

## MECHANISMS IN ENDOCRINOLOGY

# Gut microbiota in patients with type 2 diabetes mellitus

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## Abstract

Perturbations of the composition and function of the gut microbiota have been associated with metabolic disorders including obesity, insulin resistance and type 2 diabetes. Studies on mice have demonstrated several underlying mechanisms including host signalling through bacterial lipopolysaccharides derived from the outer membranes of Gram-negative bacteria, bacterial fermentation of dietary fibres to short-chain fatty acids and bacterial modulation of bile acids. On top of this, an increased permeability of the intestinal epithelium may lead to increased absorption of macromolecules from the intestinal content resulting in systemic immune responses, low-grade inflammation and altered signalling pathways influencing lipid and glucose metabolism. While mechanistic studies on mice collectively support a causal role of the gut microbiota in metabolic diseases, the majority of studies in humans are correlative of nature and thus hinder causal inferences. Importantly, several factors known to influence the risk of type 2 diabetes, e.g. diet and age, have also been linked to alterations in the gut microbiota complicating the interpretation of correlative studies. However, based upon the available evidence, it is hypothesised that the gut microbiota may mediate or modulate the influence of lifestyle factors triggering development of type 2 diabetes. Thus, the aim of this review is to critically discuss the potential role of the gut microbiota in the pathophysiology and pathogenesis of type 2 diabetes.

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## Introduction

In addition to well-established risk factors for type 2 diabetes, including genetic predisposition, poor physical activity, foetal programming and obesity (1), an altered configuration of the microbial community in our gut – the microbiota – has emerged as a new candidate that may be linked to type 2 diabetes. Trillions of micro-organisms inhabit the distal gut, where they together weigh about

1.5 kg and may be regarded as a microbial organ that carries out key functions that the human host is incapable to perform by itself. The gut microbiota includes members from all three domains of life (Bacteria, Archaea and Eukarya) as well as their viruses, but is dominated by anaerobic bacteria. More than 90% of the ~1000 prevalent bacterial species (2) can be grouped into the two

### Invited Author's profile

**Oluf Pedersen** is Professor of Molecular Metabolism at the University of Copenhagen (UCP). He is also director at the Novo Nordisk Foundation Center for Basic Metabolic Research at UCP. Prof Pedersen and his team are focused on discovering genomic variation that predisposes for common and rare cardio-metabolic disorders. Another major research effort is centred at studies of the role of the gut microbiota in metabolic health. Herein, the research team is doing quantitative metagenomics to characterise the human gut microbiome at levels of microbial genes, various taxa and derived functional potentials.



bacterial phyla Firmicutes (Gram-positive) and Bacteroidetes (Gram-negative) (3). Each individual harbours at least 160 abundant bacterial species and it has been suggested that a core gut microbiota at the functional level exists and that a set of core microbial genes is necessary for correct functioning of the gut microbial ecosystem (2). The gut microbiota protects against pathogens and helps mature and constantly educate the immune system. It also plays a role for regulation of intestinal hormone secretion and for gastrointestinal nerve activity. Furthermore, members of the gut microbiota synthesise vitamin K and several B-vitamins including folate and vitamin B12 and they produce short-chain fatty acids (SCFAs) by fermentation of otherwise non-digestible carbohydrates.

Traditionally, bacteria are studied by culture-based methods, but as it proved difficult to culture the majority of the anaerobically living commensal gut microorganisms, culture-independent methods have been developed. Culture-independent approaches take advantage of next-generation sequencing technology to study microbial DNA from faecal samples, as these generally are considered to be representative of the distal gut microbiota. DNA-based studies are dominated by two methods: targeted 16S rRNA gene sequencing and un-targeted whole-genome shotgun sequencing. The 16S rRNA gene sequencing method relies on targeted amplification and the subsequent sequencing of the phylogenetically informative marker 16S rRNA and is used to describe the bacterial community in terms of which bacteria are present, their relative abundance and their phylogenetic relationships (4, 5). While 16S rRNA gene studies may be biased by primer choice and PCR-related issues and do not provide information about bacterial genes, whole-genome shotgun sequencing, or metagenomic sequencing, bypasses these biases and limitations using direct next-generation sequencing of microbial DNA without prior amplification and thus provides a high-resolution depiction of the bacterial composition. Moreover, metagenomic sequencing enables a comprehensive description of the collective genome of the gut microbiota—the gut microbiome, and, consequently, the functional capabilities of the gut bacteria present. Based on metagenomic sequencing of European (2), Chinese, and American samples, a comprehensive microbial gene catalogue containing 9 879 896 genes – compared with ~23 000 human genes – has been recently published (6).

Alterations in the gut microbiota have been linked to the increasing prevalence of metabolic disorders including obesity, insulin resistance and type 2 diabetes. With a few

exceptions, the human studies are cross-sectional and therefore correlative, whereas the animal studies have elucidated underlying mechanisms and supported a causal role of the gut microbiota in metabolic diseases. In this review, we critically discuss the present evidence for a role of the gut microbiota in the pathophysiology and pathogenesis of type 2 diabetes and envisage where this research field is heading in the near future.

### A link to type 2 diabetes?

In 2004, the first evidence was published from studies on germ-free mice, suggesting that the gut microbiota might contribute to alterations in glucose metabolism (7). Conventionalisation of germ-free mice with microbiota from conventionally raised mice produced, despite a lower energy intake, a marked increase in body fat content and insulin resistance (7) and later germ-free mice were shown to be resistant to diet-induced obesity (8). Subsequently, several studies have investigated the role of the human gut microbiota in type 2 diabetes (for a brief overview, see Table 1). The most comprehensive studies to date used metagenomic sequencing in Chinese (9) and Swedish (10) individuals and both studies established that human type 2 diabetes was featured by a dysbiotic gut microbiota. Despite discrepancies between the two studies – which may partly reflect differences in ethnicity, diet, and intake of medication – both studies reported that patients with type 2 diabetes had less butyrate-producing bacteria (*Roseburia* species and *Faecalibacterium prausnitzii*). Earlier studies in humans as well as in mice reported that obesity and impaired glucose metabolism were associated with an altered ratio between the two major phyla in the human gut, Firmicutes and Bacteroidetes (11, 12). However, neither the Swedish nor the Chinese study confirmed these findings. Nor did they confirm that type 2 diabetes was associated with a lower diversity, which otherwise could be expected from studies in non-diabetic humans, where low gut bacteria gene richness (a marker of diversity) was associated with a high risk of obesity-related comorbidity compared with high richness (13). In a Danish study, levels of Proteobacteria were elevated in type 2 diabetic patients (14) and concordantly the abundance of *Escherichia coli* (belonging to the Proteobacteria phylum) was increased in Chinese type 2 diabetic patients (9). It may be hypothesised that these Gram-negative bacteria could shed inflammatory lipopolysaccharides (LPS) triggering the subclinical state of pro-inflammation, which is a characteristic feature of

**Table 1** Overview of studies that have applied next-generation sequencing techniques to investigate the human gut microbiota in patients with type 2 diabetes.

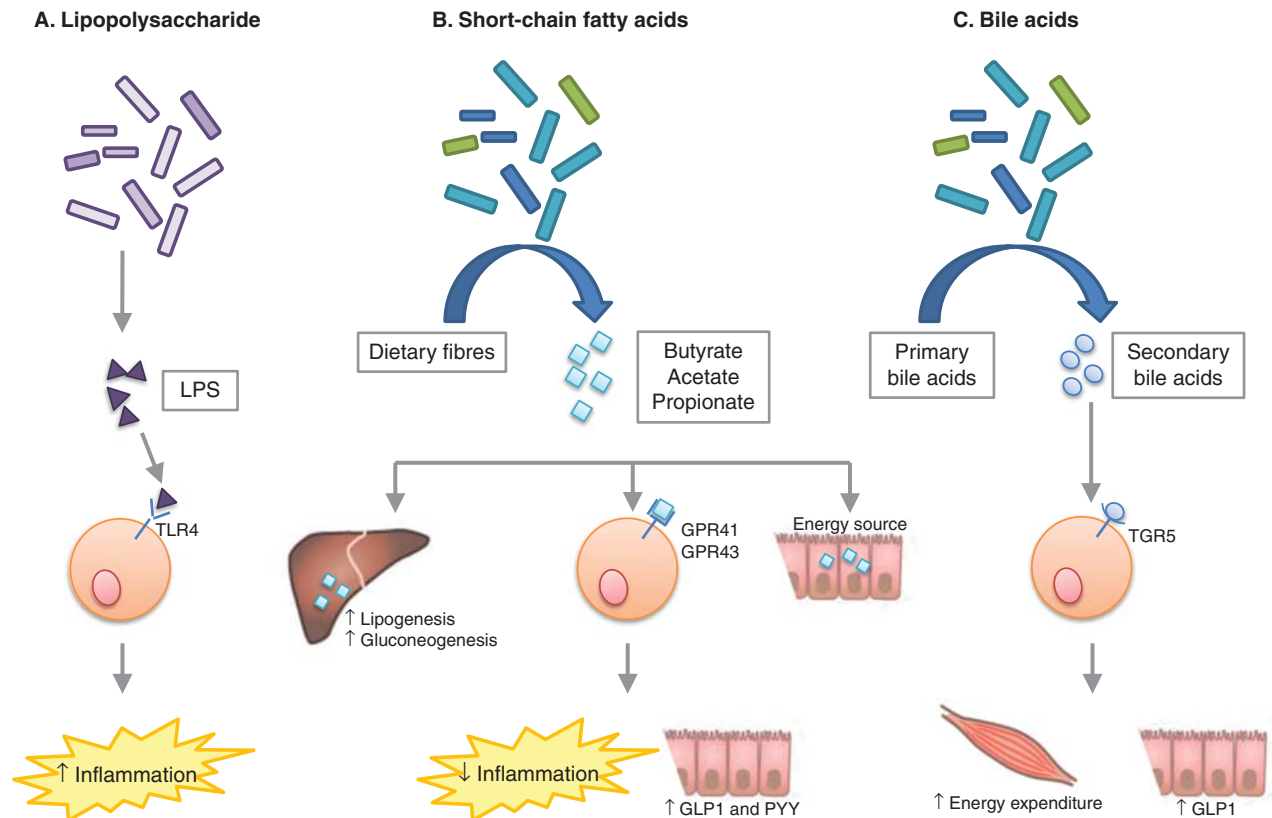
Study	Study design	Method for investigating microbiota	Number of individuals	Notes
(14)	Cross-sectional	qPCR and 16S rRNA gene sequencing in subset	$n_{\text{total}} = 36$ $n_{\text{T2D}} = 18$	Only men
(9)	Cross-sectional	Metagenomic sequencing	$n_{\text{total}} = 368$ $n_{\text{T2D}} = 183$	Age variation between cases and controls. No information on medication
(70)	Prospective	Metagenomic sequencing	$n_{\text{total}} = 6$ $n_{\text{T2D}} = 5$	Study on Roux-en-Y gastric bypass patients
(10)	Cross-sectional	Metagenomic sequencing	$n_{\text{total}} = 145$ $n_{\text{T2D}} = 53$	Only women, all post-menopausal (70 years old). Information on metformin and sulphonylurea treatment available
(71)	Cross-sectional	16S rRNA gene sequencing	$n_{\text{total}} = 121$ $n_{\text{T2D}} = 13$	Only subset have type 2 diabetes
(72)	Prospective	16S rRNA gene sequencing	$n_{\text{total}} = 12$ $n_{\text{T2D}} = 12$	Tested the effect of metformin treatment on gut microbiota in type 2 diabetic patients

both type 2 diabetes and obesity. While some of the discrepancies between studies can be explained by ethnic or dietary differences, other factors such as intake of medications are most likely to influence the bacterial composition and functional potentials. Very little information has so far been provided in any of the published studies on medication. In the Swedish study, diabetic patients treated with metformin had increased levels of Enterobacteriaceae and decreased levels of *Clostridium* and *Eubacterium*, yet the significance of these observations were not argued. However, in the light of these observations, it may be reasonable to interpret the increased abundance of *E. coli* (belonging to the Enterobacteriaceae family) in Chinese type 2 diabetic patients with caution. These preliminary findings indicate that medication – although unreported in the Chinese study – may seriously confound the previously reported signals in type 2 diabetes, suggesting that future studies should take information on drug use into account when assessing the gut microbiota. By taking advantage of the large metagenomic datasets from the Swedish and Chinese studies, respectively, it was in fact possible based on just a few metagenomic markers subjected to receiver operator curve analysis to discriminate between patients with type 2 diabetes and non-diabetic individuals with an accuracy of above 80%. It remains, however, to be established whether this discriminative power represents discrimination between individuals with type 2 diabetes and normal glucose tolerance or discrimination between individuals receiving or not receiving metformin treatment. Metformin, the first-line drug of choice for the treatment of type 2 diabetes, increases the levels of

*Akkermansia* species in high-fat diet-fed mice in parallel to its beneficial effects on glucose metabolism (15). Supplying mice with *A. muciniphila* orally has been reported to result in improvements of glucose tolerance and metabolic dysfunctions such as metabolic endotoxemia and adipose tissue inflammation (15, 16). These promising results not only suggest novel glucose-lowering mechanisms of metformin, but also provide future potential targets for altering glucose regulation by means of bacterio-therapy.

### Gut microbiota and host metabolism

The gut microbiota has been shown to interact with host metabolism leading to insulin resistance and type 2 diabetes through several mechanisms including induction of low-grade inflammation and alterations of energy homeostasis and glucose metabolism (Fig. 1). Bacterial LPSs derived from the outer membranes of Gram-negative bacteria have been intensively studied and are known to induce metabolic endotoxemia by promoting secretion of pro-inflammatory cytokines (17). Studies in both animal models and humans have shown that a high-fat diet can modulate the gut microbiota and increase circulating levels of LPSs, probably by uptake of LPSs in chylomicrons secreted from intestinal epithelial cells or through increased intestinal permeability (17, 18, 19, 20). Mice fed a diet rich in fat exhibited increased adiposity and low-grade inflammation dependent on LPS signalling, and infusion of LPSs in mice resulted in metabolic changes comparable to the high-fat feeding. Accordingly, germ-free mice developed less insulin resistance when fed a



**Figure 1**

Microbes and host metabolism. Microbes may influence host metabolism through numerous mechanisms, of which three important mechanisms are depicted. (A) Lipopolysaccharide. Lipopolysaccharide (LPS) originates from the outer membrane of Gram-negative bacteria and binds to Toll-like receptor 4 (TLR4), which activates pro-inflammatory signalling pathways resulting in low-grade inflammation and thus decreased insulin sensitivity. (B) Short-chain fatty acids. Bacteria in the colon ferment dietary fibres to short-chain fatty acids (mainly butyrate, acetate and propionate). Acetate and propionate are used as substrates for gluconeogenesis and lipogenesis in the liver, whereas butyrate is an important energy substrate for colonic mucosa cells. Moreover, short-chain fatty acids bind to the G protein-coupled receptors GPR41 and GPR43 resulting in

various effects depending on the cellular types affected. In immune cells, this signalling results in decreased inflammation and in the enteroendocrine L-cells it results in increased GLP1 and PYY levels together leading to improved insulin sensitivity. (C) Bile acids. Primary bile acids are produced by the liver and recirculated to the liver from the gut. However, gut bacteria are capable of deconjugating primary bile acids hindering their recirculation. The primary deconjugated bile acids are further metabolised by gut bacteria to secondary bile acids. Secondary bile acids bind to the G protein-coupled receptor TGR5, which results in increased energy expenditure in muscles and GLP1 secretion in the enteroendocrine L-cells, both of which lead to improved insulin sensitivity.

high-fat diet (21) and mice receiving antibiotics exhibited lower levels of circulating LPSs and TNF $\alpha$  as well as decreased insulin resistance compared with pair-fed mice (22). As a part of the immune system, Toll-like receptors (TLRs) recognise microbial molecules and activate the innate immune system. LPSs bind to and activate the TLR4/CD14 complex, which activates pro-inflammatory pathways. Other TLRs, such as TLR2 and TLR5, have also

been proposed to be part of the signalling pathways affecting the development of metabolic syndrome as observed in studies of *Thr2*- and *Thr5*-deficient mice (23, 24). Additional evidence of the importance of the crosstalk among the immune system, inflammation and metabolism was observed in the development of non-alcoholic fatty liver disease (NAFLD). Mice without the inflammasome complexes NLRP3 or NLRP5,

which are multi-protein complexes that recognise microbiota-associated molecular patterns and are involved in the pro-inflammatory pathways, exhibited compositional changes of their gut microbiota and developed NAFLD (25). Probably, the development of NAFLD is mediated by increased gut permeability and modulation of TLR4 and TLR9.

The gut microbiota also influences glucose and energy metabolism through the production of SCFAs. Butyrate, acetate, and propionate are produced through fermentation of complex polysaccharides by the colonic gut bacteria and enter the circulation. Butyrate, the main energy source of the gut epithelium, seems to possess beneficial effect on insulin sensitivity and energy balance (26), whereas acetate and propionate mainly functions as substrates for gluconeogenesis and lipogenesis in the liver. Butyrate has been demonstrated to increase secretion of GLP1 and PYY from L-cells in the colon (26, 27) and to change the intestinal transit time resulting in additional time for nutrient uptake by the host (28). The exact mechanisms by which SCFAs exert their effects are unclear, but they bind to G protein-coupled receptors (GPRs) GPR41 (FFAR3) and GPR43 (FFAR2), which are widely expressed in the intestinal mucosa, immune cells, liver, and adipose tissues (29). Signalling through these receptors mediates the SCFA-induced secretion of GLP1 and PYY as shown in *Gpr41* and *Gpr43* knockout studies on mice (30), and similarly SCFAs suppress development of inflammation by GPR43 signalling in immune cells (31). Interestingly, butyrate and propionate have recently been shown to activate intestinal gluconeogenesis through complementary mechanisms: butyrate exerted its effects via a cAMP-dependent mechanism, whereas the activation of intestinal gluconeogenesis by propionate involved GPR41 signalling (32). Thus, SCFAs may influence host glucose metabolism through regulation of intestinal gluconeogenesis. Together these findings point to pleiotropic and largely beneficial effects of SCFAs on host metabolism and also propose novel targets for treatment and prevention of type 2 diabetes.

Other signalling pathways mediating the crosstalk between the gut bacteria and glucose homeostasis have also been identified. Primary bile acids produced in the liver from cholesterol are conjugated to glycine and enter the enterohepatic circulation by re-uptake in the distal ileum. However, gut bacteria can transform primary bile acids to secondary bile acids. While primary bile acids activate the nuclear farnesoid X receptor (FXR), secondary bile acids bind to the GPR TGR5. TGR5 signalling activates GLP1 secretion from the intestinal L-cells, a signalling pathway identified to protect against diet-induced obesity (33).

When humans were treated with vancomycin for 1 week (an antibiotic that mainly affects Gram-positive bacteria such as the beneficial *Faecalibacterium prausnitzii*), the peripheral insulin sensitivity decreased (34). Interestingly, the authors found that vancomycin treatment greatly reduced levels of secondary bile acids and increased the pool of primary bile acids, signifying that bile acids function as signalling molecules. Additionally, the known beneficial effects of gastric bypass could be mediated by changes in bile acid pools as demonstrated in a vertical sleeve mouse model, where the beneficial effects on glucose metabolism were associated with changes in bile acids and gut microbiota composition, and obstruction of FXR severely reduced the ability to lower body weight and improve glucose tolerance in vertical sleeve-treated mice (35).

It is likely that the gut microbiota may exert effects on glucose and lipid metabolism through several complementary mechanisms, but, for many of these signalling pathways, a common feature is an altered intestinal integrity of the gut epithelial wall. The exact mechanism for how the gut microbiota can modulate the gut permeability is unknown, but a GLP2-dependent mechanism has been proposed as decreased permeability and reduced LPS levels were observed after an increase in endogenous GLP2 (36).

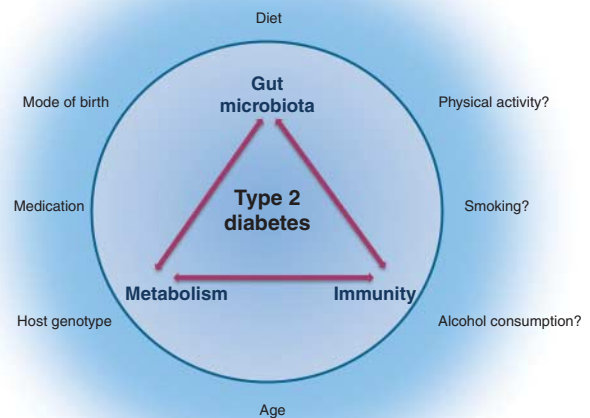
Collectively, present evidence suggests that changes in the gut microbiota from a healthy microbiota to a dysbiotic microbiota may result in a leaky gut epithelium with increased gut permeability causing absorption of macromolecules from the intestinal content triggering systemic immune alterations, altered signalling pathways affecting lipid and glucose metabolism and low-grade inflammation eventually leading to insulin resistance and possibly to type 2 diabetes. However, studies in humans have so far not been able to answer the question of whether the gut microbiota is a cause or consequence – or both – of type 2 diabetes. In order to answer this question, the first step could be implementation of well-designed prospective studies, ideally with carefully phenotyped prediabetic individuals. If such longitudinal studies support a link between the gut microbiota and future development of type 2 diabetes, microbiota in those prediabetic individuals who develop type 2 diabetes and in those who do not develop type 2 diabetes should be further tested and characterised in mechanistic mice experiments. Finally, to establish causality, proper randomised clinical intervention trials are needed in which subjects are randomised to receive treatment with health-promoting bacteria or placebo.

## Type 2 diabetes and gut microbiota: a word of caution

The composition and function of the gut microbiota are influenced by several factors that must be addressed when designing and interpreting studies examining the link between the gut microbiota and type 2 diabetes. Both host genotype and lifestyle factors such as diet, physical activity, antibiotics and other medications (for discussion of metformin see above), age and probably several additional but yet unidentified factors may simultaneously influence the gut microbiota and the risk of type 2 diabetes (Fig. 2).

Although previous studies have demonstrated that the gut microbiota of family members, including mono- and dizygotic twins, is more similar compared with unrelated subjects (37, 38), it remained unknown as to how much of the variation in the gut microbiota is determined by host genetics and how much is explained by environmental factors. Recently, however, a study of a twin cohort has reported that our microbiota signature is to some extent heritable (39). Interestingly, the abundances of some taxa were inherited with heritability estimates of 0.40, whereas others were largely environmentally determined. Noteworthy, it has been shown that the most heritable family, i.e. Christensenellaceae, was associated with a lean and healthy human phenotype (39).

Diet is probably the single most important factor influencing the gut microbiota. Studies on mice have demonstrated that changes in diet result in a rapid change in both the composition and the functional capabilities of the gut microbiota (20, 40) and several human studies have confirmed these findings. A low-fat, high-fibre diet has been linked to a more diverse gut microbiota compared with a diet rich in fat and low in fibre (41), and it has been demonstrated that the human gut microbiota is capable of adapting to a shift from a plant-based diet to an animal-based diet and *vice versa* within only 2 to 4 days (42). De Filippo *et al.* (43) compared children from Italy consuming a western diet with African children consuming a rural diet and observed substantial differences in gut microbiota between these two groups. The authors hypothesise that the gut microbiota coevolve with the diet consumed, allowing the African children to maximise energy intake from fibres and speculate that the reduced richness in the Italian children could signify that the consumption of a western diet is depriving our microbial gene pool and thus limiting the adaptive potential of our gut microbiota (43). Long-term dietary habits seem to associate with the grouping of gut bacteria into enterotypes



**Figure 2**

Gut microbiota and its surroundings. Gut microbiota, host metabolism and host immunity mutually influence each other and are individually under influence of several external factors such as diet, age, mode of birth, medication, host genotype and probably physical activity, smoking and alcohol consumption and other yet unidentified factors. These factors may – separately or simultaneously – influence the gut microbiota, host metabolism and/or host immunity and consequently alter their interdependent relationships and ultimately lead to the risk of type 2 diabetes.

(44). Enterotypes, although their existence is under intense debate (45), were originally identified as three densely populated gut bacterial communities independent of nationalities (46). The enterotype dominated by *Bacteroides* was associated with a diet rich in protein and animal fat, whereas a carbohydrate-rich diet was associated with the *Prevotella* enterotype. Following a 10-day controlled diet rich in either fat or fibres, the enterotypes did not change, indicating that long-term dietary habits shape the gut microbiome (44). An elegantly designed study where mice were transplanted with faeces from human twin donors discordant for obesity showed first that the obesity phenotype was transmissible. Secondly, the authors took advantage of the fact that mice are coprophagic and demonstrated, in cohousing experiments, that cohousing of obese and lean mice prevented development of increased adiposity and body mass. Intriguingly, it was finally shown that this phenotypic rescue was diet dependent and

occurred when the mice were fed a healthy chow but not when they were fed an unhealthy human cafeteria food-like diet (47). Interestingly, it has been lately reported that the non-caloric artificial sweetener saccharine in high daily doses is associated with impaired glucose metabolism and that this effect is most probably attributable to alterations of the composition and function of the gut microbiota (48). Together the present evidence thus suggests a strong role of long-term dietary habits in shaping the gut microbiota, while the day-to-day dietary variation might cause immediate changes to the microbiota. It has been suggested that physical activity may also exert a significant influence on the gut microbiota but, as physical activity and diet are highly correlated, physical activity has not yet been established as an independent factor influencing the gut microbiota (49). Smoking cessation has also been suggested to influence the gut microbiota but other factors than smoking cessation *per se*, such as weight gain following smoking cessation, may confound this relationship (50). Recently, it has been shown that some alcohol-dependent subjects demonstrate increased intestinal permeability and alterations of the gut microbiota at the compositional and functional level, suggesting that alcohol may also play an important role (51).

It has been speculated that the introduction of antibiotics in the early twentieth century may have contributed to the obesity epidemic and epidemiological studies have supported this notion as they have demonstrated that exposure to antibiotics in early childhood increases the risk of overweight in later childhood (52, 53, 54, 55). Compared with children of normal-weight mothers not receiving antibiotics, the risk of overweight at age 7 years was increased by 1.5-fold among children receiving antibiotics during the first 6 months of life and born to normal-weight mothers (52). For comparison, the risk of overweight was increased by twofold among children not receiving antibiotics but born to overweight mothers (52). A later study reported a 1.2-fold increased risk of obesity at 38 months of age among children receiving antibiotics before 6 months of age (55). Correspondingly, a recently published study of ~64 000 children has reported a 1.1-fold increased risk of obesity at age 2–5 years among children receiving antibiotics four or more times during their first 2 years of life, but, interestingly, no association was observed between narrow-spectrum antibiotics and obesity (53). For decades, sub-therapeutic doses of antibiotics have been used to promote growth of farm animals in industrial farming and administration of low doses of antibiotics to mice has been shown to result in an altered configuration of the

gut microbiota and increased fat mass (56). Additionally, it has been shown that low-dose penicillin administration to mice from birth enhanced the effects of high-fat diet, that the timing of exposure to antibiotics was critical and that the metabolic effects could be transferred to germ-free mice, proposing a causal role of the gut microbiota (57). Decreased insulin sensitivity following 1 week of vancomycin treatment was observed in humans following changes in gut microbiota and bile acids (34), whereas a retrospective study has reported increased adiposity following antibiotic treatment with vancomycin for infectious endocarditis (58). In contrast to these observations, exposure to antibiotics in early life among children of overweight mothers has been associated with a decreased risk of overweight in childhood (52) and studies of *ob/ob*, high-fat diet-fed and insulin-resistant mice have demonstrated improved glucose tolerance following antibiotic treatment (22, 59). Collectively, these studies may suggest that exposure to antibiotics early in life on one hand has the potential to disturb a healthy gut microbiota but, on the other hand, also has the potential to modify a disturbed microbiota towards a healthier state. Moreover, it has been speculated that there may be an exposure window during infancy when the gut is particularly susceptible to perturbation of its microbial ecology following antibiotic therapy (60).

Importantly, the composition and function of the gut microbiota are not constant during human life. During and after birth, the new-born baby is colonised by microbes from its mother and from the environment. While babies born vaginally are primarily colonised by bacteria originating from the mother's vagina and faeces, the gut microbiota of babies born by caesarean section is dominated by bacteria from the hands that touch the baby (61). Gut communities are narrow and – probably due to life events such as breastfeeding vs formula milk feeding, time of introduction of new food items, illness, travels and antibiotic treatment – highly changeable during early childhood. In adulthood, the healthy gut microbiota is characterised by stability and richness, which remains until immune function-related changes in the gut microbiota occur at advanced age (62, 63, 64). Despite this evidence, more temporal studies of the relationship between age and gut microbiota are warranted to fully understand the age-related dynamics of the gut microbiota.

Host genotype, diet, antibiotics and age may act as true confounders influencing separately the gut microbiota and the risk of type 2 diabetes or these factors may exert part of their effect on type 2 diabetes risk through effects on the gut microbiota. In any case, present evidence underlines

the critical importance of accounting for at least age, diet and medications when designing and evaluating studies of the link between the gut microbiota and type 2 diabetes, and most probably future research will disclose other regulatory factors, which will also have to be accounted for.

### The gut microbiota: hope or hype?

Over the past few years, we have seen a wealth of studies linking the gut microbiota to a variety of seemingly very discrete diseases including inflammatory bowel disease and colon cancer, obesity and type 2 diabetes as well as asthma and multiple sclerosis. Although enthusiasm and hopes are high that the gut microbiota truly represents a gold mine for new diagnostics, prognostics and therapeutics, we must ally ourselves with a sensible portion of scepticism and not get carried away. The vast majority of the studies in humans represent simple associations from which we cannot make any causal inferences and although many transplantation studies in germ-free mice support a causal role of the gut microbiota in metabolic disease, germ-free mice with an immature immune function are far from human biology and it is therefore not straightforward to translate results from studies of genetically homogenous mice living in a well-controlled laboratory environment to genetically heterogeneous humans living their daily life in a diverse environment. To establish causality, we need well-designed and statistically well-powered prospective studies where an altered microbiome is documented before the onset of disease, intervention studies and eventually proper randomised clinical trials. Moreover, studies that disentangle the interplay between the gut microbiota and the potential modifying factors such as age, sex, specific dietary components, physical activity, smoking, alcohol, medication and various infectious diseases are crucial for a scientifically rewarding interpretation of the results from gut microbiota studies.

The majority of studies published until now are based on 16S rRNA gene sequencing, but results from metagenomic studies advocate that, in addition to the composition of the gut microbiota, we need more emphasis on the microbial community functions. However, metagenomic studies generate an impressive amount of data calling for development of widely applicable bioinformatics tools to reduce and comprehend these data. In addition, the research field of gut microbiota has been highly technology driven and evolved so fast that standardised and consensus-based approaches for sample processing including preservation and storage and extraction of microbial DNA

have not been agreed upon in the pertinent international research community. Sample processing, the method used for DNA extraction and the primers used for 16S rRNA gene sequencing may all affect whether the DNA extracted and amplified from the samples accurately reflects the microbial community in the samples (65) and hopefully an international consensus will soon be reached and generally applied to facilitate comparisons across studies (66). In the near future, the focus will probably also turn towards microbial transcriptomics, proteomics and metabolomics and will include studies of other members of the gut microbial community such as bacteriophages and fungi in addition to bacteria.

Interestingly, the human microbiome encompasses thousands of genes known to code for small molecules that could act as drugs and recently a new antibiotic produced by vaginal bacteria was discovered (67). Drug-like molecules and bioactive compounds such as bacterial metabolites, neurotransmitters, immunological factors and non-coding RNAs may represent a huge reservoir of new therapeutics. Moreover, positive outcomes on metabolic health of human stool transplantation are an indication of the power of a healthy gut microbial community (68), but a refinement of the current approach, where faeces is transplanted from one person to another is warranted. Efforts are concentrated on defining the 'minimal health sustainable microbiota', i.e. the strains necessary to safely confer the desired health-promoting effect and which can be anaerobically cultured and administered safely without the risk of disease transmission from one person to another (69). One may envision that, in the future, the effect of the 'minimal microbiota' administered as slow-release encapsulated microbial cultures may be tested in randomised clinical trials together with a healthy diet also rich in natural prebiotics.

Although this research field is surrounded by a great deal of hype and media spin and although a considerable portion of scepticism definitely is appropriate when evaluating all the fascinating studies being published, the collective evidence as of today suggests that the gut microbiota may in fact act as an important mediator of a number of environmental factors triggering common diseases including type 2 diabetes. Future studies will probably unravel the underlying mechanisms and clarify what is cause, what is effect and what are the confounders?

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.



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