Glucose metabolism: Focus on gut microbiota, the endocannabinoid system and beyond

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Abstract

The gut microbiota is now considered as a key factor in the regulation of numerous metabolic pathways. Growing evidence suggests that cross-talk between gut bacteria and host is achieved through specific metabolites (such as short-chain fatty acids) and molecular patterns of microbial membranes (lipopolysaccharides) that activate host cell receptors (such as toll-like receptors and G-protein-coupled receptors). The endocannabinoid (eCB) system is an important target in the context of obesity, type 2 diabetes (T2D) and inflammation. It has been demonstrated that eCB system activity is involved in the control of glucose and energy metabolism, and can be tuned up or down by specific gut microbes (for example, Akkermansia muciniphila). Numerous studies have also shown that the composition of the gut microbiota differs between obese and/or T2D individuals and those who are lean and non-diabetic. Although some shared taxa are often cited, there is still no clear consensus on the precise microbial composition that triggers metabolic disorders, and causality between specific microbes and the development of such diseases is yet to be proven in humans. Nevertheless, gastric bypass is most likely the most efficient procedure for reducing body weight and treating T2D. Interestingly, several reports have shown that the gut microbiota is profoundly affected by the procedure. It has been suggested that the consistent postoperative increase in certain bacterial groups such as Proteobacteria, Bacteroidetes and Verrucomicrobia (A. muciniphila) may explain its beneficial impact in gnotobiotic mice. Taken together, these data suggest that specific gut microbes modulate important host biological systems that contribute to the control of energy homoeostasis, glucose metabolism and inflammation in obesity and T2D.

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1. Introduction

The gut microbiota is now considered a separate organ that is involved in the regulation of numerous physiological pathways by impacting different functions of the host [1]. Among these regulatory actions, the influence of gut microbes on energy metabolism is of particular interest, as it has been proposed to be a driving force in the pathogenesis of metabolic diseases, especially obesity. Intestinal microbes have developed a mutually beneficial relationship with their host, and can influence physiological systems by modulating gut motility, intestinal barrier homoeostasis, nutrient absorption and fat distribution [2–4]. The proof of concept for the involvement of gut bacteria in the processes of energy homoeostasis was demonstrated with germ-free mice, which lack microbiota [5]. These animals exhibit a reduced fat mass compared with their conventionally raised littermates in spite of an increased food intake. Moreover, transplantation of faecal microbiota from obese mice into lean germ-free mice has demonstrated the capacity of the bacterial ecosystem to alter host phenotype independently of either genotype or diet [6]. Several studies have confirmed this ability, including a recent one showing that the transplantation of microbiota from twins discordant for obesity partially replicated the donor’s phenotype. Transferring gut microbes from obese twin donors to germ-free mice increased their weight gain compared with mice transplanted with microbiota from lean twin donors [7]. These experiments confirm the influence of microbiota on the development of obesity, although causality between the observed changes and metabolic symptoms is still unclear, as well as the applicability of this knowledge to the diagnosis, prevention and treatment of the metabolic syndrome. Nevertheless, they do suggest a relationship between nutrition, gut microbiota and energy homoeostasis.
The present review discusses recent evidence supporting the hypothesis that gut microbiota can influence the whole of host metabolism through various mechanisms and that changes in microbiota composition can trigger changes in metabolic behaviour. Signalling and molecular pathways involved in the regulation of energy homoeostasis by the gut microbiota are varied [including short-chain fatty acids (SCFAs), the endocannabinoid (eCB) system and gut peptides], with many others that are yet to be identified or clarified.

2. Gut microbiota and metabolism: putative actors and pathways

Among the most studied bacterial metabolites that can interfere with host metabolism are the SCFAs. These products of microbiota-mediated fermentation of polysaccharides modulate levels of several gut hormones involved in glucose and energy homoeostasis, including glucagon-like peptide (GLP)-1 and ghrelin [8,9]. These metabolites circulate in the blood and, thus, can act on peripheral targets to modulate insulin sensitivity and the whole of the host’s energy metabolism [10,11]. Unfortunately, most of the pathways underlying these effects are still largely unknown, although several studies have suggested a link with members of a recently identified G-protein-coupled receptor family that includes G-protein-coupled receptors 43 (GPR43) and 41 (GPR41) [12,13]. An elegant study by De Vadder et al. [14] recently showed that SCFAs activate intestinal neoglucogenesis through a cAMP-dependent mechanism and a gut–brain neural circuit involving GPR41, thereby pointing to intestinal neoglucogenesis as a novel actor in gut microbiota ability to host interactions.

In addition to these specific metabolites, the gut bacteria are also able to interact with the host through specific cell membranes and other related molecules that activate pattern-recognition receptors (PRRs). PRRs recognize molecular patterns unique to bacteria and other microorganisms (pathogen-associated molecular patterns, or PAMPs). The most studied PRRs are the toll-like receptors (TLRs) which, when stimulated, result in an inflammatory response, cytokine production and chemokine-mediated recruitment of acute inflammatory cells [15]. Of the PAMPs, we discovered that lipopolysaccharides (LPSs), components of the cell walls of Gram-negative bacteria, contribute to the development of inflammation and insulin resistance in both obesity and type 2 diabetes (T2D) in a condition known as “metabolic endotoxaemia” (Fig. 1) [16,17]. This is associated with altered gut microbiota composition as well as increased intestinal permeability, resulting in increased plasma LPS levels (Fig. 1) [16–19]. Such increases lead to CD14/TLR4 activation, which in turn induces an inflammatory response that results in perturbations of energy homoeostasis as well as increased inflammation, hepatic steatosis, hyperinsulinaemia and insulin resistance [16]. Specifically, LPS acts first on the liver by inducing hepatic insulin resistance [16] and decreasing hepatic inflammatory responses by depleting Kupffer cells prevent high-fat-diet (HFD)-induced metabolic effects [20–22]. Taken together, these data highlight a strong relationship between gut microbiota, inflammation and metabolic perturbations.

There is also growing interest in the study of the intestinal mucus layer and its interactions with microbiota. Recently, we have demonstrated the key role played by the gut microbiota and its interaction with the mucus layer in the context of diet-induced obesity and T2D, where the numbers of Akkermansia muciniphila, mucin-degrading bacteria that reside in and abundantly colonize the mucus layer, were negatively correlated with body weight and decreased under HFD conditions (Fig. 1) [23]. Moreover, daily administration of A. muciniphila to HFD-induced obese mice for 4 weeks improved their metabolic profile by decreasing weight gain, restoring mucus-layer thickness, and counteracting metabolic endotoxaemia and insulin resistance [23]. A recent study by Shan et al. [24] confirmed that the mucus layer is not only a non-specific physical barrier, but also a complex organized structure that can deliver immunoregulatory signals that participate in gut homoeostasis. On the same topic, Kashyap et al. [25] recently revealed that modification of mucus carbohydrate composition also influences the microbiota. In a gnotobiotic mouse model that presented a severe decrease in mucus fucosylated glycans, they observed a decrease in faecal microbiota diversity compared with a control group that was associated with major changes in faecal and urinary metabolites, suggesting modification of the entire metabolism of the animals [25]. These data were further confirmed by Sommer et al. [26], who found alterations in gut microbiota composition and intestinal architecture in mice with a defect in mucus glycosylation. These findings support an important role for the mucus layer in energy homoeostasis, although the key mechanisms linking intestinal mucus production and whole-body energy homoeostasis have yet to be fully elucidated.

The gut microbiota interact with other organs. Its interactions with the liver through LPS-induced inflammation in obesity have already been alluded to, and other reports strongly suggest interactions with the brain. The presence of cross-talk between the gut microbiota and the brain was demonstrated nearly 10 years ago, when we found that changing the gut microbiota with prebiotics reduced food intake, body weight and fat-mass development in rodents; the phenomenon was also associated with an increase in the endogenous production of anorexigenic peptides, such as GLP-1 and peptide tyrosine tyrosine (PYY), and a decrease in the orexigenic peptide ghrelin [27–29]. Differences in the central expression of peptides involved in energy homoeostasis between germ-free and conventional mice have also been demonstrated [30]. The latter animals exhibited decreased central expression of proglucagon (precursor of GLP-1), and less brain-derived neurotrophic factor (BDNF) and its receptor, compared with the germ-free mice. The authors also found that the expression of food intake regulatory neuropeptides was modified, with decreases in neuropeptide Y (NPY) and Agouti-related peptide (AgRP) expression, and increases in proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) expression compared with conventional mice [30]. These studies suggest the involvement of a neural pathway that allows an exchange of information between gut microbiota and hypothalamic nuclei involved in energy homoeostasis. In
addition to interactions at the level of energy homoeostasis, and
to further underscore the close relationship between the gut and
the brain, particular pathologies such as autism and depression
have also been linked to the gut microbiota [31,32]. Indeed,
early studies showed that depression in women is associated
with increased fermentation of carbohydrates [33], suggesting a
relationship between changes in the composition or metabolic
activity of the gut microbiota and brain-related pathologies.
Independently, recent data have also shown that jejunal pro-
teins from either obese mice or insulin-resistant human subjects
impaired muscle insulin signalling, thereby promoting insulin
resistance [34]. From this it may be speculated that gut micro-
biota and its byproducts might also interact with muscles and fat
depots (Fig. 1) [16,35]. However, in spite of this wide range of
actors and interactions, it is important to remember that energy
homoeostasis is a highly complex integrated system involving
a number of pathways, and that gut microbes are neither the only
nor the most important factors in energy homoeostasis.
A study by Harley et al. [36] of two sets of C57BL/6 mice that
differed in their obesogenic responses to HFDs revealed that the
differences were not due to different gut microbiota composi-
tion. Given this result, the authors reported uncertainty as to the
involvement of gut microbiota in obesity through the expression
of species-independent metagenomic pathways.

Of the various pathways involved in the regulation of glu-
cose metabolism and energy homoeostasis, numerous studies
have been done on the eCB system. We have demonstrated that
different links between the gut microbiota and the eCB system
prompting an assessment of the physiology of the eCB system
and its links to diabetes.

3. Changing eCB system activity: a therapeutic target?

The eCB system is complex, comprising several bioac-
tive lipids and the enzymes that regulate their production and
degradation, and different types of nuclear and cell mem-
brane receptors. eCBs are synthesized on demand from cell
membrane phospholipids and immediately released from the
cell to target their receptors [37]. Anandamide (AEA) and 2-
arachidonoylglycerol (2-AG) are the best-characterized eCBs so
far. Their principal receptors are the G\textsubscript{i/o}-coupled receptors CB\textsubscript{1}
and CB\textsubscript{2}, which are also targeted by the principal active compo-
nent of Cannabis sativa, Δ\textsuperscript{2}-tetrahydrocannabinol (Δ\textsuperscript{2}-THC)
[38,39]. Several pathways are involved in both AEA and 2-
AG synthesis and degradation, making this a relatively complex
signalling system [37,40]. Monoacylglycerol lipase (MAGL) is
thought to be the primary enzyme responsible for 2-AG degra-
dation, whereas fatty acid amide hydrolase (FAAH) is thought
to be the main AEA degrading enzyme [41]. Although eCB
metabolism has mostly been studied at the level of FAAH and
MAGL, another degrading enzyme – α/β-hydrolase domain 6
(ABHD6) – has recently been implicated in 2-AG hydrolysis
[42].

Fig. 1. Cross-talk between gut microbiota and host. Obesity and type 2 diabetes (T2D) are associated with changes in the composition of gut microbiota, leading
to increases in some genera and decreases in others (arrow direction indicates either increased or decreased abundance). Some bacteria are positively or negatively
associated with obesity and T2D, depending on the study (indicated by question marks). Increased mucus degradation is associated with increased gut permeability
and metabolic endotoxaemia, triggering inflammation, macrophage infiltration of adipose tissue and insulin resistance (adapted from Delzenne et al. [111]).
Other eCB-related lipids considered part of the eCB system include palmitoylethanolamine (PEA) and oleoylethanolamine (OEA). These lipids activate non-cannabinoid receptors – namely, peroxisome proliferator-activated receptor alpha (PPARα), GPR55, GPR119 and transient receptor potential cation channel, subfamily V, member 1 (TRPV1) – and share biosynthesis and degradation pathways with AEA and 2-AG [40,43,44]. A schematic overview of eCB synthesis and degradation is shown in Fig. 2.

Over the past decade, research into eCBs has confirmed that the eCB system is a key signalling system implicated in the regulation of energy homeostasis and metabolism [45]. In fact, eCBs are widely produced in organs that contribute to energy homoeostasis (pancreas, muscle, gut, adipose tissue, liver and hypothalamus) and normally facilitate energy intake and storage in particular (Fig. 3). As eCB levels in tissues are tightly controlled by a balance between synthesis and degradation, dysregulation of this control can lead to pathological conditions such as obesity and T2D (Fig. 3). Indeed, the eCB system is severely altered in obesity, which may result in increased eCB levels and CB1 activity in the brain, liver, adipose tissue and skeletal muscles [46], and decreased levels of enzymes and receptors in other organs, such as the stomach, kidneys and heart (Fig. 3) [47]. However, we also found that eCB-related molecules such as PEA, OEA and stearyl ethanolamine (SEA) are inversely related to AEA in obesity, T2D and inflammation (Geurts L., Muccioli G.G. and Cani P.D., personal communication). CB1 receptor stimulation increases food intake (Fig. 3) [48], whereas pharmacological inhibition of CB1 decreases fat-mass expansion and reduces body weight [49–52]. The role of the CB1 receptor was confirmed several years ago using CB1 knockout (CB1−/−) mice. Without the CB1 receptor, mice were resistant to diet-induced obesity (DIO) [48–50]. However, the CB1 receptor is not the only receptor incriminated in these metabolic disturbances, as FAAH−/− mice have also shown augmented body weight and food motivation compared with their wild-type littermates [53]. More recently, the crucial role of ABHD6 was detailed by Thomas et al. [54], who found that ABDH6 was up-regulated by HFD, while ABHD6−/− mice were resistant to DIO. These data suggest that the eCB system as a whole plays a central role in the control of body weight and central appetite regulation.

eCBs have also been implicated in the development of T2D and insulin resistance, as CB1−/− mice do not display the disturbances associated with DIO. The first studies describing CB1−/− mice and pharmacological inhibition of CB1 also reported less
insulin resistance in DIO mice [49,50] and more muscle glucose uptake [55]. The role of eCBs in the control of glucose homeostasis has been reported by several studies showing the involvement of the eCB system in pancreatic islets in rodents and humans; however, it is still not clear whether CB1 receptor activation controls the insulin signalling pathways or, more directly, insulin release by β cells (Fig. 3) [56–60]. Insulin is a potent regulator of eCB metabolism in normal adipocytes [61]. Acute administration of AEA and eCB agonists in mice influences insulin sensitivity, induces glucose intolerance, and modulates insulin-induced glucose uptake and glucose transporter type 4 (GLUT4) translocation in differentiated adipocytes and skeletal muscle; these effects can be reversed by treatment with a CB1 receptor antagonist [62–65]. Blocking the CB1 receptor improves insulin sensitivity and increases whole-body insulin-dependent glucose utilization by stimulating glucose uptake in white and brown adipose tissue (WAT and BAT, respectively). eCB-induced modulation of BAT function could be one of the mechanisms by which eCBs exert their insulin-sensitizing effects [66]. Interestingly, liver-specific CB1-/ mice become obese under HFD conditions, but are resistant to hepatic steatosis and insulin resistance. These results highlight the key role of the hepatic CB1 receptor [67], and were recently confirmed by Liu et al. [68], who showed that mice with a specific overexpression of the CB1 receptor in hepatocytes became hyperinsulinemic as a result of reduced insulin clearance. Furthermore, FAAH-/- mice exhibit ectopic lipid storage in the liver, leading to an insulin-resistant state.

These results demonstrate a role for eCB players beyond the CB1 receptor and AEA [69]. Recently, we have shown that chronic stimulation with a potent CB1/CB2 receptor agonist (HU210) induces glucose intolerance in wild-type mice under physiological conditions and exacerbates glucose intolerance under HFD challenge, highlighting the specific role of the eCB system in controlling glucose homeostasis [35]. The CB2 receptor has also been implicated in glucose homeostasis, as CB2 receptor activation improves glucose tolerance in rats receiving a glucose load, mimicking the effect of CB1 receptor blockade [70]. These findings suggest that the coordinated actions of both CB1 and CB2 receptors modulate glucose homeostasis. However, the exact role of the CB2 receptor still needs further clarification.

Recent studies have unravelled the underlying mechanisms of eCB-mediated glucose metabolism dysregulation. Acute and chronic treatment of adipocytes with a CB1 receptor antagonist upregulates GLUT-4 expression, and this regulation has a transcriptional effect on both nuclear factor (NF)-κB and sterol regulatory element-binding protein (SREBP)-1 [71]. Furthermore, hepatic CB1 activation leads to accumulation of long-chain ceramides in the liver that appear to mediate eCB-induced hepatic insulin resistance [72]. An essential feature in the progression of insulin resistance may be islet inflammation, although the primary proinflammatory signal remains unidentified. In addressing this issue, a recent study revealed that peripheral blockade of the CB1 receptor delays progression of T2D and the underlying β-cell loss in Zucker diabetic fatty (ZDF) rats. This study showed that the CB1 receptors mediating these effects are located on infiltrating macrophages, and their activation induces the Nlrp3 inflammasome [73]. Nevertheless, the exact role of the CB1 receptor is complicated, as demonstrated by Li et al. [74], who found that genetic loss of the CB1 receptor in leptin-resistant (ob/ob) mice failed to suppress Ob phenotype and even worsened abnormal glucose metabolism in ob/ob mice, thereby highlighting the complex relationship

Fig. 3. The endocannabinoid (eCB) system and metabolism. This system plays a central role in the regulation of glucose homeostasis and insulin sensitivity in several peripheral organs. CB1 receptor activation induces gut permeability. Gut microbiota composition is associated with intestinal eCB content and CB1 receptor mRNA expression.
between the CB₁ receptor and leptin. Thus, focusing on the eCB system and diabetes reveals the essential roles of eCB mediators in insulin sensitivity, glycaemia and glucose uptake, although discrepancies can still be found in the literature regarding the precise mechanisms.

4. Cross-talk between eCBs and gut microbes: a key link in obesity and T2D?

To ascertain the role of eCBs as essential molecules in the metabolic alterations observed in obesity and T2D, we recently proposed that the eCB system mediates communication between adipose tissue and the gut [75,76]. These links have recently been reviewed [77,78]. In brief, we revealed that eCBs are implicated in gut barrier function and that specific changes in the tone of the eCB system in obesity and T2D lead to increased gut permeability (Figs. 1 and 3) [75]. These findings have been confirmed by two other recent studies [79,80]. More precisely, we found that modulating the gut microbiota of obese and T2D mice profoundly affected the tone of the intestinal and adipose tissue eCB system. Interestingly, changing the gut microbiota of ob/ob mice with prebiotics also reduced AEA content and CB₁ mRNA expression in adipose tissue, and improved adipose tissue metabolism, a phenotype similar to that observed following CB₁ receptor blockade [75]. Moreover, it was revealed that the lower fat mass and CB₁ mRNA levels were associated with markers of differentiation and lipogenesis in adipose tissue [75].

Recently, we demonstrated that the administration of *A. muciniphila* in mice fed either normal chow or HFD increased levels of 2-AG and associated acylglycerols [2-oleoylglycerol (2-OG) and 2-palmitoylglycerol (2-PG)] in the small intestine (Figs. 1 and 3) [23], thereby contributing to the anti-inflammatory effects and improved gut barrier function observed following treatment with these bacteria. Also, obese and T2D db/db mice showed changes in gut microbiota, which is consistent with an increased eCB system tone and inflammation in adipose tissue (Fig. 3) [76]. Chronic stimulation of the eCB system increased adipogenesis in lean mice, and induced the metabolic endotoxaemia related to the low-grade inflammation associated with obesity [16,75]. Recently, it has been proposed that adipocytes produce more AEA during adipocyte differentiation, and this AEA may be converted to prostamides by neighbouring preadipocytes, thus inhibiting adipogenesis [81]. All these data demonstrate that the eCB system is closely involved in adipose tissue metabolism, and also acts as a mediator between gut microbiota and adipose tissue.

5. Gut microbiota and T2D: a lack of consensus

One of the earliest pieces of evidence for obesity- and diabetes-related dysbiosis in mice and humans was the increase in Firmicutes-to-Bacteroidetes ratio that correlated with an increase in body mass index (BMI; Fig. 1) [6,82,83]. However, a number of recent studies, including some with human cohorts, reported no variations in this ratio between diabetic or obese patients and controls [84,85]. Abundance is a measure of the relative proportion of each bacterial phylum within an ecosystem, whereas diversity takes into account the number of bacterial phyla identified (richness) in addition to their relative abundance. Despite being useful descriptors of the bacterial ecosystem in general, neither term appears to be a reliable indicator of host diabetic status [84,86]. Although animal experiments show a clear separation between diabetic and non-diabetic subjects based on their microbiota profiles [76,87], the interindividual variability in humans most likely masks these large-scale differences [84]. Moreover, the results are not always concordant between cohorts. For example, Danish and Chinese T2D patients are clustered separately based on metagenomic data [88]. This suggests that the extrapolation of results, such as the prediction of diabetic status from microbiota data, is difficult across populations and illustrates probable population-based specificity [88]. The concept of enterotypes was initially proposed to classify human populations according to their dominant intestinal microbial features [89], but no correlations were found between enterotypes and diabetic status in the Chinese cohorts [84,90].

While the taxonomy of the microbiota alone has failed to explain their influence on the onset of the metabolic syndrome, description of bacterial metabolic functions (at the genetic level) using metagenomic shotgun sequencing in humans and mice has proved to be a reliable, complementary tool, and has revealed shifts in metabolic function related to both obesity and T2D [6]. The most prominent features of T2D-associated metagenomes are the enriched pathways related to carbohydrate metabolism and transport, branched-chain amino-acid transport and response to oxidative stress. On the other hand, pathways related to flagellar assembly, butyrate biosynthesis and vitamin metabolism were reduced in the Danish and Chinese cohorts [88,90].

Following the same reasoning, several studies have identified individual taxa as important markers of obesity and diabetes onset, although the exact roles of some of these species are not currently known. The *Bacteroides*, *Roseburia* and *Akkermansia* genera, as well as *Faecalibacterium prausnitzii*, were depleted in the T2D Chinese patients, whereas *Dorea*, *Prevotella* and *Collinsella* were found in relatively greater abundance (Fig. 1) [84]. In both humans and mice, *Prevotella*, *Akkermansia* and enterobacteria have previously been shown to vary significantly between obese and lean subjects (Fig. 1) [91–95]. The abundance of *A. muciniphila* is lower in diabetic mice and, as mentioned earlier, treatment with this bacterium by gavage improved metabolism [23]. Also, a reduction in a cluster of genes belonging to *Roseburia* and *F. prausnitzii* has been identified as a discriminant marker for the prediction of diabetic status in European women [88] and, in an obese French cohort, *F. prausnitzii* abundance was less than in control subjects, while an increase in this bacterium correlated with an improved inflammatory status [96]. However, these bacteria were increased in obese Indian children compared with the lean controls, highlighting once again the specificity of population, age and diets in phenotype–taxonomy associations [97]. In the same cohort, *Lactobacillus* abundance (species and gene clusters) was greater in T2D patients and positively correlated with blood glucose levels (Fig. 1) [97]. Metagenomic analysis of the microbiota in an obese diabetic Chinese patient undergoing a 23-week
dietary intervention showed a sharp decrease in the abundance of Enterobacteriaceae family members along with a simultaneous reduction in LPS synthesis pathways and inflammation [98]. Enterobacteria are known LPS producers and contribute to the metabolic endotoxaemia observed in the metabolic syndrome [16,99–101]. Thus, despite their very low numbers relative to the immensity of the whole ecosystem, some taxa nevertheless appear to have a strong influence on the development of obesity and its associated disorders [102].

A major characteristic of T2D is the systemic increase in oxidative stress [103]. One way to counterbalance the increase of reactive oxygen species and depletion of antioxidants is to supplement the latter through diet. Several natural substances, such as polyphenols, have antioxidant effects in addition to prebiotic effects [104,105]. Intestinal bacteria such as Bifidobacterium species also exhibit antioxidant capacities in vivo [106], and dietary interventions with probiotics containing Lactobacillus acidophilus LA5 and B. animalis subsp. lactis BB12 have improved glucose tolerance and total antioxidant status in T2D patients [107]. Thus, this mechanism may also be contributing to the reported effectiveness of prebiotics and probiotics in the reduction of metabolic syndrome symptoms [95,108–112]; nevertheless, further investigations with different strains of Lactobacillus are still warranted (Fig. 1) [113].

In addition to the use of prebiotics and probiotics, there are contradictory data to suggest that antibiotic-induced changes in gut microbiota composition may either counteract or worsen obesity and T2D. For example, we discovered that broad-spectrum antibiotic treatments abolished diet-induced body weight gain, metabolic endotoxaemia, inflammation, oxidative stress and insulin resistance in mice [17], and these data have been confirmed by others [114,115]. Such findings may support the hypothesis that dysbiosis of the intestinal microbiota is associated with obesity. Furthermore, several experiments have demonstrated the ability of antibiotics to increase the abundance of A. muciniphila in mice while reducing the incidence of diabetes (Fig. 1) [116]. On the other hand, it has been suggested that early life exposure to antibiotics may contribute to body weight gain [117]. Thus, more studies are needed to elucidate the potential of antibiotics to control microbiota to improve health, as well as to demonstrate their role in the aetiology of obesity and T2D.

Thus far, no treatments using one or several gut microbes have been used to treat T2D in humans. Although the transplantation of faecal microbiota from lean donors to obese subjects improved insulin sensitivity [118], to the best of our knowledge, this experiment has yet to be duplicated in either humans or mice. Thus, finding a treatment for T2D remains a challenge. Exercise and dietary interventions often yield only limited results, while pharmacological treatments are often associated with deleterious side effects.

6. Gastric bypass and gut microbiota: towards a consensus?

Thus far, bariatric surgery is the best treatment for T2D [119,120]. The most common technique is the Roux-en-Y gastric bypass (RYGB), which is performed on individuals with a BMI ≥ 35 kg/m². In this procedure, the stomach is reduced to a small gastric pouch, and the small intestine is bypassed through the creation of a Roux limb. The rest of the stomach and duodenum thus constitute a blind duct. In addition to sustained weight loss and BMI reduction, RYGB causes a weight-independent increase in insulin sensitivity, leading to remission of T2D in around 80% of patients [119,121].

Several mechanisms can explain the impact of RYGB on T2D and they have been regrouped under the acronym BRAVE: bile acid flow modification; reduction of stomach size; anatomical modifications and altered nutrient influx; vagal manipulation; and enteroendocrine secretion modulation [122]. Interestingly, these factors have all been shown to influence or be influenced by gut microbes. By affecting O2, pH and nutrient levels, RYGB changes the gastrointestinal ecology, leading to remodelling of the gut microbial population [123,124]. In addition, the gut bacteria themselves affect the pool of bile acids [125] and secretion of gut hormones, and may regulate host metabolism through the gut–brain axis [30,126], as detailed above.

Over the past decade, many groups have investigated whether RYGB has an impact on gut microbiota through either next-generation sequencing or quantitative polymerase chain reaction (PCR) testing. Microbial communities were compared in obese subjects that had been treated or not with RYGB [91] and in the same individuals before and after the operation [96,127,128]. These studies all found an increase in Proteobacteria after surgery, mostly members of the Enterobacteriaceae family of Gammaproteobacteria. This increase has been correlated with metabolic parameters such as reductions in BMI, adipose tissue mass, serum leptin and, in some studies, inflammation markers such as C-reactive protein [196,127].

Another common feature is a change in Firmicutes-to-Bacteroidetes ratio. Although the above-mentioned bacterial phyla were not always affected in the same way, this bacterial ratio was always systematically decreased after RYGB. Various levels of Proteobacteria, Firmicutes and Bacteroidetes were conserved, regardless of patient gender, age and medication, together with changes in BMI after surgery. However, while differences in calorie intakes were mentioned in some studies [96], there was no information on any qualitative changes in the foods ingested before and after the procedure. As with HFD and/or prebiotics, the composition of the diet can modify gut microbiota [17,18,95], and RYGB has been shown to reduce the preference for fatty meals [129]. Thus, these effects on food choices need to be taken into account when investigating the impact of bariatric surgery on gut microbes.

Other studies have shown that RYGB was accompanied by a reduction in the low-grade inflammation linking obesity with insulin resistance [96,127]. As mentioned above, the effects of metabolic endotoxaemia on inflammation and insulin resistance are associated with obesity [16]. Monte et al. [110] demonstrated that plasma LPS was reduced by 20% in patients 6 months after RYGB and correlated with weight loss, while Trøseid et al. [130] found a reduction in endotoxaemia 1 year after the operation, which was associated with an improvement in glycaemic control.
These data show a correlation between microbiota modification and T2D resolution following RYGB. Changes in gut microbes were observed from 3 to 15 months after surgery, depending on the study [91,96,127,128]. This suggests that modulation of the gut microbial community after RYGB stabilizes over time. However, it is not possible to specify the cause(s) of the changes in gut microbes, or to determine whether they are causes or consequences of changes in host physiology. Recent work by Liou et al. [131] in a murine model addressed this issue by comparing diet-induced obese mice undergoing either RYGB or a sham operation. Initially, food consumption remained the same between the two groups while energy intakes decreased in the RYGB mice, leading to weight loss after the surgery. Moreover, the RYGB mice exhibited improved glucose tolerance and insulin sensitivity compared with the weight-matched sham-operated mice, confirming the weight-independent effect of RYGB on insulin resistance.

In addition, changes in gut microbiota due to RYGB are conserved, with an increase in Proteobacteria (mainly Enterobacteriaceae) and Bacteroidetes, and a decrease in Firmicutes. Moreover, Verrucomicrobia are also increased, mainly of the Akkermansia genus, which is consistent with previous findings of the impact of A. muciniphila on glucose metabolism [1,23]. Furthermore, colonization of germ-free mice with microbiota from RYGB-treated animals led to losses in weight and fat mass, with an increasing trend towards insulin sensitivity, compared with mice colonized with microbiota from sham-operated animals. These effects may have been due to modification of SCFA production in the RYGB-colonized mice [131]. These experiments show that, at least in mice, the microbiota contribute to the impact of RYGB on host physiology.

The roles of Gammaproteobacteria and Enterobacteriaceae in the outcomes of RYGB are still controversial. Their abundance is vastly increased by the procedure in every study thus far, although recent observations suggest that these bacteria might also have a role in the development of metabolic endotoxaemia through their highly toxic LPSs [98,132]. The most striking evidence comes from a study of colonization of germ-free mice with Enterobacter cloacae, the main component of the microbiota in a morbidly obese patient, which was sufficient to induce obesity and insulin resistance after exposure to HFD. Moreover, the E. cloacae-colonized mice had high levels of serum LPS and inflammation [98]. In a follow-up study, the same animals demonstrated that the weight loss and improved insulin sensitivity after treatment with prebiotics were associated with a decrease in Enterobacteriaceae and metabolic endotoxaemia [133].

Paradoxically, RYGB is associated with both greater Gammaproteobacteria abundance and less endotoxaemia. These discordant results might be explained by the fact that different bacterial species of the same family can have diametrically opposed effects on host physiology. However, any deleterious long-term effects due to the increase in Gammaproteobacteria following RYGB cannot be ignored. Further studies are therefore required to more precisely investigate the mechanisms linking changes in gut microbiota and the improvement in metabolic parameters following bariatric surgery.

7. Conclusion

These data demonstrate that several relationships can be found between gut microbiota and glucose and energy homoeostasis. However, causality between the observed variations and metabolic symptoms is still unconfirmed. In addition, the use of prebiotics as well as gastric bypass surgery has revealed several putative metabolic targets (such as SCFAs, OEA and PEA) and bacterial species that may be viewed as putative future therapeutic targets (F. prausnitzii, A. muciniphila). Thus, investigations of the gut microbiota–host interactions and deciphering their symbiotic biological interactions constitute important areas of research towards finding new ways to treat obesity and its related diseases.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data (French abstract) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2014.02.004.

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Glossary

2-AG (2-arachidonoylglycerol): ester formed from arachidonic acid and glycerol; endogenous agonist of CB1 and CB2 receptors

ABHD6 (αβ-hydrolase domain 6): 2-AG hydrolyzing enzyme

AEA (N-arachidonoyl ethanolamine or anandamide): amide formed from arachidonic acid and ethanolamine; endogenous agonist of CB1 and CB2 receptors

CB1 (G-protein-coupled cannabinoid receptor 1): G-protein-coupled cannabinoid receptor activated by endocannabinoids such as 2-AG and AEA

CB2 (G-protein-coupled cannabinoid receptor 2): G-protein-coupled cannabinoid receptor activated by endocannabinoids such as 2-AG and AEA

Dysbiosis: imbalance in the microbiota due to the disturbance of several bacterial groups, causing a shift away from a healthy status

Enterotype: classification of a host organism based on the taxonomic structure of its microbiota [89]

Germ-free mice: gnotobiotic animals harbouring no microorganisms

Ghrelin: gut hormone with orexigenic properties

GLP-1 (glucagon-like peptide 1): potent gut peptide inducing glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion

Gnotobiotics: study and development of animal models characterized by defined microbial communities

GPR55 (G-protein-coupled receptor 55): receptor for some endocannabinoid (eCB)-related lipids

GPR119 (G-protein-coupled receptor 119): receptor for some eCB-related lipids such as OEA and 2-OG

FAAH (fatty acid amide hydrolase): NAE hydrolizing enzyme

HU210: Synthetic cannabinoid, CB1/CB2 agonist

MAGL (monoacylglycerol lipase): 2-AG hydrolyzing enzyme

Metagenome: genetic material recovered directly from an ecosystem without previous distinction between organisms

Microbiota: bacterial ecosystem colonizing the human body

NPY (neuropeptide Y)/AgRP (Agouti-related peptide): two neurotransmitters mostly known for their potent orexigenic effects

OEA (N-oleoyl ethanolamine): amide formed from oleic acid and ethanolamine, an eCB-related lipid

PEA (N-palmitoyl ethanolamine): amide formed from palmitic acid and ethanolamine, an eCB-related lipid

POMC (proopiomelanocortin)/CART (cocaine- and amphetamine-regulated transcript): two neurotransmitters mostly known for their potent anorexigenic effects

Prebiotics: selective stimulation of growth and/or action(s) of one or a limited number of microbial genus/species in the gut microbiota conferring health benefits to the host

Probiotics: microorganisms that some have claimed provide health benefits when consumed

SCFAs (short-chain fatty acids): organic acids with two to six carbon atoms produced by bacterial fermentation of indigestible fibres

SEA (N-stearoyl ethanolamine): amide formed from stearic acid and ethanolamine, an eCB-related lipid

TLRs (toll-like receptors): receptors of the innate immune system that recognize molecules broadly shared by microorganisms, but distinguishable from host molecules

TRPV1 (transient receptor potential cation channel, subfamily V, member 1): non-selective cation channel activated by endogenous ligands such as AEA