

Gut microbiota, obesity and diabetes

Elaine Patterson,^{1,2} Paul M Ryan,^{2,3} John F Cryan,^{1,4} Timothy G Dinan,^{1,5}
R Paul Ross,^{1,6} Gerald F Fitzgerald,^{1,3} Catherine Stanton^{1,2}

¹APC Microbiome Institute, University College Cork, Co. Cork, Ireland

²Food Biosciences Department, Teagasc Food Research Centre, Fermoy, Co. Cork, Ireland

³School of Microbiology, University College Cork, Co. Cork, Ireland

⁴Department of Anatomy and Neuroscience, University College Cork, Co. Cork, Ireland

⁵Department of Psychiatry and Neurobehavioural Science, University College Cork, Co. Cork, Ireland

⁶College of Science, Engineering and Food Science, University College Cork, Co. Cork, Ireland

Correspondence to

Professor Catherine Stanton, Food Biosciences Department, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland; catherine.stanton@teagasc.ie

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ABSTRACT

The central role of the intestinal microbiota in the progression and, equally, prevention of metabolic dysfunction is becoming abundantly apparent. The symbiotic relationship between intestinal microbiota and host ensures appropriate development of the metabolic system in humans. However, disturbances in composition and, in turn, functionality of the intestinal microbiota can disrupt gut barrier function, a trip switch for metabolic endotoxemia. This low-grade chronic inflammation, brought about by the influx of inflammatory bacterial fragments into circulation through a malfunctioning gut barrier, has considerable knock-on effects for host adiposity and insulin resistance. Conversely, recent evidence suggests that there are certain bacterial species that may interact with host metabolism through metabolite-mediated stimulation of enteric hormones and other systems outside of the gastrointestinal tract, such as the endocannabinoid system. When the abundance of these keystone species begins to decline, we see a collapse of the symbiosis, reflected in a deterioration of host metabolic health. This review will investigate the intricate axis between the microbiota and host metabolism, while also addressing the promising and novel field of probiotics as metabolic therapies.

INTRODUCTION

The Barker hypothesis, in which long-term metabolic outcomes are pre-programmed in the womb, has held centre stage for decades and may still remain at the heart of metabolic syndrome pathogenesis.^{1,2} However, the gut microbiota, also referred to as the hidden organ, or the organ within an organ, harbours tens of trillions of microorganisms residing in the human intestine, which are arguably as important to metabolic health as the very organs that support them. Over the last few decades, a myriad of original research publications and comprehensive review articles make it impossible to ignore the contribution of the gut microbiota towards health and disease. It is believed that the 1–2 kg of microorganisms in the human gut contain >150-fold more genes than the human genome itself, a concept that attracts researchers from gastroenterology, physiology and microbiology and one that demolishes the notion that the gut microbiota will ever again be described as a ‘forgotten organ’.

Although a very recent study has supported the possibility that the infant is first seeded with bacteria in utero from the maternal placental microbiome,³ the neonatal intestinal tract is rapidly colonised by bacteria from the mother and surrounding environment following birth. Of course, the delivery method—natural versus caesarean

section—is a strong determinant of the first real microbial colonisers of the neonatal intestinal tract.⁴ Numerous publications have described clear differences in the gut microbiota composition between babies born via caesarean section versus natural birth^{5,6} and for breast-fed versus formula-fed babies.^{7–10} The gut microbiota continues to develop throughout childhood and adolescence and becomes more stable as it is generally assumed that the gut microbiota after around 3 years of age closely resembles that of an adult.^{11,12}

The gastrointestinal (GI) tract of a healthy adult hosts around 10^2 microbial cells within the highly acidic environment of the stomach, into the duodenum and jejunum. The distal ileum contains around 10^7 – 10^8 microbial cells with the largest proportion of microbes finally residing in the colon, containing around 10^{11} – 10^{12} microbial cells. Difficulties arise in culturing the microbiota from this highly anaerobic environment, with only about 10–50% successfully culturable in the laboratory.¹³ It is only over the last few decades with the emergence of culture-independent sequencing technologies that we have gained such a vast insight into the compositional changes that exist between individuals in both healthy and diseased states. However, while compositional studies generate a large volume of data, they fail to provide direct information regarding the microbial viability or the functional potential of the populations present and so the knowledge is somewhat limited in these aspects.¹⁴ As such, much of our understanding of the role of the gut microbiota and individual microbes comes from the use of germfree animals, that is, those born and reared without exposure to microorganisms and microbial supplementation studies. Germfree animals have highlighted the importance of a gut microbiota in influencing many aspects of host biology, including metabolism,^{15,16} social development¹⁷ and immunity.¹⁸ For example, germfree animals show defects in both the development of the immune system and in immune responses. They show extensive defects in the development of gut-associated lymphoid tissue and cell-turnover rates of intestinal epithelial cells and have fewer and smaller Peyer’s patches and mesenteric lymph nodes compared with animals housed under specific pathogen-free (SPF) conditions.^{19–22} It is now fully appreciated that host–microbe interactions can together co-metabolise dietary components to produce a large array of signalling molecules that develop ‘intelligent communication systems’ in the body with beneficial impacts on health. ‘Pharmabiotics’, that is, microbially produced bioactive metabolites, such as short-chain fatty acids, conjugated fatty acids,



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exopolysaccharides and neuroactive metabolites such as γ -aminobutyric acid (GABA) and serotonin, confer health benefits on the host.^{23–25} Thus, host–microbe interactions are crucial for optimum health.

While culture-independent analyses have broadened our understanding of gut microbiota composition and germfree studies have helped determine the role of gut microbes in health, it is now apparent that an individual's microbial signature is not only unique but could be a predetermining factor for disease risk. Recent literature has linked gut microbiota composition with almost every disease known to man, from GI diseases,^{26–29} to obesity,^{30–33} diabetes,^{34–38} cancer,^{39–42} and even neurological and neurodegenerative disorders such as depression,^{43–44} autism,^{45–48} anxiety^{49–50} and Parkinson's disease.⁵¹ The demands for a clear link between the gut microbiota and disease have never been so high, and so advances in the development of methods used to analyse gene expression (metatranscriptomics), protein products (metaproteomics) and metabolic profiles (metabolomics) are proving crucial.¹⁴

This review will summarise the most relevant literature to date linking the gut microbiota with diet, obesity and diabetes and highlights some specific gut microbial compositional alterations that have been described as either potentially causal or protective towards metabolic disease.

DIET AND OBESITY

Diet shapes the gut microbiota

Before addressing the involvement of the gut microbiota towards shaping obesity and associated metabolic disorders, it is appropriate to first briefly discuss some recent literature that describes diet as a dominant contributing factor to gut microbial composition. Indeed, dietary factors have a profound impact on altering the gut microbiota of animals^{52–58} and humans.^{51–59–61} This implies that health beneficial alterations to gut microbiota composition through dietary-related changes have rapidly become attractive mechanisms through which we may prevent or alleviate diseases appearing to arise from an altered microbial composition. Dietary patterns are associated with distinct combinations of bacteria in the intestine, also called enterotypes.⁶² Considering that the role of the gut microbiota is to ferment dietary substrates, complex diets can provide a range of growth-promoting and growth-inhibiting factors for specific phylotypes.⁶³ The difficulty arises in deciphering which precise dietary constituents specifically promote the growth of health beneficial gut microbiota that maximise the production of probiotics.²³ Thus, the identification of dietary patterns or specific foods that increase bacterial diversity and promote the growth of beneficial bacteria that produce high levels of bioactive metabolites, is the ultimate research goal.

Western-style diets or various combinations of high fat (HF) diets, such as HF-high-sucrose and HF-low-plant-polysaccharide have profound effects on gut microbiota composition of animals and humans, often with conflicting results. Such HF-dietary-related gut microbial alterations often correlate with deleterious metabolic health effects.^{38–60–64} For example, one recent study in overweight and obese human subjects demonstrated the effects of three different dietary regimens on gut microbiota composition and health outcomes.⁶⁵ They found that subjects consuming what was considered to be the least healthiest dietary regimen, characterised by the high consumption of confectionary and sugary drinks and the lowest consumption of fruits, yoghurt and water, had a significantly greater inflammatory profile compared with those consuming the healthiest dietary regimen, characterised by the lowest

consumption of confectionary and sugary drinks and the highest consumption of fruits, yoghurts and soups.⁶⁵ Interestingly, although the data did not highlight any significant changes to the seven gut microbial groups studied between the groups, the healthiest dietary regimen was associated with the highest microbial gene cluster or the highest microbial diversity/richness.⁶⁵ This is indeed a significant finding as the importance of microbial diversity has previously been identified as a significant factor influenced by diet and exercise⁶⁶ and which is also decreased during overweight and obese disease states.⁶⁷ Fat type is also a strong determinant of inflammation and gut microbiota composition. Diets rich in saturated fat (lard) are associated with increased white adipose tissue (WAT) inflammation and metabolic disease, while diets rich in polyunsaturated fatty acids (fish oil) can counteract inflammation to promote a lean and metabolically healthy phenotype.^{68–69} In fact, mice fed fish oil have increased levels of taxa from the genera *Lactobacillus* and *Akkermansia* (discussed in more detail later) while mice fed lard have increased levels of taxa related to *Bilophila*.⁶⁹ *Bilophila wadsworthia* has previously been shown to exacerbate colitis in genetically susceptible models.⁷⁰ While previous studies have shown that microbial factors can directly contribute to WAT inflammation through Toll-like receptor (TLR) signalling,^{71–72} dietary and host factors are also strong TLR ligands and so whether gut microbial factors initiate TLR signalling with an effect on health is uncertain. Caesar *et al* proved that lard-induced WAT inflammation is mediated through gut microbial activation of TLR4. *Trif*^{−/−} and *Myd88*^{−/−} (adaptor proteins necessary for interpreting TLR signalling) mice were protected against lard-induced WAT inflammation and impaired insulin sensitivity. Furthermore, the authors discovered that serum lipopolysaccharide (LPS) levels were higher in mice fed lard compared with those fed fish oil, indicating that microbial factors are present in the periphery that affect WAT inflammation. The impact of gut microbial factors on lard-induced WAT inflammation was further elucidated by comparing the effects of lard and fish oil in conventionally raised and germfree mice. These results demonstrated an adiposity-independent link between the gut microbiota and WAT inflammation, which led the authors to conclude that microbially derived products act as mediators of inflammation through TLR signalling. This highlights the gut microbiota as independent factors aggravating inflammation following saturated fat feeding.⁶⁹

The data of Clarke *et al*⁶⁶ demonstrate the importance of dietary variation and protein consumption, coupled with exercise, towards shaping gut microbial composition. In this study, protein consumption positively correlated with high microbial diversity (22 distinct phyla) and athletes with a low body mass index had significantly higher levels of *Akkermansia*, previously shown to inversely correlate with obesity in both mice and humans.^{73–74} These results highlight the importance of dietary macronutrients and exercise on positively shaping the gut microbiota composition. Fascinating discoveries have been made from the recently developed multi-taxon Insertion Sequencing (INSeq) method, which monitors the genetic factors that enable members of the gut microbial community to flourish within this niche.⁷⁵ This approach can be used to understand the mechanisms (gene-level characterisation) that determine gut microbial fitness and perturbation through diet, disease and clinical treatment. Using this approach, Wu *et al*⁷⁵ revealed a strain-specific and diet-specific fitness determinant (arbinoxylan utilisation locus) in *Bacteroides cellulosilyticus* WH2 that is critical for the organism's fitness during HF/simple-sugar feeding. What's more, supplementation of drinking water with arbinoxylan

(cereal-derived hemicellulose) in mice consuming this diet selectively increased the abundance of *B. cellulosilyticus*.⁷⁵ Thus, by increasing our understanding of how host genotypic and phenotypic factors affect gut microbial niches, multi-taxon INSeq can provide gene-level characterisation of species and strain-specific fitness determinants that enable therapeutic interventions (eg, prebiotic supplementation) to manipulate gut microbial community structure and function in health.

It is, however, often very difficult to determine the extent by which dietary factors affect gut microbiota composition, aside from genetic factors. While we appreciate that the component members of the gut microbiota can be stable for years,⁷⁶ the community structure or the relative abundances of each member is highly dynamic.⁷⁷ To this end, Carmody *et al*⁷⁸ described the role of host genetics in driving dietary-related alterations in gut microbiota composition. This study proved that diet is a predominant environmental factor over host genetics in shaping gut microbiota composition. This theory was based on previous studies whereby monozygotic twins failed to possess more similar gut microbiota profiles when compared with dizygotic twins at various ages from infancy to adulthood, suggesting that host genetic factors play a minor role in altering the human gut microbiota.^{30–79} The results showed that consumption of a HF-high-sugar diet reproducibly altered the gut microbiota in five inbred mouse strains, mice deficient for genes relevant to host–microbial interactions (*MyD88*^{-/-}, *NOD2*^{-/-}, *ob/ob* and *Rag1*^{-/-}) and in >200 outbred strains.⁷⁸ Thus, the study concluded that diet is a dominant environmental factor that alters the gut microbial community universally in the host, despite genetic variation. Furthermore, dietary-related gut microbial changes occurred within an average of 3.5 days and were reversible.⁷⁸ Indeed, the interactions between host genetics and environmental conditions have recently been described as confounding factors in the development of metabolic syndrome. Ussar *et al*⁸⁰ discovered that the phenotype of the 129SvEv/Tac (129T) mouse strain from Taconic Farms, usually susceptible to developing diet-induced obesity and enlarged livers on HF-diet but with normal insulin sensitivity and mild glucose intolerance, could be altered through environmental normalisation over at least three generations to become ‘metabolic syndrome resistant’, similar to the phenotype of the genetically related 129SvEv/ImJ (129J) strain from Jackson Laboratories. Environmental normalisation of the 129T strain remodelled the microbiota, and these changes correlated with a metabolic resistant phenotype.⁸⁰ It must be noted that environmental normalisation of the C57Bl/6J and the 129J strains had no significant effect on metabolic phenotype, despite a changed gut microbiota.⁸⁰ This study is crucial in describing the importance of dietary, genetic and microbial interactions on the development of metabolic syndrome.

If diet is a dominant environmental factor driving gut microbial changes, then Bolnick *et al*⁸¹ have demonstrated that these changes are sex dependent. Laboratory fish, laboratory mice and humans have all demonstrated sex-dependent, diet–microbiota associations. Interestingly, this means that diet affects gut microbiota differently in males and females. From a clinical perspective, if the intention is to take advantage of the dominant effect dietary factors have on shaping a more healthful gut microbiota, perhaps future therapies ought to be sex specific.

The gut microbiota shape obesity

On a global scale, projection estimations have predicted that the obesity epidemic rose from 400 million obese adults in 2005 to >700 million in 2015, a trend that is set to continue towards

2030.^{82–83} Characterised by an excess accumulation of body fat and a low-grade systemic inflammatory tone, behavioural patterns including dietary trends remain the underlying cause of this imbalance of energy input versus expenditure. Convenient, easily accessible, energy-dense foods and overnutrition represent major starting points altering lipid metabolism, systemic inflammation and the gut microbiota. Although studies highlighting the impact of dietary patterns and overnutrition on gut microbiota composition are plentiful, few decipher a clear link between the microbial changes observed and the mechanisms involved. In addition, the definition of this ‘microbial signature’ of obesity is ever changing, each new study describing conflicting compositional results to the last. Nonetheless, microbial intervention or the promotion/inhibition of the growth of certain species are attractive proposals for the future prevention of this obesity epidemic.

Turnbaugh *et al*⁸⁴ performed one of the first studies that definitively linked the gut microbiota with weight gain, caused by an increase in the energy-harvesting capabilities of the ‘obese-microbiota’. The gut microbiome possesses glycoside hydrolase enzymes, not found in the human genome and that are crucially involved in hydrolysing and fermenting a wide variety of dietary polysaccharides that enhance host energy status.^{85–86} The microbially derived energy from these otherwise indigestible complex polysaccharides are in the form of short-chain fatty acids (SCFA) (acetate, butyrate and propionate, predominantly). SCFAs are crucial to host health and are the principal energy source for colonocytes as well as playing a key role in the prevention and treatment of metabolic and bowel disorders and certain types of cancer.^{87–91} Germfree mice develop less body fat than conventionally raised mice and germfree mice colonised with the gut microbiota of conventional mice, despite increasing their food intake and decreasing their metabolic rate.⁹² Furthermore, germfree mice colonised with an ‘obese-microbiota’ isolated from genetically obese *ob/ob* mice had a greater percentage increase in body fat than those colonised with a ‘lean-microbiota’, despite no significant differences in energy consumption or initial body fat/weight between the groups.⁸⁴ In this setting, the germfree mouse model has provided invaluable insights into the link between gut microbiota and body fat accumulation in the host.

At the compositional level, obesity is associated with changes in abundance ratios of two of the most dominant phyla (constituting >90% of known phylogenetic categories), namely Firmicutes and Bacteroidetes (figure 1). However, while some studies have described increases in the proportion of Firmicutes to Bacteroidetes in the obese phenotype, compared with normal weight individuals³⁰ and weight loss is said to reduce the Firmicutes to Bacteroidetes ratio in human subjects,³¹ these findings are not universal^{32–53} and so the usefulness of the Firmicutes:Bacteroidetes ratio as a compositional biomarker for obesity remains unclear.

The low-grade inflammatory state characteristic of obesity can be further exacerbated by microbiota-associated inflammatory processes (figure 1). Cani *et al*^{71–93} first described the concept of metabolic endotoxemia (ie, increased plasma LPS) in a series of experiments in mice. The first finding was that both fat feeding and a HF diet increased plasma LPS levels by two-fold and five-fold, respectively.⁷¹ It was also observed that subcutaneous infusions of LPS into mice increased insulin resistance and obesity similar to that seen after feeding the HF diet.⁷¹ This positive correlation between HF feeding, genetic obesity (*ob/ob*), type 2 diabetes (T2D) (*db/db*) and metabolic endotoxemia has since been confirmed in mice.^{94–95} Furthermore,

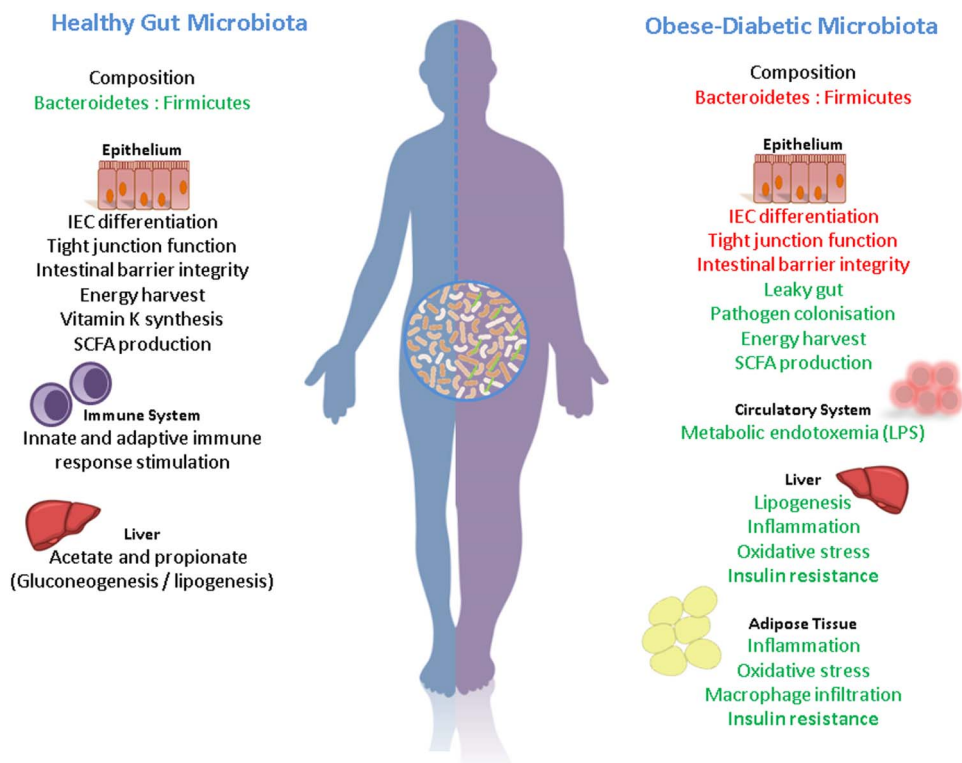


Figure 1 Compositional and functional alterations in the healthy gut microbiota versus the obese-diabetic microbiota. The metabolic processes in peripheral organs leading to increased adiposity, inflammation, oxidative stress, insulin resistance and lipogenesis are associated with the altered microbiota profile associated with the obese-diabetic phenotype. IEC, intestinal epithelial cell; LPS, lipopolysaccharide; SCFA, short chain fatty acid.

probiotic,⁹³ prebiotic⁹⁶ and antibiotic⁹⁴ therapies have improved metabolic inflammatory parameters in HF diet fed and *ob/ob* mice. In a healthy state, the translocation of LPS, through the intestinal epithelium, is restricted as it damages the integrity of the intestinal barrier. This gut barrier dysfunction observed in the obese state may represent an open door for microbes and microbial-derived LPS endotoxin to enter systemic circulation, increasing gut permeability^{94 97–99} and paracellular permeability by disrupting tight-junction proteins that link epithelial cells together (eg, claudin, occludin and zonula occludens).¹⁰⁰ Mechanistically, increased gut permeability in obese mice may have been associated with an alteration in the expression, localisation and distribution of two tight-junction proteins (occludin and zonula occludens 1) in the small intestine.^{94 95 97 99} Thus, microbial-associated metabolic endotoxemia appears to represent one of the most important links between the gut microbiota, intestinal permeability and the low-grade inflammatory state linked with obesity.

Ridaura *et al*¹⁰¹ recently described a fascinating link between diet, gut microbial composition and obesity. Their cohort of female twin pairs, discordant for obesity, provided a unique opportunity to examine the interrelations between obesity, associated metabolic disorders, diet and the gut microbiota. Transplantation of human gut microbiota from each member of discordant twin pairs for obesity permitted the donors' communities to be replicated in germfree mice, that is, transplantation of a human 'obese-microbiota' from one co-twin transmitted an increase in total body and fat mass, as well as other obesity-associated metabolic phenotypes with corresponding faecal bacterial cultures in germfree mice.¹⁰¹ Furthermore, cohousing mice transplanted with this human 'obese-microbiota' with mice transplanted with a human 'lean-microbiota'

prevented the development of obesity.¹⁰¹ Invasion of specific members of the Bacteroidetes phylum from the transplanted mice with the 'lean-microbiota' to their 'obese-microbiota' littermates correlated with the prevention of obesity development.¹⁰¹ In addition, following consumption of a low-fat, high-fibre diet, the 'obese-microbiota' failed to colonise lean mice as efficiently as it had in mice consuming a HF, low-fibre diet when cohoused with mice transplanted with human 'lean-microbiota'.¹⁰¹ The results of this study highlight both diet and environment as crucial factors involved in altering the microbiota to affect the metabolic phenotype in the host.

Compositional differences are evident in the obese relative to the lean state.⁶⁷ With obese individuals harbouring lower bacterial richness and characterised by greater adiposity, with increased insulin resistance, dyslipidemia and higher rates of systemic inflammation (increased C reactive protein), when compared with high bacterial richness individuals.⁶⁷ Importantly, the significant differences in bacterial richness and thus microbial communities/metabolic capabilities between obese and lean groups in this study were based on 46 genera. *Bacteroides*, *Parabacteroides*, *Ruminococcus*, *Campylobacter*, *Dialister*, *Porphyromonas*, *Staphylococcus* and *Anaerostipes* were more dominant in subjects of low bacterial richness with an obese phenotype, while *Faecalibacterium*, *Bifidobacterium*, *Lactobacillus*, *Butyrivibrio*, *Alistipes*, *Akkermansia*, *Coproccoccus* and *Methanobrevibacter* were more prevalent in subjects with high bacterial richness and a lean phenotype.⁶⁷ These compositional data permitted a closer look to be taken at the microbial metabolic capabilities of individuals with high and low bacterial richness. It was concluded from the vast amount of data that subjects with low bacterial richness and an obese phenotype had (1) a reduction in butyrate-producing bacteria (2), a reduction in

hydrogen and methane production (3), an increase in mucus degradation and (4) an increase in the potential to manage oxidative stress.⁶⁷ Thus, it appears that obese individuals have lower bacterial richness and harbour a microbiota that predisposes them to further inflammation.

It is clear that obesity is associated with an altered gut microbiota that worsens this debilitating disease. Diversity is key to increasing the metabolic capabilities of the gut microbiota in order to prevent worsening metabolic endotoxemia, mucus degradation and oxidative stress. In the vast gut microbial ecosystem, two residents have been the subject of extensive research over the last number of years due to their negative association with the obese phenotype. Intriguing data have supported an antiobese movement of these two species, so much so that alleviating obesity and associated metabolic disorders through increasing their abundances has become a real opportunity.

Akkermansia muciniphila

A. muciniphila is a mucin-degrading Gram-negative bacterium residing in the mucus layer of intestinal epithelial cells and that constitutes about 3–5% of the gut microbiota biomass in humans.¹⁰² Murine⁷³ and human⁷⁴ studies have demonstrated an inverse correlation between *A. muciniphila* and overweight, obesity and diabetes. Everard *et al*^{73–99} reported convincing data describing a potential protective function of *A. muciniphila* in murine models of obesity. They describe a comprehensive data set highlighting an inverse correlation between genetic and dietary models of obesity and gut concentrations of *A. muciniphila*.⁷³ Furthermore, prebiotic supplementation with oligofructose (dietary fibre) dramatically increased the abundance of *A. muciniphila* in genetically obese mice⁹⁹ and restored the decreased basal levels of *A. muciniphila* associated with obesity, improving metabolic function and endotoxemia.⁷³ Such studies suggest that *A. muciniphila* plays a role in controlling fat storage, adipose tissue inflammation and glucose metabolism.

A recent *ex vivo* model based on mouse ileal organoids has provided some novel data linking the gut microbiota and the host.¹⁰³ Lukovac *et al*¹⁰³ reported that ileal organoid expression of fasting induced adipocyte factor, G-protein coupled receptor (Gpr)-43, histone deacetylases and peroxisome proliferator-activated receptor- γ were modulated by *A. muciniphila* and the SCFA, propionate.¹⁰⁴ Thus, it is clear that *A. muciniphila* and its SCFA regulate transcription factors and genes involved in cell cycle control, lipolysis and satiety.¹⁰³ Previously, the effect of *A. muciniphila* on cell survival, more specifically in cell death receptor signalling within ileal tissue, has also been described *in vivo* in germfree mice.¹⁰⁵

Early life therapy in the non-obese diabetic (NOD) mouse model of diabetes with antibiotics increased the relative abundance of *A. muciniphila* with an improvement to the diabetic phenotype.¹⁰⁶ In addition, Dubourg *et al*¹⁰⁷ demonstrated a dramatic colonisation of the human gut microbiota by the phylum Verrucomicrobia following broad-spectrum antibiotic treatment. Interestingly, all Verrucomicrobia phylotypes were represented by *A. muciniphila*.¹⁰⁷ This shift in gut microbial composition highlights the susceptibility of some bacterial populations following antibiotic therapy and the susceptibility of other more resistant microorganisms to thrive under antibiotic pressure.¹⁰⁸ In a follow-up study, Caputo *et al*¹⁰⁹ assembled the whole genomes of *A. muciniphila* isolated directly from the stool of the patients who partook in the previous study using a metagenomic approach.

Despite the confounding evidence suggesting the anti-inflammatory effects of *A. muciniphila* in obesity,⁷³ diabetes¹⁰⁶

and colitis,¹¹⁰ others have failed to demonstrate such health benefits. Higher abundances of *A. muciniphila* have been observed in healthy controls in some human gut microbiota studies.³⁸ In addition, the Chinese MGWAS study reported that 337 of the 2176 *A. muciniphila*-related genes were more abundant in T2D patients compared with healthy controls.³⁷ It has also been suggested that *A. muciniphila* could facilitate intestinal inflammation through mucin degradation as previously shown in *Salmonella typhimurium*-infected mice.¹¹¹ Such conflicting results highlight the need for further studies to decipher the precise role of *A. muciniphila* in inflammatory-related diseases and to clarify previous discrepancies.

Faecalibacterium prausnitzii

Faecalibacterium prausnitzii is also an abundant intestinal microbe comprising approximately 4% of the mainly luminal microbiota.¹⁰⁵ Patients with inflammatory symptoms of inflammatory bowel disease (IBD), obesity and related metabolic dysfunctions harbour reduced abundances of *F. prausnitzii*.^{37–38, 74, 112–114} Some recent functional studies have described increased *F. prausnitzii* in overweight subjects who underwent a fasting programme over 1 week followed by supplementation of a probiotic formula for 6 weeks.¹¹⁴ Dietary supplementation with a type 3-resistant starch in the SATIN project altered the gut microbiota composition in overweight human subjects by enriching taxa involved in starch degradation (*Ruminococcus bromii*) and butyrate production (*Eubacterium rectale*) and increasing *F. prausnitzii*.¹¹⁵ This study describes the association between microbial fermentation of resistant starch and satiety.¹¹⁵ Furthermore, *F. prausnitzii* has shown protective effects in both acute¹¹² and chronic¹¹⁶ inflammatory responses in chemically induced models of inflammation. Considering the low-grade inflammatory tone, symptomatic of IBD and often associated with obesity, Martin *et al* recently investigated the protective effects of *F. prausnitzii* in a preclinical murine model of colitis.¹¹⁷ Importantly, decreased intestinal permeability was a feature following *F. prausnitzii* supplementation in mice chemically subjected to chronic low-grade inflammation and gut dysfunction compared with controls.¹¹⁷ Results from this study prompted the authors to describe *F. prausnitzii* as a potential novel probiotic that can be successfully used in the treatment of gut dysfunction and inflammation. Although the authors propose that the protective effects of *F. prausnitzii* could at least be in part due to the enhancement of tight junction proteins in the epithelial cells, promoting barrier function, further studies are needed to determine the precise role of *F. prausnitzii* in obesity and inflammatory-related metabolic diseases.

The endocannabinoid system and obesity

The endocannabinoid (eCB) system has been proposed to be involved in the regulation of energy homeostasis, appetite¹¹⁸ and gut barrier function¹¹⁹ via the microbiota–gut–brain axis during obesity.¹²⁰ The eCB system is composed mainly of the bioactive lipids anandamide (AEA; an *N*-arachidonylethanolamine), 2-arachidonoylglycerol (2-AG) (synthesised locally in the GI tract), the proteins that regulate their production/degradation and the cannabinoid receptors CB₁ and CB₂, through which they signal. While both AEA and 2-AG are CB₁ and CB₂ ligands, CB₁ is expressed mainly in the liver, pancreas, adipose tissue and the peripheral and central nervous systems.¹²¹ CB₂ is expressed mainly in the immune cells, in addition to the brain, pancreas and adipose tissue.¹²² In short, CB₁ activation reduces gastric motility while CB₂ activation reduces an inflammatory response. Interestingly, obesity is

associated with an increase in eCB system tone¹²³ and an altered expression of CB₁. For example, CB₁ receptor knockout mice have upregulated eCB signalling in a diet-induced obese mouse model.¹²⁴ Muccioli *et al*¹²⁵ have shown in genetically obese and T2D mice that CB₁ receptor antagonists decreased gut permeability. Furthermore, by altering gut microbiota composition through prebiotic feeding, colonic CB₁ mRNA expression is reduced and antibiotic treatment also decreased the expression of the CB₁ receptor in the colon.¹²⁵ These results correlate with a reduction in colonic AEA (endogenous CB₁ ligand), an increase in fatty acid amine hydrolase (the main enzyme in degradation of AEA) and reduced plasma LPS levels.¹²⁵ This suggests that the gut microbiota indeed play a role in regulating obesity and obesity-associated metabolic endotoxemia through the eCB system.

The gut microbiota and type 1 diabetes

Unlike T2D, type 1 diabetes (T1D) is primarily caused by a genetic disposition to pancreatic β -cell autoimmunity,¹²⁶ in which inflammatory T cells are acknowledged to play a central role.¹²⁷ Among the genetic loci identified in T1D patients, it is specific human leucocyte antigen (HLA) genotypes that have the strongest identified genetic factor to date.^{128–129} However, twin and family studies have shown that only a fraction of genetically predisposed individuals will go on to develop T1D,^{130–132} and with the incidence of T1D increasing steadily by about 3–4% over the past few decades, particularly among young children in developed countries,¹³³ environmental factors seemingly play a stronger role than once anticipated towards triggering this autoimmune response. The impact of environmental factors on the increasing incidence and decreasing age of diagnosis of children with lower-risk class II HLA genes have been described.¹³⁴ Infant feeding practices, diet and viruses are all relevant environmental triggers shown to play a role in disease onset. Moreover, risk of T1D onset in childhood is higher in children delivered by caesarean section,¹³⁵ where there is also an altered gut microbiota composition.⁴

Genetically induced, that is, the NOD mouse and the bio-breeding diabetes prone (BBDP)/diabetes-resistant (BBDR) and chemically induced, that is, streptozotocin (STZ) and alloxan-induced T1D animal models all describe convincing data linking an altered microbiota with disease. Wen *et al*¹³⁶ previously described the importance of the gut microbiota and its interactions with the host innate immune system in modulating T1D onset. MyD88 is an adaptor for multiple innate immune receptors, including TLRs involved in microbial sensing. It is the MyD88 signalling pathway that is required for autoimmune diabetes development in NOD mice under SPF conditions.¹³⁶ The knockout of MyD88 in NOD mice completely protects against T1D development.¹³⁶ Furthermore, heterozygous MyD88^{-/-} NOD mice, which normally develop robust diabetes, are protected from disease development when exposed from birth to the gut microbiota of a MyD88 knockout NOD donor.¹³⁶ Thus, disease progression in the NOD mouse is partly driven by an exaggerated innate immune response to symbiotic microbiota and altering microbial composition can curtail this response and prevent disease. Exposure to bacterial antigens and infections has also been shown to decrease the risk of NOD mice developing T1D.¹³⁷

Further evidence for the role of the gut microbiota in T1D arises from the studies of BBDP and BBDR rodents. Interestingly, treating BBDP mice with antibiotics decreased their risk of developing T1D.¹³⁸ Focusing on the proportions of bacteria found in BBDP rats at disease onset, probiotic-like

microbiota such as bifidobacteria and lactobacilli were lower while *Bacteroides*, *Ruminococcus* and *Eubacterium* were higher compared with BBDR rats.¹³⁹ However, pre-T1D onset the gut microbiota of BBDP rats was significantly different to that of BBDR rats¹³⁹ and so it is difficult to conclude from this study whether microbial alterations were causal or consequential of disease since changes were observed before and after disease onset. Recently, we described distinct microbial alterations in STZ-induced T1D rats over time.¹⁴⁰ In this study, rats were injected with a single dose of the pancreatic β -cell toxin STZ and T1D was apparent following 1 week. Although this model was severe, given the lack of insulin therapy throughout the 5 weeks of disease, with blood glucose levels between 500 and 600 mg/dL in diabetic rats, it does provide a comprehensive account of the microbial alterations that occurred following disease onset and progression over time.¹⁴⁰ Most notably, T1D onset was associated with a shift in the Bacteroidetes: Firmicutes ratio, while at the genus level increased proportions of lactic acid producing, *Bifidobacterium* and *Lactobacillus* were identified at the later stages of T1D progression. Coincidentally, T1D increased caecal lactate levels and decreased caecal butyrate levels compared with healthy controls. It has been suggested that the imbalance of bacteria that produce SCFA can affect gut permeability and cause T1D. Brown *et al*³⁵ have described the importance of butyrate in intestinal permeability during T1D. Heightened gut permeability has been demonstrated as one of the phenomena that precedes the clinical onset of T1D in both animal models of autoimmune diabetes as well as in patients with T1D and pre-diabetic individuals.^{141–145} It is the fate of lactate that is crucial in determining intestinal health as conversion to butyrate results in mucin synthesis^{146–149} and tighter junctions^{150–151} while conversion to other SCFA such as acetate and propionate does not induce mucin synthesis.³⁵ In addition, butyrate contributes to colonic health through its anti-inflammatory properties^{152–154} and decreases bacterial transport across metabolically stressed epithelial cells,¹⁵³ thus preventing the development of the so-called ‘leaky gut’. Furthermore, the STZ-induced T1D rats demonstrated a reduced microbial diversity after just 1 week of disease onset that did not recover over the duration of the trial.¹⁴⁰ Previously described are the links between a reduced microbial diversity and obesity-associated metabolic diseases.⁶⁷

The altered gut microbiota associated with T1D has also been reported in numerous human studies. A 4-matched case-control study in Finland reported that the gut microbiota differed between children who were healthy and those with autoimmune disorders.¹⁵⁵ A higher level of Bacteroidetes relative to Firmicutes approximately 6 months after birth was observed in those who eventually developed T1D and suggested that this ratio of Bacteroidetes to Firmicutes increased over time in autoimmune cases, but declined in those who did not develop T1D.¹⁵⁵ The authors concluded that the ratio of Bacteroidetes to Firmicutes could be an early diagnostic marker of pending autoimmunity problems. Such case-control studies have associated a higher *Bacteroides* abundance with autoimmunity, with increased *Bacteroides ovatus* and *Bacteroides uniformis* and decreased *Bacteroides fragilis* in T1D patients.^{34–36 155} Furthermore, butyrate producers such as *Faecalibacterium* and *Roseburia* and mucin degraders *Prevotella* and *Akkermansia* were all found in greater proportions in healthy controls compared with cases and have been described as protective against T1D.^{34–36 155} A recent German study, however, found no differences in the *Bacteroides* abundance between children who developed anti-islet cell autoimmunity and healthy controls between 6 months of age and 3 years.¹⁵⁶ They did, however,

observe an increase in the abundance of the phylum Verrucomicrobia, of which *Akkermansia* is the only known gut microbial member.^{105 156} Previously, Brown *et al*³⁵ suggested that a gut microbiota abundant with butyrate producers, increases mucin production, increases tight junction assembly, and thus, increases epithelial cell integrity. Therefore, with increased mucin production, a favourable niche exists for mucin degraders such as *A. muciniphila* that could possibly be used as an indicator of gut integrity.³⁵ A very recent study has extensively examined the gut microbiota composition and diversity of 33 infants genetically predisposed to T1D.¹⁵⁷ The authors acknowledged the limitations of the study being that all children in their cohort carry T1D-predisposing HLA alleles and are restricted to the countries of Finland and Estonia; nonetheless, their results are extremely novel in linking the gut microbiota with disease.¹⁵⁷ The study reported a 25% reduction in gut microbial diversity in T1D patients compared with seroconverters (those positive for at least two of the five autoantibodies analysed; insulin autoantibodies, glutamic acid decarboxylase antibodies, islet antigen-2 antibodies, zinc transporter eight antibodies and islet cell antibodies) and non-converters. Importantly, this was associated with alterations to both phylogenetic microbial composition and metabolic pathways in T1D diagnosed children throughout the study period.¹⁵⁷ In addition, these shifts occurred prior to disease onset but after seroconversion and were specific to T1D progressors but not seen in seroconverters without disease.¹⁵⁷ Remarkably, this novel data set describes a gut microbial profile that precedes T1D development but that is not found in seroconverters without disease or non-converters. Such results highlight a unique opportunity to exploit gut microbial profiles in distinguishing between T1D progressors from non-progressors and so has major implications for the future treatment of disease, based on microbial profiles.

The gut microbiota and T2D

It is estimated that >80% of patients with T2D are overweight and increased body weight is now recognised as the greatest risk factor of T2D, aside from genetic and lifestyle factors. Numerous studies have focused on the relationship between obesity and the gut microbiota; however, with T2D now generally considered an attribute to obesity, observational studies linking glucose intolerance and insulin resistance associated with T2D with the gut microbiota are somewhat lacking. Once again, inflammatory pathways underscore the evolution of insulin resistance.¹⁵⁸ Patients with metabolic syndrome and T2D exhibit a remarkable endotoxemia.^{159 160} Recent studies have suggested that LPS may be crucially involved in T2D-associated inflammation. Serino *et al*¹⁶¹ reported an increase in gut permeability and endotoxemia with a specific gut microbial profile in mice receiving a HF diet until they became diabetic. In line with this, Amar *et al*^{162 163} described the concept of 'metabolic infection' to define the contribution of the gut microbiota in the endotoxemia-associated inflammation coupled with insulin resistance in T2D. Blood levels of certain bacterial DNA (>85% derived from Proteobacteria) were found to be enhanced in pre-diabetes.¹⁶³ Microbially derived endotoxin could, therefore, play a significant role in insulin resistance associated with T2D.

Qin *et al*³⁷ were among the first authors to complete a significant metagenome-wide association study in T2D. High-throughput sequencing on a cohort of Chinese patients with T2D demonstrated some interesting correlations between the clinical data and gut microbial composition.³⁷ While butyrate-producing bacteria (Clostridiales sp. SS3/4, *E. rectale*, *F. prausnitzii*, *Roseburia intestinalis*, among others) were

enriched in healthy control subjects, patients with T2D exhibited an altered gut microbial profile, marked by decreased butyrate-producing bacteria such as *R. intestinalis* and *F. prausnitzii*.³⁷ Interestingly, the T2D gut microbial profile was colonised by opportunistic pathogens such as *Bacteroides caccae*, various Clostridiales, *Escherichia coli* and the sulfate-reducing species *Desulfovibrio*.³⁷ The functional capacity of the T2D gut microbial profile was linked with enrichment in the membrane transport of sugars, oxidative stress responses, branched chain amino acid transport, sulfate reduction and decreased butyrate biosynthesis.³⁷ In total, >3% of the gut microbial genes differed between healthy controls and patients with T2D.³⁷

In a follow-up study, Karlsson *et al*⁷⁴ reported compositional changes linked with T2D in a Scandinavian cohort of postmenopausal women. The data presented in this cohort again revealed significant decreases in butyrate producers *R. intestinalis* and *F. prausnitzii* in T2D women compared with women with impaired glucose tolerance.⁷⁴ Both cohorts also demonstrated increases in *Lactobacillus* species associated with T2D.^{37 74} As already discussed, butyrate plays a significant role in the maintenance of intestinal epithelial cell integrity with important functions in the prevention of 'leaky gut' associated with diabetes. Therefore, the role of SCFA, particularly butyrate and butyrate-producing bacteria, is crucial for health in obesity and diabetes. As studies in T2D consistently reveal that production of SCFAs, especially butyrate, is impaired, it is reasonable to assume that such mechanisms may contribute to the low-grade inflammation observed in such disorders.¹⁶⁴

Although plentiful in compositional data, both Chinese and Scandinavian cohorts lacked data determining whether T2D-specific medication may or may not have influenced gut microbial profiles of patients or whether such medications could have 'corrected' the altered microbial ecosystem associated with T2D. Recent promising data described a role for metformin, one of the most widely prescribed T2D therapeutic agents, towards improving the gut microbial profile in T2D.¹⁶⁵ Following 6 weeks of metformin treatment in HF-diet fed mice, both the glycaemic profile and microbial profile (29 genera) was altered, when compared with HF-fed controls.¹⁶⁵ Interestingly, HF-fed mice treated with metformin showed higher abundances of the mucin-degrading bacterium *Akkermansia* that correlated with increased mucin-producing goblet cells.¹⁶⁵ Furthermore, oral administration of *A. muciniphila* to HF-diet fed mice in the absence of metformin, significantly enhanced glucose tolerance and attenuated adipose tissue inflammation.¹⁶⁵ This improvement in glucose tolerance associated with *A. muciniphila* had also previously been identified.⁷³ The results of Shin *et al* were further confirmed a few months later by Lee and Ko,¹⁶⁶ who demonstrated an increase in *A. muciniphila* following metformin treatment with a negative correlation between glycaemia and *A. muciniphila* abundance. In line with this, Zhang *et al*³⁸ reported that the abundances of *Verrucomicrobiaceae* and *A. muciniphila* were significantly reduced in patients with pre-diabetes compared with healthy controls. Thus, *A. muciniphila* may contribute to the antidiabetic effect of metformin, suggesting that pharmacological manipulation of the gut microbiota in favour of *Akkermansia* may be a potential treatment for T2D. These studies describe attractive data sets that highlight the possibility of gut microbial manipulation to alleviate T2D and describes a new mechanism for the therapeutic effect of metformin in treating T2D.

Following on from this, one recent study dissected 784 available human gut genomes from the MetaHIT project, novel Danish MetaHIT genomes, cohorts of female Swedish patients

with T2D and Chinese patients with T2D to demonstrate how metformin impacted on gut microbial profiles.¹⁶⁷ For these 784 gut metagenomes, taxonomic and functional profiles were determined. Using the available data sets and controlling for metformin, the authors identified a unified signature of gut microbiome shifts in T2D, characterised by a depletion in butyrate-producing taxa.¹⁶⁷ It was shown that gut microbial function was altered following metformin treatment such that intestinal lipid absorption and LPS-triggered local inflammation was reduced, providing a competitive advantage to *Escherichia* species, possibly triggering a positive feedback loop that contributed to the taxonomic changes.¹⁶⁷ *Akkermansia* was also found at similar abundances in non-diabetic control and metformin-treated diabetic patients.¹⁶⁷ Furthermore, functional analysis demonstrated significantly enhanced butyrate and propionate production potential in metformin-treated patients. This is of significance due to the potential of these SCFA to trigger intestinal gluconeogenesis.^{168 169} Thus, characterisation of a metformin-associated human gut microbiome highlighted novel microbial mechanisms that contribute to the beneficial effects of the drug on host metabolism and provided evidence for the importance of controlling for metformin treatment once reporting functional and compositional shifts in the human microbiome.

What's more, microbial manipulation to treat insulin resistance has previously been described, whereby microbial faecal transplant from lean donors to male insulin-resistant subjects significantly improved peripheral insulin resistance associated with an altered small intestinal microbial composition.¹⁷⁰

PROBIOTICS AS DIABETIC THERAPIES

It is now well established that there is enormous potential for human metabolic health in the manipulation of the gut microbiome.¹⁷¹ We have already discussed the inverse relationship between gut microbiota diversity and diabetic state; however, it is likely that certain keystone microbial taxa, alongside diversity, may be at the centre of this association. Aside from diet, probiotic administration currently represents the most effective and safest means of selectively altering the gut microbiome, ultimately with the intention to improve host health.^{78 172}

Probiotics for T1D

It is well established that the microbiome can have a central role in the progression of T1D.¹⁷³ However, it also appears to be true that we can alter the microbiome to delay onset or manage T1D pathogenesis. Three main targets have emerged for probiotic therapy in T1D; reduction or redirection of autoimmunity, increased β -cell proliferation and decreased β -cell apoptosis (figure 2).

The redirection of T-cell differentiation towards T_{reg} cells by probiotics, commensals and their metabolites has received some attention. A probiotic cocktail of three *Lactobacillus plantarum* strains was observed to significantly reduce inflammatory cytokines interferon (IFN) γ and tumour necrosis factor- α production, while increasing anti-inflammatory interleukin-10 production in a mouse model of autoimmunity.¹⁷⁴ The zwitterionic polysaccharide of *B. fragilis* (PSA) has also been implicated in a similar mechanism of anti-inflammatory protection for autoimmune disease-induced mice.^{175 176} One study investigating the ability of two *Lactobacillus* spp. isolated from the GI tract of T1D-resistant mice found that administration of a *Lactobacillus johnsonii* delayed or prevented the onset of the disease in a BioBreeding model.¹⁷⁷ The strain elicited this

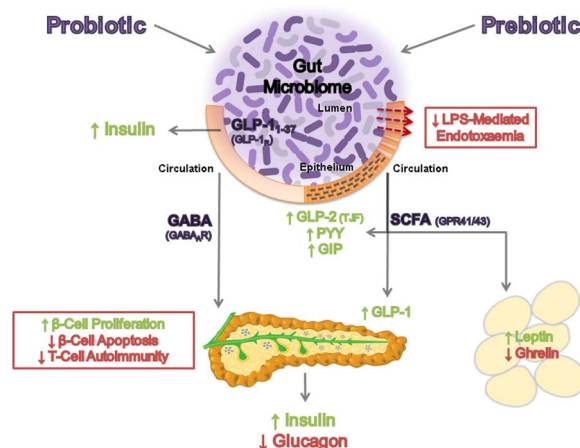


Figure 2 Putative mechanisms of action through which prebiotics and probiotic bacteria can impact on host metabolic health in type 1 and type 2 diabetes. Green and red texts indicate hormones, systems and actions that are upregulated and downregulated, respectively. LPS, lipopolysaccharide.

response by reducing host IFN γ and iNOS levels, while also increasing claudin expression.

Conversely, some members of the gut microbiota have been shown to accelerate T1D.¹⁷⁸ Segmented filamentous bacteria (SFB) are a Gram-positive, strict anaerobe taxonomic lineage within the Clostridiaceae family, which occupy the GI tract.¹⁷⁹ These SFB are found tightly associated with the intestinal wall and, as a result, interact with the host immune system causing considerable differentiation towards inflammatory T_H17 cells in the small intestinal lamina propria.¹⁸⁰ While this type of immunostimulation can be extremely important to the development of a healthy host immune system,¹⁸¹ this same action can exacerbate autoimmunity in others that are genetically susceptible to T1D.¹⁸² Just one probiotic strain has previously been described as displaying anti-SFB activity in an immunocompromised mouse model.¹⁸³ However, the mechanistic features proposed by the authors are those common to many probiotics—direct pathogen exclusion, antimicrobial activity or indirect activity through host immunostimulation^{184 185}—suggesting that SFB may be a potential target for probiotic therapy in T1D.

GABA is a metabolite produced from glutamate by the glutamate decarboxylase (GAD) pathway of many lactic acid bacteria,¹⁸⁶ as a cellular response to acidic stress.¹⁸⁷ In the host, GABA is produced by β -cells and acts as the main inhibitory neurotransmitter in the enteric and parasympathetic nervous systems, where it is involved in pain perception and stress.¹⁸⁸ However, GABA has also been proposed as a potential therapy in T1D pathogenesis.^{189 190} GABA acts on β -cell receptors, $GABA_A$ R and $GABA_B$ R, to increase insulin production and β -cell proliferation, while also reducing β -cell apoptosis.¹⁹¹ In addition, $CD4^+$ T cells have also been shown to harbour $GABA_A$ R, allowing the amino acid to interact with the immune system to effectively inhibit the inflammatory process involved in the progression of T1D.^{192 193} While these studies have investigated the use of injected or parenteral delivery of pure GABA, none have applied GABA-producing probiotics to a T1D model. The research to date investigating the bioactivity of GABA-producing probiotics has been solely focused on the gut-brain axis.¹⁹⁴ However, selected probiotics can produce physiologically relevant levels GABA in faecal fermentations¹⁸⁶ and, as such, should be considered potential T1D therapies warranting

further investigation. One such study recently conducted within our group investigated the potential of an efficient GABA-producing *Lactobacillus brevis* in modifying host metabolism (Marques *et al*, in press). Following 5 weeks of probiotic intervention, healthy rat serum insulin was increased by 70% when compared with controls, while all other metabolic parameters were not significantly altered.

An intriguing novel avenue that is currently being explored in probiotic research is the engineering of commensal bacteria that can deliver bioactive molecules capable of managing or correcting a disease state.¹⁹⁵ Due to their safety and the association that probiotics have with host intestinal mucosa, this system has been of considerable interest for vaccine delivery (for review, see Wells and Mercenier^{186 196}). Such treatments have been coined as synthetic signalling therapeutics.¹⁹⁷ In a recent study, a recombinant probiotic *Lactobacillus gasseri* ATCC 33323 capable of secreting the inactive full length form of glucagon-like peptide-1 (GLP-1)(1-37) was designed.¹⁹⁸ GLP-1 is a conserved mammalian peptide produced by neuroendocrine L-cells, which can lower postprandial glucose levels in T2D by stimulating insulin production and attenuating glucagon release.¹⁹⁹ GLP-1 also acts systemically on the pancreas, liver, heart, stomach and brain, impacting on gastric emptying, satiety and lipid absorption and metabolism.^{200 201} As such, a plethora of drugs have been developed as GLP-1R agonists or blockers (gliptins) of DPP-4, the enzyme that cleaves active GLP-1 in circulation.²⁰² Although GLP-1-based therapies have been entirely targeted towards the treatment of T2D, it has become clear that this full-length form of the peptide may have significant potential as a T1D therapy. GLP-1 was originally identified in its extended 1–37 form through cDNA cloning,²⁰³ although it was thought to have little bioactivity compared with the truncated GLP-1 (7–37 and 7–36), later discovered to be naturally produced by human β -cells.²⁰⁴ However, a recombinant GLP-1(1-37) producing *E. coli* has previously been shown to stimulate the production of 1 ng insulin per millilitre of media in Caco-2 cells.²⁰⁵ Furthermore, the peptide has also been implicated in the conversion of crypt-located small intestinal epithelial cells to insulin⁺ β -like-cells *ex vivo*.²⁰⁶ Following STZ-induction of T1D in mice, Duan *et al* demonstrated that twice daily administration of a high dose (1.6×10^{10} CFU/kg) of this recombinant GLP-1(1-37) producing probiotic for 90 days resulted in ~30% restoration of insulin-production capabilities and significantly improved glucose metabolism. It was confirmed by immunofluorescent microscopy that this activity was through differentiation of intestinal crypt epithelial cells to β -like cells. Thus, GI tract-derived probiotics may serve as drug delivery or signalling systems, which could have downstream implications for diabetes.^{207 208}

Probiotics for T2D

Much of the initial research around probiotics as T2D therapies has been through non-targeted approaches, assessing the ability of different lactobacilli and bifidobacteria to attenuate the effects of the disease state without any genuine molecular hypothesis prior to experimentation.^{209 210} More recent studies have progressed to implicating reduced inflammation, as a direct result of improved tight junction function preventing metabolic endotoxemia, as the source of the probiotic effect in diabetes therapy (figure 2).^{162 211 212} This attenuation of low-grade inflammation undoubtedly plays a central role in T2D therapy; however, the manner in which this is achieved by probiotic bacteria is complex. The most compelling theories of the T2D–microbiome interaction have come from the Delzenne and Cani

group of the University of Louvain (for review, see Cani *et al*).^{162 204 206–214} The systematic research of this group has uncovered several metabolic targets for probiotic therapies, some of which we have previously discussed already.

The first and most thoroughly characterised targets for probiotic-mediated T2D therapy are the SCFA. SCFA—primarily acetate, butyrate and propionate—are thought to interact with the host enteroendocrine system by binding to GPR41 and GPR43.^{214 215} These GPCRs in turn upregulate the expression of gut hormone precursor peptide proglucagon,²¹⁶ GLP-1,²¹⁷ GLP-2,⁹⁵ gastric inhibitory polypeptide (GIP) and peptide YY (PYY) levels,²¹⁸ as well as adipocyte-derived leptin,²¹⁹ while downregulating ghrelin.²²⁰ As mentioned previously, GLP-1-based therapies have demonstrated great efficacy in treating patients presenting T2D, as well as dyslipidemia. However, microbiota-mediated modulation of these other gut hormones also has significant implications for metabolic health, affecting gut permeability, satiety, gastric emptying and food intake.²²¹ Most recently, SCFAs have also been shown to act upon a cAMP-dependent mechanism, increasing intestinal gluconeogenesis,¹⁶⁸ which is thought to contribute to satiety.²²² While some studies have reported contrasting effects, this is likely a result of the varying actions of specific SCFA. As we have discussed previously, it appears that butyrate may be of most significance in the correction of metabolic endotoxemia disease states through proliferation of colonic epithelial cells and tight junction function.¹⁵¹ The importance of butyrate-producing bacteria in T2D development has been demonstrated by a recent human faecal transplant study.¹⁷⁰ This research implicates enteric butyrate-producing bacteria, such as *R. intestinalis*, *Faecalibacterium* spp. and *Eubacterium hallii*, as potential probiotics for the alleviation of metabolic endotoxemia-induced T2D.²²³ There is also evidence that more classical probiotics, such as the commercial probiotic cocktail VSL#3, can offer complete correction of weight gain and glucose intolerance associated with diet-induced T2D in mice.²²⁴ Moreover, a recent transcriptomics study in elderly patients demonstrated the ability of *Lactobacillus rhamnosus* GG to promote the relative expression of butyrate-producing *Roseburia* and *Eubacterium* genes within the metagenome.²²⁵ This could offer a more intriguing mechanism for the anti-inflammatory effects attributed to traditional probiotics in T2D therapy.

The second proposed target for T2D probiotic therapy is the eCB system, already discussed in relation to obesity.²²⁶ CB₁ is involved in gut permeability and modulation of the gut microbiota appears to yield similar responses to CB₁ antagonists, upregulating expression and correcting the localisation of intestinal epithelial tight junction proteins, occludin and zonula occludens1.²²⁷ Furthermore, supplementation with 2×10^8 CFU/day of live *A. muciniphila* increased ileum 2-AG and 2-palmitoylglycerol levels in mice fed a HF diet, which had the knock-on effect of reducing serum LPS content.⁷³ The manner in which *A. muciniphila* alters eCB levels remains unclear, but the correlation is apparent and this may offer a mechanism of action for the beneficial metabolic effects attributed to the strain.

Finally, eCB-like lipids, such as *N*-oleoylethanolamine, 2-oleoylglycerol (2-OG) and linoleoylethanolamine have been shown to interact with GPR119, a receptor that stimulates the host enteroendocrine system.²²⁸ Live *A. muciniphila* administration has demonstrated potential in altering 2-OG in mice fed a HF diet, contributing to tight junction function and significantly improving glucose metabolism, through stimulation of intestinal L-cell GLP-2 and GLP-1 production, respectively.⁷³ In addition,

the aforementioned increase in 2-AG production has been shown to potentiate anti-inflammatory effects from 2-OG, which could have further implications for correction of T2D.²²⁹

Additionally, GABA has also demonstrated potential in the treatment of T2D *ex vivo*²³⁰ and *in vivo*²³¹ through the same pathways targeted for T1D. The former study, in which human pancreatic islets were excised, demonstrated that the GABA signalling pathway was hindered in T2D compared with healthy controls.²³⁰ However, GABA_BR agonist CPG55845 proved capable of stimulating insulin release from islets of both T2D and healthy control islets. Interestingly, Tian *et al*²³¹ found that GABA therapy in a diet-induced obese model of T2D improved fasting glucose, glucose tolerance and insulin sensitivity through action on peripheral GABA_AR-expressing immune cells. The study found that this in turn promoted a CD4⁺Fox3⁺ T_{reg} differentiation tone and, as a result, reduced T2D-associated inflammation. The potential for GABA-producing probiotics as T2D therapies certainly warrants preclinical investigation.

LIMITATIONS AND RISKS OF MICROBIOME-MEDIATED THERAPIES

While this review has highlighted the major advances and promising outcomes of current microbiome-metabolism research, it is important to comment on several limitations and potential risks associated with the science. In terms of limitations, as is the case for all personalised medicines, what works for me may not work for you. That is to say that an individual's genetics, specific diet, drug intake and endogenous microbiota may greatly impact on the efficacy of certain microbiome-mediated therapies. Moreover, some interventions may not only be ineffective in combating an individual's disease state, they may even be an additional risk factor. Certain avant-garde bacterial therapy procedures have demonstrated great potential in combating grave diseases: for example, the use of faecal microbiota transplants (FMTs) in the treatment of *Clostridium difficile* infection. However, with such efficacy comes risk. There have been several anecdotal reports and a single case study reporting increased weight gain following FMT from overweight donors,²³² mirroring preclinical outcomes. In addition, there has even been speculation that transplantation from a mentally unfit donor could induce the same phenotype in the recipient. Although it is unlikely that single or even multistrain bacterial therapies, which have undergone strenuous preclinical and clinical investigation, would transfer such deleterious attributes. However, it certainly warrants attention, and microbiome-mediated therapies such as FMT will require significant regulation in order to reduce safety risks and ensure whole system host health.

CONCLUSION

The obesity epidemic has become a thorn in the side of a 21st-century global health crisis. Modern sequencing technologies have demonstrated that we are more microbe than man, in fact we always were, but the vast data now available to prove this are unarguable. The relationship between microbe and man is symbiotic, but all good relationships need work. And so we must nurture and *feed* ourselves healthy by indirectly *feeding* our microbes healthy too. The compelling evidence described in this review suggests that diet and environment can shape gut microbial composition and, in turn, gut microbial composition can have direct effects on human health. The ultimate goal is to permanently switch off this microbiota-influenced 'trip switch'

determining metabolic endotoxemia in the host. This may be ambitious right now, but it is clear that certain strains of bacteria (and the pharmabiotics they produce) can have very positive effects on host metabolic health. Although these effects may seem a small solution to this epidemic, the future development of probiotics as medicines to treat metabolic disease or indeed prevent their onset is a promising proposal. The data described in this review bring us ever closer to discovering an 'antiobese microbiota', one that could prevent this obesity epidemic from polluting future global health.

Main messages

- ▶ While the gut microbiota is unique and essential for development, its composition can be influenced by many factors including health status and environment.
- ▶ 'Pharmabiotics' are bioactive microbial metabolites that confer a health benefit to the host.
- ▶ The gut microbiota are a modifiable target with the potential to progress or prevent the development of the metabolic syndrome.

Current research questions

- ▶ Can we eat ourselves healthy by feeding specific gut microbes?
- ▶ Is it possible to transplant a healthful 'lean-microbiota' to an obese host with the effect of alleviating symptoms of the metabolic syndrome?
- ▶ Can specific strains of bacteria be used as future therapies against diabetes or could potential disease-provoking strains be knocked out of the gut microbial ecosystem?

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Self assessment questions

Please answer true or false to the below.

1. Metabolic endotoxemia is characterised by excess lipopolysaccharide in the blood.
2. The endocannabinoid system is comprised of an array of signalling peptides.
3. Akkermansia can improve host insulin resistance in mice.
4. The pharmabiotic γ -aminobutyric acid functions as an excitatory neurotransmitter in the central nervous system.
5. Prebiotics are live microorganisms that may offer a therapeutic means of managing metabolic health.

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Answers

1. True
2. False
3. True
4. False
5. False