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REVIEW

Gut microbiota and obesity

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Abstract The human intestine harbors a complex bacterial community called the gut microbiota. This microbiota is specific to each individual despite the existence of several bacterial species shared by the majority of adults. The influence of the gut microbiota in human health and disease has been revealed in the recent years. Particularly, the use of germ-free animals and microbiota transplant showed that the gut microbiota may play a causal role in the development of obesity and associated metabolic disorders, and lead to identification of several mechanisms. In humans, differences in microbiota composition, functional genes and metabolic activities are observed between obese and lean individuals suggesting a contribution of the gut microbiota to these phenotypes. Finally, the evidence linking gut bacteria to host metabolism could allow the development of new therapeutic strategies based on gut microbiota modulation to treat or prevent obesity.

Keywords Microbiome · Gnotobiotic models · Metabolic syndrome · Intestinal permeability · Antibiotics · Probiotics · Prebiotics · Fecal transplant

Abbreviations

AMPK	AMP-activated protein kinase
ANGPTL4	Angiopoietin-like 4
BSH	Bile salt hydrolase
DIO	Diet-induced obesity
eCB	Endocannabinoïd
FFAR	Free fatty acid receptor
FMT	Fecal microbiota transplant
GF	Germ-free
GI	Gastro-intestinal
GLP	Glucagon-like peptide
HFD	High-fat diet
HGC	High gene count
IBD	Inflammatory bowel disease
LGC	Low gene count
LPL	Lipoprotein lipase
LPS	Lipopolysaccharides
PYY	Peptide YY
SCFA	Short chain fatty acid
TLR	Toll-like receptor
TMA	Trimethylamine

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Introduction

Obesity is a major health concern, whose incidence is increasing dramatically in both industrialized and developing countries [1]. Nowadays, more than 500 million people are obese worldwide leading to considerable economical costs as well as public health challenge [2]. Indeed, obesity predisposes individuals to a number of diseases including diabetes, cardiovascular diseases, non-alcoholic fatty liver disease, cancer, and some immune-related disorders. Several genes have been implicated in the determination of body weight, but this genetic susceptibility may only explain a small fraction of obesity, and cannot explain the rise in incidence of this pathology. Therefore, obesity arises from complex interactions between genes and environmental factors such as diet, food

components and/or way of life, and results from a long-term positive imbalance between energy intake and expenditure with excessive increase in body fat [3]. Overall, the complex pathways that lead to development of overweight and its consequences are not thoroughly understood and recent studies have suggested that the gut microbiota (the trillions of bacteria that normally reside within the human gastrointestinal (GI) tract) should be factored into this equation [4]. Indeed, there is growing evidence that the gut microbiota and its bacterial genome (the microbiome) affect nutrient acquisition, energy regulation and fat storage [5]. These findings raise the possibility that the gut microbiota plays a role in regulating host energy metabolism and may contribute towards the development of obesity and associated metabolic diseases. The objective of this review is to provide an overview of this emerging field related to the role of the gut microbiota in obesity and metabolic disorders.

The gut microbiota

Development and stability of the gut microbiota

Foetuses are thought to be sterile in uteri although low levels of bacterial translocation through the placental circulation may provide an elementary microbiota before birth [6]. During birth, bacteria from the mother and the surrounding environment colonize the infant's gut rapidly. The composition of this microbiota depends on various factors including mode of birth (cesarean section or vaginal delivery), antibiotic treatment, feeding (breastfeeding or formula) or sanitation of the environment [7]. This microbiota changes during the first years of life, under the control of different factors including developmental changes in the gut environment, the host genotype, and the introduction of solid foods, and a more complex and stable community, close to the adult microbiota, is established at approximately 3 years of age [7, 8]. In adults, the gut microbiota remains remarkably constant slightly fluctuating around an individual core of stable colonisers. Then, alterations occur at old age when digestive physiology and diet changes [9]. Nevertheless, dietary factors or antibiotic treatments can lead to transient changes. For example, short-term treatment in humans with a single dose of oral antibiotics alters the gut microbiota for as long as 4 weeks before it tends to recover its original composition [10]. Moreover, some bacterial species are not recovered even several months after treatment leading to a reduced diversity following repeated antibiotic exposures [11]. Similarly, changes in diet lead to modification of the gut microbiota composition. Diet provides nutrients for both the host and the microbiota whose bacterial species can be favoured or disadvantaged by dietary substrates. Therefore, a study showed that diet changes in mice could explain 57 % of the total structural variation in gut microbiota, whereas genetic mutation accounted for no more than 12 % [12]. In humans, the gut microbiota in twin pairs and their mothers has been characterized to assess the impact of genotype and environmental exposures. It revealed that family members share more similar microbiotas than unrelated individuals, but that monozygotic twins have a similar degree of variance than dizygotic twins, indicating that early environmental exposures are key determinants of adult gut bacterial community [13]. Interestingly, this study also showed that if each microbiota is different in terms of bacterial composition (no bacterial species shared by all 154 individuals), there was a wide array of shared microbial genes named the "core microbiome", reflecting evolutionary convergence of unrelated bacterial species. The final composition of the microbiota is, therefore, unique and specific to each individual [14] and a recent study demonstrated that individuals could be uniquely identified based on their microbiomes alone [15]. Nonetheless, the factors guiding this feature are still a matter of debate.

Composition of the gut microbiota

In humans, the gut microbiota is a complex and dynamic ecosystem that has coevolved with its host [16] and represents approximately 1 kg of our body weight. It is now recognized that the communities of microbes in our gut function as an organ with many metabolic, immunologic and endocrine-like actions that influence human health [17]. A large fraction of the gut bacteria still remains impossible to culture so that our understanding of this microbiota has been a long time limited by technical issues. In the 1980s, Pace and co-workers introduced a new culture-independent method to identify bacteria, based on the sequencing of the 16S rRNA gene [18]. The development of these molecular techniques makes now possible a reliable assessment of the gut microbiota. Although Eukaryota and Archaea domains are also present in the intestine, Bacteria clearly predominate. Adult humans are colonized by microbes from nine divisions (deep evolutionary lineages) of Bacteria and at least one division of Archaea [19]. This represents only a small fraction of the more than 70 bacterial and 13 archaeal divisions known in the biosphere. Moreover, three bacterial divisions, the Firmicutes (Gram-positive), Bacteroidetes (Gram-negative) and Actinobacteria (Gram-positive), dominate the adult human gut microbiota and account for more than 90 % of all bacteria, whereas *Methanobrevibacter smithii*, a hydrogen-consuming methanogen, dominates the Archaea domain [20]. These results were obtained from fecal samples which

correspond to the colonic population. However, the specific density and type of bacteria in the GI tract are influenced by environmental variations through the gut, such as pH, oxygen, and nutrient availability. Hence, the bacterial concentration is higher in the lower portion of the GI tract, and if this gut section is largely populated by anaerobic bacteria, aerobic bacteria dominate in the proximal gut. Nevertheless, molecular analyses revealed that the same bacteria are present in the different sections of the gut and that only the relative abundance of the different species varies [21].

Increased sequencing and bioinformatics capacities have allowed entering into a new era, through the Human Microbiome Project [22, 23], not only giving access to the type of bacterial species present but also to their gene content [24]. Using these techniques, it is now estimated that the human GI tract harbors approximately 10^{14} microorganisms (ten times more cells than the whole human body) and that each gut microbiota is composed of 500–1000 distinct bacterial species [20, 25]. Moreover, the MetaHit consortium has published a catalog of nearly 10 million non-redundant genes obtained from sequencing fecal samples from 1267 individuals [26] indicating that the human microbiome contains at least 100 times as many genes as the human genome [26–28]. Each individual hosts on average 500–600,000 bacterial genes, about half of which is shared by most individuals (functional core). However, many functions are worn by the intestinal microbiota in a fraction of humans only and could contribute greatly to the metabolic diversity observed in the human population. It was further observed that the human population may be grouped into three distinct clusters, based on gut microbiota composition [29]. This concept of enterotypes is based on co-occurring of bacterial species, with enterotypes being dominated by *Bacteroides*, *Prevotella*, and *Ruminococcus*, respectively. Although divergences in enterotypes were first found to be independent of geography, age, gender, or body mass index, differences in long-term diet pattern have been associated with these enterotypes [30]. Finally, although the concept of enterotypes is still debated [31], it can be important for making associations between gut microbiota and health and defining what constitutes healthy and dysbiotic bacterial community.

Gut microbiota functions

The gut microbiota performs essential functions that the human body cannot carry out, resulting in a symbiotic relationship. The gut microbiota is, therefore, critical for maintaining normal GI and immune functions, and efficient digestion of nutrients [32, 33]. For example, the microbiota ferments otherwise indigestible food components, synthesizes vitamins and other essential micronutrients, metabolizes dietary toxins and carcinogens, converts cholesterol and bile acids, assures the maturation of the immune system, affects the growth and differentiation of enterocytes, regulates intestinal angiogenesis, and protects against enteric pathogens [34]. In the last decades, relationships between the gut microbiota and human health have been established. Moreover, imbalances in its composition (i.e., dysbiosis) have been associated with immune disorders, susceptibility to infections, and more recently to several non-intestinal pathologies including cardiovascular diseases, obesity, diabetes, liver or even brain diseases [35–38]. The current knowledge of the relationship between the gut microbiota, obesity and associated diseases is presented in the next parts of this review.

Gut microbiota and obesity

Evidences from germ-free animal models

The first evidence on the role of the gut microbiota on host adiposity came from studies in germ-free (GF) animals, i.e., animals devoid of bacteria and bred in sterile isolators. In 1983, Wostmann and colleagues observed that GF rodents require 30 % more calories to maintain their body mass than conventional ones (possessing their own microbiota) [39]. The potential mechanisms accounting for this observation remained unclear until recent landmark studies, preeminently originating from Jeffrey Gordon's laboratory at Washington University, Saint-Louis, MO, USA. Indeed, they pioneered the investigation of gut microbiota as a factor influencing fat storage and obesity. They first found that conventionally raised mice have 42 % more total body fat and 47 % more gonadal fat than GF mice, although GF mice consumed more food [40]. They next demonstrated that colonization of GF mice with a cecum-derived microbiota from conventional mice produces a 60 % increase in body fat mass within 2 weeks. The increase in body fat was accompanied by insulin resistance, adipocyte hypertrophy, and increased levels of circulating leptin and glucose [40]. This was partly explained by the capacity of the gut microbiota to degrade undigestible polysaccharides into monosaccharides which could be absorbed leading to increased hepatic lipogenesis in the host. Moreover, inoculation of a gut microbiota suppresses the intestinal expression of angiopoietin-like 4 (ANGPTL4), a circulating inhibitor of lipoprotein lipase (LPL). Conventionalization leads, therefore, to increased adipocyte LPL activity and then to increased cellular uptake of fatty acids and adipocyte triglycerides accumulation [40]. The physiologic importance of ANGPTL4 was further established by the demonstration that GF

ANGPTL4$^{-/-}$ mice have the same degree of adiposity as their conventional counterparts. Finally, in contrast to conventional mice, GF mice fed a high-fat, sugar-rich diet are protected from diet-induced obesity (DIO) [41]. This lean phenotype was associated with increased skeletal muscle and liver levels of phosphorylated AMP-activated protein kinase (AMPK) [41]. AMPK is an enzyme that is conserved from yeast to humans and functions as a fuel gauge that monitors cellular energy status and stimulated fatty acid oxidation in peripheral tissue. Hence, the gut microbiota may suppress skeletal muscle fatty acid oxidation through a metabolic pathway involving phosphorylation of AMPK. It was further observed that GF mice receiving a high-fat diet (HFD) showed enhanced insulin sensitivity with improved glucose tolerance and reduced insulinemia in comparison to conventional mice. This was associated with a reduced hypercholesterolemia, a moderate accretion of hepatic cholesterol and an increase in fecal cholesterol excretion suggesting an altered cholesterol metabolism in GF mice [42]. Nevertheless, this resistance to diet-induced obesity may depend on mice genetic background and diet composition as C3H GF mice were not found resistant to the obesogenic effect of a low-sucrose, lard-based high-fat diet, while resistant to high sucrose, palm oil-based high-fat diet [43]. Also, similar body weights and adiposity were observed in GF and conventional Fischer 344 rats [44]. In these rats, GF status was associated with increased intestinal ANGPTL4 and reduced hepatic lipogenesis as well as increased adipocyte size suggesting that impact of a gut microbiota on fat storage may be more complex than proposed by pioneer papers.

Role of bacterial fermentation in host energy harvest and appetite regulation

An important mechanism that can explain differences in body fat between conventional and GF mice is the increase in energy harvest from the food due to the fermentation of the gut microbiota. Indeed, the gut microbiota is able to process complex dietary plant polysaccharides, otherwise inaccessible to humans, to monosaccharides and short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate. These SCFAs represent an important energy source for our body as they can provide approximately 10 % of the daily energy supply in omnivores and up to 70 % in herbivores [45]. Butyrate is the preferred source of energy for colonic epithelial cells and increases the density of capillaries underlying the small intestine villus epithelium [46]. Absorbed propionate and acetate are delivered to hepatocytes where they can be used for gluconeogenesis and lipogenesis, respectively. However, SCFAs act not only as energy substrates for the host, but also as signaling molecules, influencing energy intake and metabolism [47]. Therefore, they are ligands for at least two G-protein-coupled receptors, free fatty acid receptor 2 (FFAR2 or GPR43) and free fatty acid receptor 3 (FFAR3 or GPR41). These GPCRs are mainly expressed by gut epithelial cells, in particular enteroendocrine cells. FFAR2 is preferentially activated by acetate, and FFAR3 by butyrate, whereas propionate activates both receptors [48]. FFAR2$^{-/-}$ mice were found to resist to diet-induced obesity indicating that FFAR2 could promote fat storage [49]. Similarly, it has been shown that GF FFAR3$^{-/-}$ mice colonized by a microbiota gained less fat mass than their wild-type littermates [50]. The authors proposed that in the absence of FFAR3 signaling, the plasma level of peptide YY (PYY) is reduced leading to an increased gut motility and reduced energy harvest from the diet. Therefore, FFAR2 and FFAR3 would be modulators of host energy balance through effects dependent upon the gut microbiota. These receptors may also control eating behavior as increased production of SCFAs due to fiber administration leads to increased satiety and reduced food intake [51–53]. These effects are mediated by increases in the satietogenic gut peptides PYY and glucagon-like peptide (GLP) 1 together with decrease in orexigenic ghrelin [54]. Moreover, butyrate and propionate may reduce appetite via induction of leptin expression from adipocytes [55]. Other pathways may be involved in SCFAs outcomes as butyrate and propionate were also shown to protect mice against diet-induced obesity via FFAR3-independent mechanism [56]. Finally, SCFAs produced by the microbiota may constitute a fine tune of the host metabolism by the regulation of energy harvest, fat storage and appetite.

The influence of antibiotics on obesity

If the gut microbiota plays a role in obesity, modulation of this bacterial community should have an impact on obesity development. Antibiotics are known to disrupt microbiota composition and while a rapid recovery is observed following short-term antibiotic treatment, pervasive effects may be obtained after repeated antibiotic perturbations [10, 11]. Seventy years ago, it was first shown that administration of low doses of antibiotics resulted in promotion of growth in chicks [57]. This effect was confirmed in mammalian livestock (cows, pigs, and sheep), and antibiotics have been used to promote weight gain in farm animals for over 60 years. Moreover, antibiotics have no growth-promoting effects in GF chicken [58] indicating that changes in the microbiota of treated animals are responsible for these effects. Interestingly, increases in body mass are obtained only when antibiotic exposure occurs early in life [59] which has been confirmed in mice more recently [60, 61]. Indeed, mice that received low-dose

penicillin treatment at birth had higher body weight gain than their counterparts that were exposed at weaning [61]. Notably, these effects in mice lasted into adulthood weeks after antibiotic treatment was stopped indicating that even transient perturbations early in life can cause long-term effects [62]. Finally, GF mice colonized with the microbiota from low-dose penicillin-treated mice gained more fat mass than mice colonized with the microbiota from control mice, demonstrating that the shifted microbiota itself possesses the capacity to trigger obesity [61]. Nevertheless, the effect of antibiotic exposure in obesity development is dependent on the dose of antibiotics. Indeed, high doses of antibiotics resulted in reduced fat mass and insulin resistance in mice models of obesity [63]. It can be assumed that these high doses of antibiotics reduce considerably the bacterial population and, therefore, the capability of the gut microbiota to extract calories from the diet, then mimicking the conditions observed in GF animals. In humans, a growth-promoting effect of antibiotic treatments has been reported in the 1950s [64, 65], but these studies have been ignored until the recent demonstration of a link between the gut microbiota and obesity. Therefore, epidemiological studies from different countries have been recently launched to evaluate the impact of antibiotic treatment in infancy on risk of obesity development. The first published results seem to confirm that exposure to antibiotics in early life is associated with an increased body mass index [66–69]. This suggests that the massive use of antibiotics in the last decades could be involved in the parallel increase in prevalence of obesity in the western countries.

Gut microbiota and obesity: the dysbiosis concept

Dysbiosis is defined as the condition of having microbial imbalances associated with a pathology. In accordance with this definition, it has been shown, firstly in mice, that obesity can be associated with an altered gut microbiota. Ley et al. analyzed 5088 bacterial 16S rRNA gene sequences from fat ob/ob, lean ob/+ and wild-type mice fed the same polysaccharide-rich diet [70]. They revealed that obese animals have a 50 % reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes [70]. Ob/ob mice also harbored more methanogenic Archaea, which may increase the efficiency of bacterial fermentation via removal of H_2. Similar differences in the gut microbiota of lean versus obese humans were observed in initial studies by the same team [71]. Indeed, it was found that obese people had lower Bacteroidetes and more Firmicutes than did lean control subjects. Moreover, the Bacteroidetes to Firmicutes ratio approached a lean phenotype after 52 weeks of diet-induced weight loss. Together, these results obtained in mice and humans suggested that obesity alters the nature of the gut microbiota and raised the possibility that manipulation of the gut microbiota towards a lower Firmicutes/Bacteroidetes ratio could be a new strategy for treating obese people. However, if several studies confirmed an increased Firmicutes/Bacteroidetes ratio in obese individuals [72–74], others did not report any differences in the abundance of Firmicutes and Bacteroidetes in obese and lean subjects, or even found an opposite relationship [75–78]. Therefore, these phylum-wide changes in the gut microbiota composition cannot be currently considered as a biomarker for obesity. Changes in microbiota composition at lower taxonomic levels have also been associated with obesity and genera or even specific bacterial species abundance may better define the dysbiosis associated with obesity than Firmicutes/Bacteroidetes ratio. Moreover, if several bacterial genera have been found increased or decreased in obese patients, bacterial species belonging to these genera may follow an opposite trend suggesting a species or even strain-dependent effect. As an example, if a higher level of *Lactobacillus* has been found in obese patients than in controls [72], some species belonging to this genus (*L. reuteri*) have been indeed associated with obesity whereas others (*L. casei*, *L. plantarum*) have been found associated with weight loss in humans and animals [79]. Several studies also reported an association between obesity and lower populations of bifidobacteria [74, 75, 80], but only a few bifidobacterial species have been proved to exert anti-obesogenic effects in animal models [81]. Also, potential opportunistic pathogens have been frequently associated with obesity and a few bacterial strains have been proposed as markers or even contributors to obesity. For instance, *Staphylococcus aureus* was found more abundant in the gut of overweight children and pregnant women [75, 80, 82]. Similarly, increased populations of Enterobacteriaceae have been described in overweight pregnant women as compared to normal weight [74]. Further, a bacterium belonging to this family, *Enterobacter cloacae* strain B29, has been isolated from the gut microbiota of an obese human. B29 constituted almost 35 % of his gut microbiota before dietary intervention, but became undetectable after the volunteer lost 51.4 kg of his 174.8 kg initial weight. Strikingly, this bacterium was shown to cause obesity when introduced into high-fat diet-fed germ-free mice [83]. Conversely, *Faecalibacterium prausnitzii*, a butyrate producer with known anti-inflammatory properties [84], has been found decreased in morbidly obese subjects with diabetes [73], similarly to its low abundance in patients with inflammatory bowel disease (IBD) [85]. Moreover, it was negatively associated with plasma levels of inflammatory cytokines. Similarly, *Akkermansia muciniphila* is inversely related to fasting plasma glucose levels, visceral fat accumulation, and adipocyte diameter in subcutaneous

adipose tissue in obese humans [86]. In addition, feeding mice with *A. muciniphila* reduces body weight gain, fat mass development, and low-grade inflammation and restores gut barrier function [87]. Altogether these results suggest that specific bacterial species, or a combination of these species, may contribute directly to obesity development or protection. However, it is still impossible to define what an "obese" microbiota is due to a variety of co-founding factors (including heterogeneity in genotype, diet, lifestyle…) that exist within the human population, and it is likely that identical gut microbiota may have a different influence on obesity development in the heterogeneous human population. Also, we can hypothesize that the contribution of the gut microbiota to obesity may rather depend on the genes present in the microbiome and on the metabolites produced than on taxonomic composition. The development of high-throughput sequencing techniques makes it now possible to get access to the whole gene content of the gut microbiota. This could allow the identification of metabolic functions that can be overrepresented in the microbiome of obese individuals. Accordingly, it was first showed that the cecal microbiome of genetically obese ob/ob mice contains more genes involved in the hydrolysis of indigestible polysaccharides leading to the hypothesis that the ob/ob microbiome has increased capacity to harvest energy from the diet [88]. Consistently, fecal energy content was reduced and the amounts of cecal SCFAs were increased in ob/ob vs wild-type mice. In obese humans, genes involved in phosphotransferase system, in carbohydrate metabolism and in membrane transport, were found increased whereas genes involved in transcription, nucleotide metabolism and cofactors and vitamin metabolism were found depleted [13, 89]. Analysis of SCFAs also suggests that the fermentation activity of the gut microbiota is higher in obese individuals, propionate being the most increased SCFA [90]. Recently, a bimodal distribution of microbial gene counts leading to the stratification of the population as either "low gene count" (LGC) or "high gene count" (HGC) has been identified [91]. This microbial gene richness was associated with body weight, fat mass, inflammation, glucose and lipid metabolism. Strikingly, dietary restriction in overweight or obese patients was less efficient in LGC than in HGC individuals in terms of body weight loss, improvement of insulin sensitivity and decrease of inflammation [92]. These results suggest that a decreased bacterial diversity may be a feature of the "obese microbiota" as it has been described for other disease states [93]. In conclusion, it appears that obesity is associated with a gut microbiota differing from a lean microbiota in terms of composition, diversity, metabolic activity, and gene contents. However, the association studies described in this paragraph do not inform whether this dysbiosis is a cause or a consequence of obesity.

Causative role in obesity: evidences from gut microbiota transplant experiments

If the gut microbiota plays a causal role in obesity development, transplanting different gut microbiota to GF mice should lead to different weight gain and adiposity. Consistently, Turnbaugh et al. first transplanted cecal microbiota from lean and ob/ob mice to GF wild-type recipient. They found that after only 2 weeks, mice harboring the microbiota from obese mice gained more fat compared to the mice inoculated with the gut microbiota from lean donors [88], supporting a causal role of these bacteria in the pathogenesis of obesity. They further raised an important question. Are the differences in the composition of the gut microbiota a consequence of host genotype or the hyperphagic state, as it is well known that ob/ob mice consume more food than their wild-type littermates. To further investigate this question, the same team developed a model of Western diet-induced obesity (DIO) to study the interrelationship between gut microbiota, diet and energy balance [94]. They showed that DIO produced a bloom in a single uncultured clade within the Firmicutes, named Erysipelotrichi. Moreover, this group of bacteria was reduced following dietary manipulations that limit weight. Similar to transplantation of the ob/ob microbiota, transplantation of a DIO gut microbiota to GF recipients promoted greater fat gain than transplants from lean donors. Metagenomic sequencing also revealed that the Western diet microbiome is enriched in pathways involved in import and fermentation of simple sugars and host glycans [94]. To determine whether human gut microbiota may also be able to cause obesity in mice, transplantations of adult human fecal microbiota to GF mice have been performed. In a first study, these humanized gnotobiotic mice were then fed a low fat or a high-fat diet. Finally, GF recipients were colonized with cecal microbiota from humanized donors fed either diet, and were kept on the low fat diet. Mice colonized with the microbiota from HFD-fed donors gained significantly more adiposity than mice colonized with the microbiota from low fat diet-fed donors [95]. More recently, the same group inoculated the GF mice with gut microbes from four pairs of female twins, each in which one person was obese and the other had a healthy weight. Mice that received the obese humans' microbes gained more body fat, put on more weight, and showed increased level of markers of metabolic disorders [96]. Because mice are coprophagic, cohousing is widely used to investigate the impact of sharing microbial communities on the host phenotype. Strikingly, cohousing mice

associated with the human obese or lean microbiota prevents increased adiposity, and it was further demonstrated that bacteria from the lean mice were able to invade the obese microbiota, the best colonizers among the lean communities belonging to the Bacteroidetes phylum [96]. Whether these species were responsible for the lean-like state remains to be proven but it indicates that bacterial species within the human gut microbiota may have the capacity to protect from obesity.

Interestingly, these fecal transplant experiments have also demonstrated that the gut microbiota may play a causal role in the development of the metabolic disorders associated with obesity. Toll-like receptor 5 (TLR5) is predominantly expressed basolaterally by intestinal epithelial cells and serves to detect motile bacteria that breach the epithelial monolayer and limit their dissemination. It was first described that TLR5$^{-/-}$ mice present increased adiposity, elevated serum triglycerides and cholesterol levels, as well as mild loss of glucose tolerance, insulin resistance, hyperlipidemia and hypertension. Remarkably, the transfer of TLR5$^{-/-}$ cecal contents into wild-type GF mice recapitulated most aspects of the metabolic syndrome phenotype [97]. Similarly, another study demonstrated that gut microbiota determines development of Non-alcoholic fatty liver disease in mice [37]. First, conventional mice were fed an HFD for 16 weeks and two of them were selected based on their opposite response to HFD. Although both mice were the same weight, one displayed low fasting glycemia and weak steatosis (Non-Responder). The other one displayed insulin resistance and marked steatosis (Responder). Two groups of GF mice were transplanted with the gut microbiota of the two selected mice. After being fed an HFD, only the mice associated with the Responder microbiota developed fasting hyper-glycemia and hyper-insulinemia as well as hepatic macrovesicular steatosis [37]. These experiments demonstrating a causal role of the gut microbiota in obesity and associated metabolic disorders raise the question whether gut microbiota transfer in humans could be a new way to treat obesity and metabolic syndrome as recently shown in rats [98]. This question will be developed in the section dedicated to the "Therapeutic potential of the gut microbiota".

Gut microbiota, intestinal permeability and inflammation: the LPS hypothesis (and beyond)

Obesity is associated with a low-grade inflammation, which has been implicated in the development of the metabolic syndrome and insulin resistance [99]. The origin of this inflammation is unclear and Cani et al. have proposed that the lipopolysaccharide (LPS, a membrane component of Gram-negative bacteria) is the triggering factor of the early development of inflammation and metabolic diseases [100]. This hypothesis is based on the following points: (1) LPS triggers the secretion of proinflammatory cytokines when it binds to the complex of CD14 and TLR4 at the surface of immune cells [101], (2) LPS is continuously produced within the gut through lysis of Gram-negative bacteria and is physiologically carried into intestinal capillaries though a TLR4-dependent mechanism [102], (3) LPS is transported from the intestine by a mechanism facilitated by chylomicrons freshly synthesized from epithelial intestinal cells in response to fat feeding [103]. Cani et al. first demonstrated that mice fed an HFD for 2–4 weeks exhibited a significant increase in plasma LPS that they named metabolic endotoxemia [100]. Interestingly, it has been also reported that patients with type 2 diabetes and patients with non-alcoholic fatty liver disease had higher LPS levels than control humans [104, 105]. It should be noted that these levels of LPS are 10–50 times lower than values seen in septicemia or other infections. Cani et al. also showed that continuous subcutaneous low-rate infusion of LPS mimicked the HFD phenotype including excessive weight gain, hyperglycemia, steatosis, adipose tissue macrophages infiltration and insulin resistance in mice. Finally, to demonstrate the causal link between LPS and the development of metabolic diseases, they challenged LPS receptor (CD14) knock out mice with an HFD or LPS infusion [100]. CD-14 deficient mice were protected from metabolic disease induced by both high-fat feeding and LPS infusion. These results were corroborated by studies showing that TLR4-deficient mice are resistant to the development of DIO [106, 107]. They also suggest that GF mice could be protected from DIO through the lack of LPS in their gut. To further assess the contribution of the gut microbiota in the development of these metabolic disorders, they used intestinal-focused antibiotic treatment in high-fat fed or genetically obese ob/ob mice. Drastic changes in the gut microbiota through antibiotic treatment completely blunted the metabolic endotoxemia, and the related metabolic disorders (e.g., macrophages infiltration, glucose intolerance and insulin resistance) [63, 108]. They also showed that high-fat feeding increased gut permeability and changed gut microbiota composition with a reduction of *Bacteroides*, *Clostridium coccoides* group and bifidobacteria [108]. Interestingly, bifidobacteria have been shown to reduce intestinal LPS levels and to improve gut barrier function in mice [109, 110]. To determine if the metabolic disorders observed during high-fat feeding can be attributed to the decrease of bifidobacteria, they used prebiotic dietary fibers to specifically increase the gut bifidobacteria in high-fat fed mice. They confirmed that mice fed an HFD exhibit a higher endotoxemia, a phenomenon completely abolished through dietary supplementation with the prebiotic fibers [111]. Moreover, in prebiotic-treated mice, bifidobacteria positively correlated with improved glucose tolerance and

negatively correlated with metabolic endotoxemia and body weight gain [111]. More recently, they deciphered one of the mechanisms explaining how these specific changes in the gut microbiota improved metabolic endotoxemia. They found that the modulation of gut microbiota controls and increases endogenous production of the intestinotrophic peptides GLP-2, and consequently improves tight junction integrity and gut barrier functions by a GLP-2-dependent mechanism [112]. They also identified the endocannabinoid (eCB) system as determinant of gut barrier function. More specifically, they proposed that the eCB system mediates communication between adipose tissue and the gut microbiota. Accordingly, modulating the gut microbiota of obese and diabetic mice profoundly affected the tone of the intestinal and adipose tissue eCB system and improved adipose tissue metabolism [113, 114]. Finally, they further showed that MyD88 (myeloid differentiation primary response gene 88), a central adaptor molecule of most of the TLRs, acts as a sensor involved in the interaction between nutrients, gut microbes and the host during DIO [115]. Indeed, they first revealed that inducible intestinal epithelial cell-specific deletion of MyD88 partially protects against DIO, diabetes and inflammation. Remarkably, the gut microbiota of these MyD88-deleted mice protected GF recipients after fecal transplant. Finally, it appears that the gut barrier function may be important in the crosstalk between gut bacteria and host metabolism, and lps are probably only one of the players linking the gut microbiota, the gut permeability and metabolic disorders. Accordingly, it was also shown that HFD leads to increased bacterial DNA in ileal mucosa, blood and mesenteric adipose tissue in mice [116]. Then, a 9-year longitudinal study revealed that blood bacterial DNA at baseline was higher among participants who presented with abdominal adiposity and developed diabetes during the 9-year period than among those who did not [117] suggesting that translocation of entire bacteria and the existence of a tissue microbiota could be another contributor to metabolic syndrome [118].

Influence of the gut microbiota on plasma lipids

Dyslipidemia is commonly associated with obesity and metabolic syndrome and besides its influence on weight gain and metabolic disorders, it has been shown for a long time that the gut microbiota impacts the lipid metabolism of the host. As an example, pioneer studies by Wostmann et al. in the 1960s demonstrated that GF animals absorb dietary cholesterol more efficiently than conventional controls, but display reduced plasma cholesterol [119, 120]. Nevertheless, this topic was not studied thoroughly until recently when comparison of GF and conventional mice fed an HFD showed that GF mice display increased fecal lipid excretion and reduced plasma free fatty acid and liver triglyceride levels [42]. Interestingly, this study also revealed that GF mice have reduced cholesterolemia, an increase in fecal cholesterol excretion, and a moderate accretion of hepatic cholesterol confirmed by an up-regulation of cholesterol biosynthesis genes in the liver, suggesting that the gut microbiota alters cholesterol metabolism in the host [42]. This influence of the gut microbiota on cholesterol metabolism was further confirmed using MS-based lipidomics of serum, white adipose tissue, and liver of GF and conventional mice [121]. This impaired cholesterol metabolism could be related to the lack of cholesterol conversion by the intestinal bacteria. Indeed, even if only a few cholesterol-reducing bacteria have been isolated from human or animal gut [122, 123], the conversion of cholesterol to the saturated product coprostanol by intestinal microorganisms was established during the 1930s. Coprostanol is poorly absorbed by the human intestine and an inverse relationship has been observed between serum cholesterol levels and the coprostanol-to-cholesterol ratio in human feces [124]. Hence, conversion of cholesterol to coprostanol by the gut may lead to decreased cholesterol absorption and then cholesterolemia [125]. Besides cholesterol, comparison of GF and conventional mice also showed that the gut microbiota modified a number of lipid species in the serum, adipose tissue, and liver, with its greatest effect on triglyceride and phosphatidylcholine species [121]. The latter is of importance regarding the role of the gut microbiota in cardiovascular diseases. Indeed, dietary choline and phosphatidylcholine are converted to trimethylamine (TMA) by gut microbes. Then, the absorbed TMA is metabolized to trimethylamine-N-oxide, a proatherosclerotic metabolite, by hepatic flavin monooxygenases [126, 127]. Gut microbiota could also regulate serum lipids by taking part in bile acid metabolism. Bile salts are highly effective detergents that promote solubilization and absorption of dietary lipids throughout the intestine. The bile acids that escape the enterohepatic circulation undergo bacterial metabolism in the colon leading to over twenty different secondary bile acids in human feces [128]. Also, deconjugation of bile acids through the enzyme bile salt hydrolase (BSH) may alter plasma cholesterol levels. Briefly, glycine or taurine is liberated from the steroid moiety of the molecule, resulting in the formation of free bile acids which are less efficiently reabsorbed than their conjugated counterparts. Hence, deconjugated bile acids are more readily excreted within the feces than conjugated bile acids. Cholesterol, being a precursor of bile acids, is broken down to replace the processed bile salts leading to a reduction in serum cholesterol [129].

Therapeutic potential of the gut microbiota

Probiotics and prebiotics

Probiotics are defined by the Food and Agricultural Organization and the World Health Organization as "live microorganisms which when administered in adequate amounts, confer a beneficial health effect on the host". Whereas probiotics are used for decades in agriculture for their growth-promoting effects, several studies have demonstrated that probiotics may ameliorate obesity and associated metabolic disorders both in animal models and in humans. In particular, probiotics containing *Bifidobacterium* were shown to exert beneficial effects in HFD fed mice and rats [81, 130–135], mainly through increased gut barrier function, leading to reduced bacterial translocation and endotoxemia, and improvement of inflammation, insulin sensitivity, fat accumulation as well as cholesterol and triglyceride serum levels. Similarly, probiotics containing *Lactobacillus* strains have been shown to be effective in reducing body fat mass and improving lipid profiles and glucose homeostasis in animal models of obesity [136–147]. Proposed mechanisms include conjugated linoleic acid production, BSH activity, stimulation of fatty acids oxidation, or inhibition of lipoprotein lipase activity. Well-controlled studies in humans are scarce but recent studies suggested that *Lactobacillus gasseri* may decrease body weight and abdominal adiposity and improve postprandial serum lipid responses in overweight human subjects [148–150]. Finally, a meta-analysis based on 17 randomized clinical trials in humans, 51 studies on farm animals and 14 experimental models highlighted the strain-dependent effect of *Lactobacillus* containing probiotics on weight management. Therefore, *Lactobacillus acidophilus* administration resulted in a significant weight gain in humans and in animals. *Lactobacillus fermentum* and *Lactobacillus ingluviei* were associated with weight gain in animals. Conversely, *Lactobacillus plantarum* was associated with weight loss in animals and *Lactobacillus gasseri* was associated with weight loss both in obese humans and in animals [79]. In conclusion, although encouraging results emerge from rodents experiments, the efficacy of probiotics remains highly debatable and their therapeutic used for obesity management has not yet been recommended [151].

Prebiotics are defined as non-digestible polysaccharides that promote "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 benefit to the host" [152]. The most studied prebiotics are the inulin and various types of fructo-oligosaccharides and galactooligosaccharides and numerous studies in animal models showed that they modify gut microbial composition, enhancing the growth of beneficial *Bifidobacteria* and *Lactobacillus* [111, 153–155]. However, more recent studies revealed that prebiotics affect many more bacterial taxa than previously thought [156, 157]. Interestingly, this microbiota modulation is commonly associated with a reduction in body weight, body fat and adipocyte size. These effects are mediated through decreased food intake and appetite, as well as reduced fatty acid storage [154, 155, 158–160]. Furthermore, the improvement of gut barrier integrity [112, 154] leads to better glucose tolerance and insulin sensitivity. In humans, beneficial effects of prebiotics on glycemia and insulinemia have been largely confirmed whereas impacts on body weight, fat mass and satiety have not been consistently observed and are still matter of debate [161–165].

Fecal microbiota transplant

Fecal microbiota transplant (FMT) refers to infusion of a fecal suspension from a healthy individual into the GI tract of another person to cure a specific disease. Transplantation of stool for the treatment of GI disease was first reported in the fourth century in China by Ge Hong, who described the use of human fecal suspension by mouth for patients who had food poisoning or severe diarrhea [166]. Sixteen centuries later, its first clinical use was for the treatment of pseudomembranous colitis and was reported in 1958 in a four-patient case series [167]. In the recent years, FMT has gained an increasing interest as an effective treatment strategy for severe recurrent *Clostridium difficile* infection with global success rate over 80 % [168]. Moreover, early experience suggests that FMT could be used for other GI and non-GI diseases associated with microbial dysbiosis and whose aetiologies are uncertain, including IBD, irritable bowel syndrome or metabolic diseases. Indeed, as metabolic phenotypes can be transmitted to GF mice via gut microbiota transplant, it can be postulated that FMT may be effective to improve lipid and glucose homeostasis. In a first pilot study, intestinal microbiota was transferred from lean human donors to recipients with metabolic syndrome via a postpyloric enteral feeding tube. Patients who received microbiota from lean donors had an increase in peripheral insulin sensitivity 6 weeks after FMT in comparison with peripheral insulin sensitivity prior to FMT, although the body weights and adiposity were not modified. This was associated with increased gut bacterial diversity, as well as increase in the amount of the butyrate producer *Eubacterium hallii* [169]. Therefore, if the legal framework and the standardization of the FMT are needed and if more well-designed randomized controlled trials in the context of metabolic diseases should be performed, this

study suggests that FMT might be a new way to improve obesity and associated metabolic disorders in the future.

Conclusions and future challenges

Gut microbiota is now viewed as a novel factor involved in body weight management. The gut microbiota may, therefore, participate to energy metabolism through energy harvest from the diet, regulation of fat storage, regulation of lipogenesis, or regulation of fatty acid oxidation. Further, differences in the composition of gut microbiota in obese humans and mice suggest that different microbes or community may influence body weight differently. Although the cause–effect relationships of the gut microbiota with obesity remain unclear, the rapid developments in high-throughput techniques may make it possible to unravel the real impact of the gut microbiota on host's metabolism. Multidisciplinary research in this field will be helpful to provide evidence-based data and to shed light on the roles of specific sets of microbes. The next step will be the discovery of pharmacological, dietary or fecal transplant interventions to modify the gut microbiota in such a way that prevents or treats metabolic disorders and/or obesity.

References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA, Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwari P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Merriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX, Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang X, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ, Gakidou E (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384(9945):766–781. doi:10.1016/S0140-6736(14)60460-8
2. Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL (2011) The global obesity pandemic: shaped by global drivers and local environments. Lancet 378(9793):804–814. doi:10.1016/S0140-6736(11)60813-1
3. Hill JO (2006) Understanding and addressing the epidemic of obesity: an energy balance perspective. Endocr Rev 27(7):750–761
4. Clavel T, Desmarchelier C, Haller D, Gerard P, Rohn S, Lepage P, Daniel H (2014) Intestinal microbiota in metabolic diseases: from bacterial community structure and functions to species of pathophysiological relevance. Gut Microbes 5(4):544–551. doi:10.4161/gmic.29331
5. Rosenbaum M, Knight R, Leibel RL (2015) The gut microbiota in human energy homeostasis and obesity. Trends Endocrinol Metab 26(9):493–501. doi:10.1016/j.tem.2015.07.002
6. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J (2014) The placenta harbors a unique microbiome. Sci Transl Med 6(237):237ra265. doi:10.1126/scitranslmed.3008599
7. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO (2007) Development of the human infant intestinal microbiota. PLoS Biol 5(7):e177
8. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE (2011) Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci USA 108(Suppl 1):4578–4585. doi:10.1073/pnas.1000081107
9. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL, Rosenbaum M, Gordon JI (2013) The long-term stability of the human gut microbiota. Science 341(6141):1237439. doi:10.1126/science.1237439
10. Dethlefsen L, Huse S, Sogin ML, Relman DA (2008) The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol 6(11):e280. doi:10.1371/journal.pbio.0060280
11. Dethlefsen L, Relman DA (2011) Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci USA 108(Suppl 1):4554–4561. doi:10.1073/pnas.1000087107
12. Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, Mao Y, Zhang X, Pang X, Wei C, Zhao G, Chen Y, Zhao L (2010) Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME J 4(2):232–241. doi:10.1038/ismej.2009.112
13. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI (2009) A core gut microbiome in obese and lean twins. Nature 457(7228):480–484
14. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489(7415):220–230. doi:10.1038/nature11550
15. Franzosa EA, Huang K, Meadow JF, Gevers D, Lemon KP, Bohannan BJ, Huttenhower C (2015) Identifying personal microbiomes using metagenomic codes. Proc Natl Acad Sci USA 112(22):E2930–2938. doi:10.1073/pnas.1423854112
16. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI (2008) Evolution of mammals and their gut microbes. Science 320(5883):1647–1651
17. O'Hara AM, Shanahan F (2006) The gut flora as a forgotten organ. EMBO Rep 7(7):688–693. doi:10.1038/sj.embor.7400731

18. Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40:337–365
19. Gérard P (2011) Le microbiote intestinal: composition et fonctions. Phytothérapie 9(2):72–75
20. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005) Diversity of the human intestinal microbial flora. Science 308(5728):1635–1638
21. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 104(34):13780–13785
22. Hsiao WW, Fraser-Liggett CM (2009) Human Microbiome Project–paving the way to a better understanding of ourselves and our microbes. Drug Discov Today 14(7–8):331–333
23. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007) The human microbiome project. Nature 449(7164):804–810
24. Frank DN, Pace NR (2008) Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol 24(1):4–10
25. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. Science 312(5778):1355–1359
26. Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, Arumugam M, Kultima JR, Prifti E, Nielsen T, Juncker AS, Manichanh C, Chen B, Zhang W, Levenez F, Wang J, Xu X, Xiao L, Liang S, Zhang D, Zhang Z, Chen W, Zhao H, Al-Aama JY, Edris S, Yang H, Wang J, Hansen T, Nielsen HB, Brunak S, Kristiansen K, Guarner F, Pedersen O, Dore J, Ehrlich SD, Meta HITC, Bork P, Wang J, Meta HITC (2014) An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol 32(8):834–841. doi:10.1038/nbt.2942
27. Dethlefsen L, McFall-Ngai M, Relman DA (2007) An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 449(7164):811–818
28. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464(7285):59–65
29. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Dore J, Antolin M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Merieux A, Melo Minardi R, M'Rini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P (2011) Enterotypes of the human gut microbiome. Nature 473(7346):174–180. doi:10.1038/nature09944
30. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD (2011) Linking long-term dietary patterns with gut microbial enterotypes. Science 334(6052):105–108. doi:10.1126/science.1208344
31. Knights D, Ward TL, McKinlay CE, Miller H, Gonzalez A, McDonald D, Knight R (2014) Rethinking "enterotypes". Cell Host Microbe 16(4):433–437. doi:10.1016/j.chom.2014.09.013
32. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI (2005) Host-bacterial mutualism in the human intestine. Science 307(5717):1915–1920
33. Hooper LV, Midtvedt T, Gordon JI (2002) How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr 22:283–307
34. Gérard P, Bernalier-Donadille A (2007) Les fonctions majeures du microbiote intestinal. Cahiers de Nutrition et de Diététique 42:S28–S36
35. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, Versalovic J, Young V, Finlay BB (2012) Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe 12(5):611–622. doi:10.1016/j.chom.2012.10.012
36. Duca F, Gerard P, Covasa M, Lepage P (2014) Metabolic interplay between gut bacteria and their host. Front Horm Res 42:73–82. doi:10.1159/000358315
37. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, Martin P, Philippe C, Walker F, Bado A, Perlemuter G, Cassard-Doulcier A, Gérard P (2013) Intestinal microbiota determines development of nonalcoholic fatty liver disease in mice. Gut 62(12):1787–1794
38. Mayer EA, Tillisch K, Gupta A (2015) Gut/brain axis and the microbiota. J Clin Invest 125(3):926–938. doi:10.1172/JCI76304
39. Wostmann BS, Larkin C, Moriarty A, Bruckner-Kardoss E (1983) Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats. Lab Anim Sci 33(1):46–50
40. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 101(44):15718–15723
41. Backhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci USA 104(3):979–984
42. Rabot S, Membrez M, Bruneau A, Gérard P, Harach T, Moser M, Raymond F, Mansourian R, Chou CJ (2010) Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J 24(12):4948–4959
43. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M (2010) Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr 104(6):919–929. doi:10.1017/S0007114510001303
44. Swartz TD, Sakar Y, Duca FA, Covasa M (2013) Preserved adiposity in the Fischer 344 rat devoid of gut microbiota. FASEB J 27(4):1701–1710. doi:10.1096/fj.12-221689
45. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA (2008) Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat Rev Microbiol 6(2):121–131
46. Stappenbeck TS, Hooper LV, Gordon JI (2002) Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci USA 99(24):15451–15455
47. Conterno L, Fava F, Viola R, Tuohy KM (2011) Obesity and the gut microbiota: does up-regulating colonic fermentation protect against obesity and metabolic disease? Genes Nutr 6(3):241–260. doi:10.1007/s12263-011-0230-1

48. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, Pike NB, Strum JC, Steplewski KM, Murdock PR, Holder JC, Marshall FH, Szekeres PG, Wilson S, Ignar DM, Foord SM, Wise A, Dowell SJ (2003) The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem 278(13):11312–11319. doi:10.1074/jbc.M211609200
49. Bjursell M, Admyre T, Goransson M, Marley AE, Smith DM, Oscarsson J, Bohlooly YM (2011) Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. Am J Physiol Endocrinol Metab 300(1):E211–220. doi:10.1152/ajpendo.00229.2010
50. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JI (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci USA 105(43):16767–16772
51. Cani PD, Dewever C, Delzenne NM (2004) Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br J Nutr 92(3):521–526
52. Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, Delzenne NM (2009) Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. Am J Clin Nutr 90(5):1236–1243. doi:10.3945/ajcn.2009.28095
53. Tarini J, Wolever TM (2010) The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. Appl Physiol Nutr Metab 35(1):9–16. doi:10.1139/H09-119
54. Delzenne NM, Neyrinck AM, Cani PD (2011) Modulation of the gut microbiota by nutrients with prebiotic properties: consequences for host health in the context of obesity and metabolic syndrome. Microb Cell Fact 10(Suppl 1):S10. doi:10.1186/1475-2859-10-S1-S10
55. Soliman MM, Ahmed MM, Salah-Eldin AE, Abdel-Aal AA (2011) Butyrate regulates leptin expression through different signaling pathways in adipocytes. J Vet Sci 12(4):319–323
56. Lin HV, Frassetto A, Kowalik EJ Jr, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, Marsh DJ (2012) Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One 7(4):e35240. doi:10.1371/journal.pone.0035240
57. Moore PR, Evenson A et al (1946) Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. J Biol Chem 165(2):437–441
58. Coates ME, Fuller R, Harrison GF, Lev M, Suffolk SF (1963) A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Br J Nutr 17:141–150
59. Gaskins HR, Collier CT, Anderson DB (2002) Antibiotics as growth promotants: mode of action. Anim Biotechnol 13(1):29–42. doi:10.1081/ABIO-120005768
60. Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitler I, Li H, Alekseyenko AV, Blaser MJ (2012) Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 488(7413):621–626. doi:10.1038/nature11400
61. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, Li H, Gao Z, Mahana D, Zarate Rodriguez JG, Rogers AB, Robine N, Loke P, Blaser MJ (2014) Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell 158(4):705–721. doi:10.1016/j.cell.2014.05.052
62. Cox LM, Blaser MJ (2015) Antibiotics in early life and obesity. Nat Rev Endocrinol 11(3):182–190. doi:10.1038/nrendo.2014.210
63. Membrez M, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, Corthesy I, Mace K, Chou CJ (2008) Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. FASEB J 22(7):2416–2426
64. Haight TH, Pierce WE (1955) Effect of prolonged antibiotic administration of the weight of healthy young males. J Nutr 56(1):151–161
65. Ozawa E (1955) Studies on growth promotion by antibiotics. II. Results of aurofac administration to infants. J Antibiot 8(6):212–214
66. Ajslev TA, Andersen CS, Gamborg M, Sorensen TI, Jess T (2011) Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. Int J Obes (Lond) 35(4):522–529. doi:10.1038/ijo.2011.27
67. Bailey LC, Forrest CB, Zhang P, Richards TM, Livshits A, DeRusso PA (2014) Association of antibiotics in infancy with early childhood obesity. JAMA Pediatr 168(11):1063–1069. doi:10.1001/jamapediatrics.2014.1539
68. Murphy R, Stewart AW, Braithwaite I, Beasley R, Hancox RJ, Mitchell EA, Group IPTS (2014) Antibiotic treatment during infancy and increased body mass index in boys: an international cross-sectional study. Int J Obes (Lond) 38(8):1115–1119. doi:10.1038/ijo.2013.218
69. Trasande L, Blustein J, Liu M, Corwin E, Cox LM, Blaser MJ (2013) Infant antibiotic exposures and early-life body mass. Int J Obes (Lond) 37(1):16–23. doi:10.1038/ijo.2012.132
70. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 102(31):11070–11075
71. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444(7122):1022–1023
72. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D (2009) Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and methanogens in anorexic patients. PLoS One 4(9):e7125. doi:10.1371/journal.pone.0007125
73. Furet JP, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL, Mariat D, Corthier G, Dore J, Henegar C, Rizkalla S, Clement K (2010) Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. Diabetes 59(12):3049–3057. doi:10.2337/db10-0253
74. Santacruz A, Collado MC, Garcia-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, Marti-Romero M, Lopez RM, Florido J, Campoy C, Sanz Y (2010) Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 104(1):83–92. doi:10.1017/S0007114510000176
75. Collado MC, Isolauri E, Laitinen K, Salminen S (2008) Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr 88(4):894–899
76. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ (2008) Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes (Lond) 32(11):1720–1724
77. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J (2011) Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient

absorption in humans. Am J Clin Nutr 94(1):58–65. doi:10.3945/ajcn.110.010132
78. Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD (2010) Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring) 18(1):190–195
79. Million M, Angelakis E, Paul M, Armougom F, Leibovici L, Raoult D (2012) Comparative meta-analysis of the effect of *Lactobacillus* species on weight gain in humans and animals. Microb Pathog 53(2):100–108. doi:10.1016/j.micpath.2012.05.007
80. Kalliomaki M, Collado MC, Salminen S, Isolauri E (2008) Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr 87(3):534–538
81. Yin YN, Yu QF, Fu N, Liu XW, Lu FG (2010) Effects of four Bifidobacteria on obesity in high-fat diet induced rats. World J Gastroenterol 16(27):3394–3401
82. Kozyrskyj AL, Kalu R, Koleva PT, Bridgman SL (2015) Fetal programming of overweight through the microbiome: boys are disproportionately affected. J Dev Orig Health Dis. doi:10.1017/S2040174415001269
83. Fei N, Zhao L (2013) An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. ISME J 7(4):880–884. doi:10.1038/ismej.2012.153
84. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottiere HM, Dore J, Marteau P, Seksik P, Langella P (2008) Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA 105(43):16731–16736
85. Cao Y, Shen J, Ran ZH (2014) Association between *Faecalibacterium prausnitzii* reduction and inflammatory bowel disease: a meta-analysis and systematic review of the literature. Gastroenterol Res Pract 2014:872725. doi:10.1155/2014/872725
86. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, Kayser BD, Levenez F, Chilloux J, Hoyles L, Consortium MI-O, Dumas ME, Rizkalla SW, Dore J, Cani PD, Clement K (2015) Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut. doi:10.1136/gutjnl-2014-308778
87. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD (2013) Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci USA 110(22):9066–9071. doi:10.1073/pnas.1219451110
88. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444(7122):1027–1031
89. Greenblum S, Turnbaugh PJ, Borenstein E (2012) Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci USA 109(2):594–599. doi:10.1073/pnas.1116053109
90. Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD (2010) Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring) 18(1):190–195. doi:10.1038/oby.2009.167
91. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jorgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clement K, Dore J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T, Bork P, Wang J, Ehrlich SD, Pedersen O, Guedon E, Delorme C, Layec S, Khaci G, van de Guchte M, Vandemeulebrouck G, Jamet A, Dervyn R, Sanchez N, Maguin E, Haimet F, Winogradski Y, Cultrone A, Leclerc M, Juste C, Blottiere H, Pelletier E, LePaslier D, Artiguenave F, Bruls T, Weissenbach J, Turner K, Parkhill J, Antolin M, Manichanh C, Casellas F, Boruel N, Varela E, Torrejon A, Guarner F, Denariaz G, Derrien M, van Hylckama Vlieg JE, Veiga P, Oozeer R, Knol J, Rescigno M, Brechot C, M'Rini C, Merieux A, Yamada T (2013) Richness of human gut microbiome correlates with metabolic markers. Nature 500(7464):541–546. doi:10.1038/nature12506
92. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, Almeida M, Quinquis B, Levenez F, Galleron N, Gougis S, Rizkalla S, Batto JM, Renault P, Dore J, Zucker JD, Clement K, Ehrlich SD, Blottiere H, Leclerc M, Juste C, de Wouters T, Lepage P, Fouqueray C, Basdevant A, Henegar C, Godard C, Fondacci M, Rohia A, Hajduch F, Weissenbach J, Pelletier E, Le Paslier D, Gauchi JP, Gibrat JF, Loux V, Carre W, Maguin E, van de Guchte M, Jamet A, Boumezbeur F, Layec S (2013) Dietary intervention impact on gut microbial gene richness. Nature 500(7464):585–588. doi:10.1038/nature12480
93. Mondot S, de Wouters T, Dore J, Lepage P (2013) The human gut microbiome and its dysfunctions. Dig Dis 31(3–4):278–285. doi:10.1159/000354678
94. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 3(4):213–223
95. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI (2009) The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 1 (6):6ra14
96. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341(6150):1241214. doi:10.1126/science.1241214
97. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT (2010) Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 328(5975):228–231
98. Di Luccia B, Crescenzo R, Mazzoli A, Cigliano L, Venditti P, Walser JC, Widmer A, Baccigalupi L, Ricca E, Iossa S (2015) Rescue of fructose-induced metabolic syndrome by antibiotics or faecal transplantation in a rat model of obesity. PLoS One 10(8):e0134893. doi:10.1371/journal.pone.0134893
99. Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444(7121):860–867
100. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R (2007) Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56(7):1761–1772
101. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC (1990) CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 249(4975):1431–1433
102. Neal MD, Leaphart C, Levy R, Prince J, Billiar TR, Watkins S, Li J, Cetin S, Ford H, Schreiber A, Hackam DJ (2006) Enterocyte TLR4 mediates phagocytosis and translocation of

bacteria across the intestinal barrier. J Immunol 176(5):3070–3079
103. Vreugdenhil AC, Rousseau CH, Hartung T, Greve JW, van 't Veer C, Buurman WA (2003) Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. J Immunol 170(3):1399–1405
104. Creely SJ, McTernan PG, Kusminski CM, Fisher M, Da Silva NF, Khanolkar M, Evans M, Harte AL, Kumar S (2007) Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 292(3):E740–747
105. Harte AL, da Silva NF, Creely SJ, McGee KC, Billyard T, Youssef-Elabd EM, Tripathi G, Ashour E, Abdalla MS, Sharada HM, Amin AI, Burt AD, Kumar S, Day CP, McTernan PG (2010) Elevated endotoxin levels in non-alcoholic fatty liver disease. J Inflamm (Lond) 7:15
106. Davis JE, Gabler NK, Walker-Daniels J, Spurlock ME (2008) Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. Obesity (Silver Spring) 16(6):1248–1255
107. Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, Araujo EP, Vassallo J, Curi R, Velloso LA, Saad MJ (2007) Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 56(8):1986–1998
108. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57(6):1470–1481
109. Wang Z, Xiao G, Yao Y, Guo S, Lu K, Sheng Z (2006) The role of bifidobacteria in gut barrier function after thermal injury in rats. J Trauma 61(3):650–657
110. Griffiths EA, Duffy LC, Schanbacher FL, Qiao H, Dryja D, Leavens A, Rossman J, Rich G, Dirienzo D, Ogra PL (2004) In vivo effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. Dig Dis Sci 49(4):579–589
111. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 50(11):2374–2383
112. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58(8):1091–1103. doi:10.1136/gut.2008.165886
113. Geurts L, Lazarevic V, Derrien M, Everard A, Van Roye M, Knauf C, Valet P, Girard M, Muccioli GG, Francois P, de Vos WM, Schrenzel J, Delzenne NM, Cani PD (2011) Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. Front Microbiol 2:149. doi:10.3389/fmicb.2011.00149
114. Muccioli GG, Naslain D, Backhed F, Reigstad CS, Lambert DM, Delzenne NM, Cani PD (2010) The endocannabinoid system links gut microbiota to adipogenesis. Mol Syst Biol 6:392. doi:10.1038/msb.2010.46
115. Everard A, Geurts L, Caesar R, Van Hul M, Matamoros S, Duparc T, Denis RG, Cochez P, Pierard F, Castel J, Bindels LB, Plovier H, Robine S, Muccioli GG, Renauld JC, Dumoutier L, Delzenne NM, Luquet S, Backhed F, Cani PD (2014) Intestinal epithelial MyD88 is a sensor switching host metabolism towards obesity according to nutritional status. Nat Commun 5:5648. doi:10.1038/ncomms6648
116. Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermudez-Humaran LG, Smirnova N, Berge M, Sulpice T, Lahtinen S, Ouwehand A, Langella P, Rautonen N, Sansonetti PJ, Burcelin R (2011) Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol Med 3(9):559–572. doi:10.1002/emmm.201100159
117. Amar J, Serino M, Lange C, Chabo C, Iacovoni J, Mondot S, Lepage P, Klopp C, Mariette J, Bouchez O, Perez L, Courtney M, Marre M, Klopp P, Lantieri O, Dore J, Charles M, Balkau B, Burcelin R, Group DESIRS (2011) Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept. Diabetologia 54(12):3055–3061. doi:10.1007/s00125-011-2329-8
118. Burcelin R, Serino M, Chabo C, Garidou L, Pomie C, Courtney M, Amar J, Bouloumie A (2013) Metagenome and metabolism: the tissue microbiota hypothesis. Diabetes Obes Metab 15(Suppl 3):61–70. doi:10.1111/dom.12157
119. Wostmann BS (1973) Intestinal bile acids and cholesterol absorption in the germfree rat. J Nutr 103(7):982–990
120. Wostmann BS, Wiech NL (1961) Total serum and liver cholesterol in germfree and conventional male rats. Am J Physiol 201:1027–1029
121. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, Islam S, Felin J, Perkins R, Boren J, Oresic M, Backhed F (2010) The gut microbiota modulates host energy and lipid metabolism in mice. J Lipid Res 51(5):1101–1112. doi:10.1194/jlr.M002774
122. Gérard P, Lepercq P, Leclerc M, Gavini F, Raibaud P, Juste C (2007) Bacteroides sp. strain D8, the first cholesterol-reducing bacterium isolated from human feces. Appl Environ Microbiol 73(18):5742–5749
123. Parmentier G, Eyssen H (1974) Mechanism of biohydrogenation of cholesterol to coprostanol by Eubacterium ATCC 21408. Biochim Biophys Acta 348(2):279–284
124. Sekimoto H, Shimada O, Makanishi M, Nakano T, Katayama O (1983) Interrelationship between serum and fecal sterols. Jpn J Med 22(1):14–20
125. Gérard P (2009) GI tract: microbial metabolism of steroids. In: Timmis KN (ed) Microbiology of hydrocarbons, oils, lipids, and derived compounds, vol 4. Springer, Heidelberg, pp 3133–3140
126. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472(7341):57–63
127. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL (2013) Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 368(17):1575–1584. doi:10.1056/NEJMoa1109400
128. Gérard P (2014) Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens 3(1):14–24. doi:10.3390/pathogens3010014
129. Joyce SA, Shanahan F, Hill C, Gahan CG (2014) Bacterial bile salt hydrolase in host metabolism: potential for influencing gastrointestinal microbe-host crosstalk. Gut Microbes 5(5):669–674. doi:10.4161/19490976.2014.969986
130. Kondo S, Xiao JZ, Satoh T, Odamaki T, Takahashi S, Sugahara H, Yaeshima T, Iwatsuki K, Kamei A, Abe K (2010) Antiobesity effects of Bifidobacterium breve strain B-3 supplementation in a mouse model with high-fat diet-induced obesity. Biosci Biotechnol Biochem 74(8):1656–1661. doi:10.1271/bbb.100267
131. An HM, Park SY, Lee do K, Kim JR, Cha MK, Lee SW, Lim HT, Kim KJ, Ha NJ (2011) Antiobesity and lipid-lowering effects of *Bifidobacterium* spp. in high fat diet-induced obese rats. Lipids Health Dis 10:116. doi:10.1186/1476-511X-10-116

132. Chen J, Wang R, Li XF, Wang RL (2012) Bifidobacterium adolescentis supplementation ameliorates visceral fat accumulation and insulin sensitivity in an experimental model of the metabolic syndrome. Br J Nutr 107(10):1429–1434. doi:10.1017/S0007114511004491
133. Chen JJ, Wang R, Li XF, Wang RL (2011) Bifidobacterium longum supplementation improved high-fat-fed-induced metabolic syndrome and promoted intestinal Reg I gene expression. Exp Biol Med 236(7):823–831. doi:10.1258/ebm.2011.010399
134. Cano PG, Santacruz A, Trejo FM, Sanz Y (2013) Bifidobacterium CECT 7765 improves metabolic and immunological alterations associated with obesity in high-fat diet-fed mice. Obesity (Silver Spring) 21(11):2310–2321. doi:10.1002/oby.20330
135. Moya-Perez A, Neef A, Sanz Y (2015) *Bifidobacterium pseudocatenulatum* CECT 7765 reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. PLoS One 10(7):e0126976. doi:10.1371/journal.pone.0126976
136. Lee HY, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH (2006) Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. Biochim Biophys Acta 1761(7):736–744. doi:10.1016/j.bbalip.2006.05.007
137. Lee K, Paek K, Lee HY, Park JH, Lee Y (2007) Antiobesity effect of trans-10, cis-12-conjugated linoleic acid-producing *Lactobacillus plantarum* PL62 on diet-induced obese mice. J Appl Microbiol 103(4):1140–1146. doi:10.1111/j.1365-2672.2007.03336.x
138. Hamad EM, Sato M, Uzu K, Yoshida T, Higashi S, Kawakami H, Kadooka Y, Matsuyama H, Abd El-Gawad IA, Imaizumi K (2009) Milk fermented by *Lactobacillus gasseri* SBT2055 influences adipocyte size via inhibition of dietary fat absorption in Zucker rats. Br J Nutr 101(5):716–724. doi:10.1017/S0007114508043808
139. Aronsson L, Huang Y, Parini P, Korach-Andre M, Hakansson J, Gustafsson JA, Pettersson S, Arulampalam V, Rafter J (2010) Decreased fat storage by *Lactobacillus paracasei* is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4). PLoS One 5(9). doi:10.1371/journal.pone.0013087
140. Okubo T, Takemura N, Yoshida A, Sonoyama K (2013) KK/Ta mice administered *Lactobacillus plantarum* strain no. 14 have lower adiposity and higher insulin sensitivity. Biosci Microbiota Food Health 32(3):93–100. doi:10.12938/bmfh.32.93
141. Fak F, Backhed F (2012) *Lactobacillus reuteri* prevents diet-induced obesity, but not atherosclerosis, in a strain dependent fashion in Apoe$^{-/-}$ mice. PLoS One 7(10):e46837. doi:10.1371/journal.pone.0046837
142. Kim SW, Park KY, Kim B, Kim E, Hyun CK (2013) *Lactobacillus rhamnosus* GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. Biochem Biophys Res Commun 431(2):258–263. doi:10.1016/j.bbrc.2012.12.121
143. Wang LX, Liu K, Gao DW, Hao JK (2013) Protective effects of two Lactobacillus plantarum strains in hyperlipidemic mice. World J Gastroenterol 19(20):3150–3156. doi:10.3748/wjg.v19.i20.3150
144. Tomaro-Duchesneau C, Saha S, Malhotra M, Jones ML, Labbe A, Rodes L, Kahouli I, Prakash S (2014) Effect of orally administered *L. fermentum* NCIMB 5221 on markers of metabolic syndrome: an in vivo analysis using ZDF rats. Appl Microbiol Biotechnol 98(1):115–126. doi:10.1007/s00253-013-5252-8
145. Park KY, Kim B, Hyun CK (2015) *Lactobacillus rhamnosus* GG reverses insulin resistance but does not block its onset in diet-induced obese mice. J Microbiol Biotechnol 25(5):753–757
146. Martin FP, Wang Y, Sprenger N, Yap IK, Lundstedt T, Lek P, Rezzi S, Ramadan Z, van Bladeren P, Fay LB, Kochhar S, Lindon JC, Holmes E, Nicholson JK (2008) Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. Mol Syst Biol 4:157. doi:10.1038/msb4100190
147. Park YH, Kim JG, Shin YW, Kim SH, Whang KY (2007) Effect of dietary inclusion of Lactobacillus acidophilus ATCC 43121 on cholesterol metabolism in rats. J Microbiol Biotechnol 17(4):655–662
148. Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M, Tsuchida T (2010) Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr 64(6):636–643. doi:10.1038/ejcn.2010.19
149. Kadooka Y, Sato M, Ogawa A, Miyoshi M, Uenishi H, Ogawa H, Ikuyama K, Kagoshima M, Tsuchida T (2013) Effect of *Lactobacillus gasseri* SBT2055 in fermented milk on abdominal adiposity in adults in a randomised controlled trial. Br J Nutr 110(9):1696–1703. doi:10.1017/S0007114513001037
150. Ogawa A, Kadooka Y, Kato K, Shirouchi B, Sato M (2014) *Lactobacillus gasseri* SBT2055 reduces postprandial and fasting serum non-esterified fatty acid levels in Japanese hypertriacylglycerolemic subjects. Lipids Health Dis 13:36. doi:10.1186/1476-511X-13-36
151. Floch MH (2014) Recommendations for probiotic use in humans—a 2014 update. Pharmaceuticals 7(10):999–1007. doi:10.3390/ph7100999
152. Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, Wolvers D, Watzl B, Szajewska H, Stahl B, Guarner F, Respondek F, Whelan K, Coxam V, Davicco MJ, Leotoing L, Wittrant Y, Delzenne NM, Cani PD, Neyrinck AM, Meheust A (2010) Prebiotic effects: metabolic and health benefits. Br J Nutr 104(Suppl 2):S1–63. doi:10.1017/S0007114510003363
153. Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, Possemiers S, Van Holle A, Francois P, de Vos WM, Delzenne NM, Schrenzel J, Cani PD (2011) Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes 60(11):2775–2786. doi:10.2337/db11-0227
154. Neyrinck AM, Possemiers S, Druart C, Van de Wiele T, De Backer F, Cani PD, Larondelle Y, Delzenne NM (2011) Prebiotic effects of wheat arabinoxylan related to the increase in bifidobacteria, Roseburia and Bacteroides/Prevotella in diet-induced obese mice. PLoS One 6(6):e20944. doi:10.1371/journal.pone.0020944
155. Parnell JA, Reimer RA (2012) Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. Br J Nutr 107(4):601–613. doi:10.1017/S0007114511003163
156. Everard A, Lazarevic V, Gaia N, Johansson M, Stahlman M, Backhed F, Delzenne NM, Schrenzel J, Francois P, Cani PD (2014) Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity. ISME J 8(10):2116–2130. doi:10.1038/ismej.2014.45
157. Respondek F, Gerard P, Bossis M, Boschat L, Bruneau A, Rabot S, Wagner A, Martin JC (2013) Short-chain fructo-oligosaccharides modulate intestinal microbiota and metabolic parameters of humanized gnotobiotic diet induced obesity mice. PLoS One 8(8):e71026. doi:10.1371/journal.pone.0071026

158. Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G, Delzenne NM (2005) Involvement of endogenous glucagon-like peptide-1(7-36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. J Endocrinol 185(3):457–465. doi:10.1677/joe.1.06100
159. Cani PD, Neyrinck AM, Maton N, Delzenne NM (2005) Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. Obes Res 13(6):1000–1007. doi:10.1038/oby.2005.117
160. Chassaing B, Miles-Brown JP, Pellizzon M, Ulman E, Ricci M, Zhang L, Patterson AD, Vijay-Kumar M, Gewirtz AT (2015) Lack of soluble fiber drives diet-induced adiposity in mice. Am J Physiol Gastrointest Liver Physiol 00172:02015. doi:10.1152/ajpgi.00172.2015
161. Cani PD, Joly E, Horsmans Y, Delzenne NM (2006) Oligofructose promotes satiety in healthy human: a pilot study. Eur J Clin Nutr 60(5):567–572. doi:10.1038/sj.ejcn.1602350
162. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP, Delzenne NM (2013) Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut 62(8):1112–1121. doi:10.1136/gutjnl-2012-303304
163. Genta S, Cabrera W, Habib N, Pons J, Carillo IM, Grau A, Sanchez S (2009) Yacon syrup: beneficial effects on obesity and insulin resistance in humans. Clin Nutr 28(2):182–187. doi:10.1016/j.clnu.2009.01.013
164. Kellow NJ, Coughlan MT, Reid CM (2014) Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. Br J Nutr 111(7):1147–1161. doi:10.1017/S0007114513003607
165. Parnell JA, Reimer RA (2009) Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr 89(6):1751–1759. doi:10.3945/ajcn.2009.27465
166. Aroniadis OC, Brandt LJ (2013) Fecal microbiota transplantation: past, present and future. Curr Opin Gastroenterol 29(1):79–84. doi:10.1097/MOG.0b013e32835a4b3e
167. Eiseman B, Silen W, Bascom GS, Kauvar AJ (1958) Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery 44(5):854–859
168. Kassam Z, Lee CH, Yuan Y, Hunt RH (2013) Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 108(4):500–508. doi:10.1038/ajg.2013.59
169. Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 143(4):913–916 e917. doi:10.1053/j.gastro.2012.06.031