic potency of a pomegranate peel extract rich in polyphenols in a mouse nutritional model of obesity associated with hypercholesterolaemia and inflammatory disorders. Supplementation with pomegranate peel extract was able to modulate the gut microbiota in favour of bifidobacteria without significantly modifying *Bacteroides-Prevotella* spp. (P=0.07 versus mice fed a high-fat diet), *Lactobacillus* spp. or *Roseburia* spp. (Neyrinck *et al.*, 2012b). This prebiotic effect was accompanied by a lower expression of key inflammatory

components in the colon and visceral adipose tissue. However, it did not significantly modify body weight gain, glycaemia, glucose tolerance and inflammatory serum markers (Neyrinck *et al.*, 2013). The changes in gut microbiota due to treatment with pomegranate peel extract were accompanied by an improvement in atherogenic markers, such as LDL cholesterol in high-fat diet-induced obesity (Neyrinck *et al.*, 2013).

Resveratrol is a natural phytoalexin produced by various plants, such as red grapes (*Vitis vinifera* L.), peanuts (*Arachis* spp.), berries (*Vaccinium* spp.) and *Polygonum cuspidatum* that exerts antioxidant and anti-inflammatory properties. One study showed that resveratrol increased GLP-1 production and required the GLP-1 receptor to mediate its anti-diabetic effect in high-fat diet-induced diabetic mice. The mechanism through which GLP-1 secretion is restored may be linked to a change in intestinal microbiota and inflammation. Denaturing gradient gel electrophoresis profiles clearly showed that resveratrol normalised the strongly modified caecal bacterial composition of animals fed a high-fat diet after a 5 week treatment. Specifically, bands corresponding to *Parabacteroides johnsonii*, *Alistipes putredinis* and *B. vulgatus* were induced by high-fat treatment and disappeared with resveratrol supplementation (Dao *et al.*, 2011). Those results suggest that polyphenol extracts can confer positive health impacts associated with gut microbiota modulation and may be a natural alternative for preventing obesity and cardiovascular diseases.

We postulate that a strong interaction exists between gut microbiota and polyphenols based on the following considerations: (1) polyphenol gut microbiota metabolites are often better absorbed than the parent food phenolics; (2) these microbial metabolites have specific biological effects that extend those of the food phenolic compounds; and (3) polyphenols can modulate the gut microbiota and promote development of some bacterial groups. This is a major topic of innovative research in the fields of obesity and type 2 diabetes.

5. Conclusions

The global epidemic of overweight and obesity is becoming a major health problem in many parts of the world. Although this area of science has been investigated for many years, most of the projects performed in an academic setting or within individual drug discovery projects in the private sector have failed to fill the gap of knowledge regarding the mechanisms responsible for these multifactorial and complex conditions. The current paradigm in basic science, drug discovery and development emphasises an improved understanding of physiopathology and etiopathology to provide medical solutions. The growing evidence that the gut microbiota is a novel partner that should be considered in inter-organ crosstalk has led to the discovery of various interactions between host and gut microbes. Identifying the different microbiota-to-host signalling pathways that are recruited to trigger the onset of metabolic diseases may offer new templates and therapeutic targets. The endocannabinoid and apelinergic systems are both involved in regulation of several common metabolic features (i.e. appetite regulation, inflammation, glucose homeostasis, adipose tissue functions) and may be considered future targets. Targeted approaches with a specific focus on non-digestible nutrients (e.g. inulin-type fructans, AX and polyphenols) that can modify the gut microbiome appear to be a promising area of research for designing nutritional interventions to improve the metabolic profiles observed in obesity and type 2 diabetes.

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