Corrected: Author Correction

Probiotics and prebiotics in intestinal health and disease: from biology to the clinic

Mary Ellen Sanders[1], Daniel J. Merenstein[2], Gregor Reid[3], Glenn R. Gibson[4] and Robert A. Rastall[4]*

Abstract | Probiotics and prebiotics are microbiota-management tools for improving host health. They target gastrointestinal effects via the gut, although direct application to other sites such as the oral cavity, vaginal tract and skin is being explored. Here, we describe gut-derived effects in humans. In the past decade, research on the gut microbiome has rapidly accumulated and has been accompanied by increased interest in probiotics and prebiotics as a means to modulate the gut microbiota. Given the importance of these approaches for public health, it is timely to reiterate factual and supporting information on their clinical application and use. In this Review, we discuss scientific evidence on probiotics and prebiotics, including mechanistic insights into health effects. Strains of *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* have a long history of safe and effective use as probiotics, but *Roseburia* spp., *Akkermansia* spp., *Propionibacterium* spp. and *Faecalibacterium* spp. show promise for the future. For prebiotics, glucans and fructans are well proven, and evidence is building on the prebiotic effects of other substances (for example, oligomers of mannose, glucose, xylose, pectin, starches, human milk and polyphenols).

The request in 2000 by the Argentinian government that the Food and Agriculture Organization of the United Nations form an expert panel to evaluate the health and nutritional properties of probiotics in food precipitated the re-emergence of a concept long part of human history. International recognition of the concept of probiotics, and coalescence around a definition of probiotic offered by this expert consultation[1], established an important consensus foundation. The definition of probiotic decided by the consultation retained the essence of historical definitions offered over previous decades. It was intentionally broad, to encompass a wide variety of microorganisms, hosts, benefits, target sites and product types. It has stood the test of time and was reaffirmed, but grammatically corrected, in 2014 to the consensus definition of probiotics, which is: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[2].

There are many studies that have investigated how microorganisms are integrated into life processes and defined ways that beneficial microorganisms — both commensal and externally applied — affect physiological homeostasis and host function[3]. On the horizon is the promise of newly constructed recombinant strains and promising novel microbial species, which await testing in vivo. However, as these advances develop, we should recognize actionable evidence that is currently available. As is discussed in this Review, convincing evidence exists for some established probiotics, which should be incorporated into health management. This incorporation includes complementary use with pharmaceutical agents, foods and lifestyle. Education of consumers, practitioners and regulators will facilitate appropriate use and point out needs for further research, which will hopefully include exploration of how to reach the individuals at greatest need with affordable and reliable probiotic products[4].

Prebiotics, first defined in 1995 (REF.[5]), have been used to manipulate microorganisms in the host to improve measurable health outcomes. An update to the definition of prebiotics published in 2017 as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" was made necessary by the need to clarify what did and did not constitute a prebiotic substance in the face of scientific advances[6]. The desire to optimize, for improved health, the microbial world associated with humans has led to the development of compounds targeting an ever-expanding group of microorganisms and benefits that are derived through them. No longer are prebiotics seen simply as boosters of the growth of bifidobacteria and lactobacilli but are now recognized for their effects on system-wide metabolic and physiological

[1]*International Scientific Association for Probiotics and Prebiotics, Centennial, CO, USA.*

[2]*Department of Family Medicine, Georgetown University Medical Center, Washington, DC, USA.*

[3]*Lawson Research Institute, and Western University, London, Ontario, Canada.*

[4]*Department of Food and Nutritional Sciences, University of Reading, Reading, UK.*

**e-mail: g.r.gibson@reading.ac.uk*

https://doi.org/10.1038/s41575-019-0173-3

Key points

- The human gut microbiota is integral to health and is associated with a variety of diseases.
- Therapeutic and prophylactic effects of some probiotics and prebiotics for a variety of gut-related disorders might be, at least in part, mediated through modification of the microbiota and/or its function.
- Probiotic microorganisms act via a variety of means, including modulation of immune function, production of organic acids and antimicrobial compounds, interaction with resident microbiota, interfacing with the host, improving gut barrier integrity and enzyme formation.
- Prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit; prebiotic effects include defence against pathogens, immune modulation, mineral absorption, bowel function, metabolic effects and satiety.
- Use of some probiotics and prebiotics is justified by robust assessments of efficacy, but not all products have been validated; the goal is evidence-based use by healthcare professionals.

readouts[6]. Although the intestine remains the gateway to most of these effects, it is not the exclusive gateway. The extent to which prebiotics can affect microbial communities of the urogenital tract, oral–nasal areas and skin is now the subject of intense exploration[7].

This Review describes the current understanding of probiotic and prebiotic mechanisms of action, provides important examples of clinical studies on probiotic and prebiotic applications, and discusses current knowledge on mechanisms at the heart of these effects.

Human gut microbiome

The human gut is predominantly inoculated at birth. Microbial diversity develops as feeding and dietary patterns mature. By the age of 3–5 years the microbiota resembles that in the adult[8]. Because of variations in pH, substrate concentration, Eh (redox potential, activity of electrons) and transit time, microbial numbers vary between different anatomical regions of the gut[9]. The stomach harbours fewer microorganisms than the small and large intestines[10]. Studies using metagenomic approaches have highlighted the complex inter-relationship between human resident intestinal microbiota and mammalian metabolism[11]. Through the process of fermentation, anaerobic gut bacteria metabolize substrates to form end products such as organic acids and gases[12]. The main precursors for fermentation are dietary carbohydrates, proteins and lipids, as well as indigenous secretions such as mucin. This anaerobic metabolism contributes positively towards host daily energy requirements and homeostasis in the gut[13]. Ideally, the human host lives in harmony with its complex gut microbiota in a state that promotes physiological resilience[14]. However, dysbiosis can result from challenges such as medications, infections, ageing, lifestyle, surgery and poor nutrition[14,15].

In humans, a range of acute and chronic disorders can be a consequence of perturbation of gut microbial communities[16–18]. On a chronic basis, inflammatory bowel disease (IBD), obesity and irritable bowel syndrome (IBS) have all been linked to intestinal bacteria and their activity[10]. This aspect opens up the possibility of influencing the microbiota to reduce disease risk, fortify homeostasis and, in some cases, improve therapeutic status. Diet is a principal driver of gut fermentation and therefore can greatly influence functionality of the indigenous microbiota[19]. Prebiotics are a popular dietary approach to the modification of the gut microbiota to improve host health[6], as they are affordable, effective, safe and accessible.

Probiotics

As the concept of probiotics evolved over the past decades, the assumption was that their effects would be mediated through direct interaction with commensal microbiota. According to some early definitions, probiotics function "by contributing to [the host's] intestinal microbial balance"[20] or "by improving the properties of the indigenous microflora"[21]. However, in the current consensus definition of probiotics, the effects of probiotics are not considered to be only microbiota-mediated, and, indeed, other types of mechanism are known. This idea that probiotics function in ways that might act beyond affecting the colonizing microbiota opens the door to a wider range of probiotic possibilities, encouraging innovation in the field.

Much of our knowledge on probiotic mechanisms is based on research using in vitro, animal, cell culture or ex vivo human models. FIGURE 1 shows known mechanisms distributed among various probiotic strains. Not all mechanisms have been confirmed in humans nor do they exist in every probiotic strain. Although multiple mechanisms are probably co-expressed in a single probiotic, the importance of any given mechanism will depend on many factors. For example, in an inflamed intestine, the ability to downregulate inflammatory mediators and increase epithelial barrier function might be most important[22,23], whereas the ability to increase short-chain fatty acids (SCFAs) and hydration in the colon could be more important to normalizing intestinal motility[24].

Research elucidating mechanisms of probiotics has often relied on in vitro or animal studies. Probiotics are not unique in this regard. Animal studies have not always translated to humans[25]; notable examples are probiotics for Crohn's disease and mental health function[26,27]. Furthermore, there are inherent differences among probiotic strains; for example, one probiotic (in this case in conjunction with a prebiotic) was found to markedly reduce sepsis in infants[28], whereas a different formulation failed to prevent necrotizing enterocolitis in very preterm infants[29].

The historic concept of 'colonization resistance'[30], the situation whereby native gut microbiota occupy host tissues to exclude infection by potential pathogens (resident or invading), is another mechanism attributed to probiotics[31]. Expression of colonization resistance is probably a sum outcome of the functioning of many of these different mechanisms in concert. Indeed, many host factors could affect the ultimate expression of health effects imparted by a probiotic, including properties of baseline microbiota. Although few data exist, one study showed that probiotic persistence in the gut is linked to the properties of the baseline microbiota. Persistence of *Bifidobacterium longum* subsp. *longum* AH1206 in the human gut was predicted by low abundance in the host

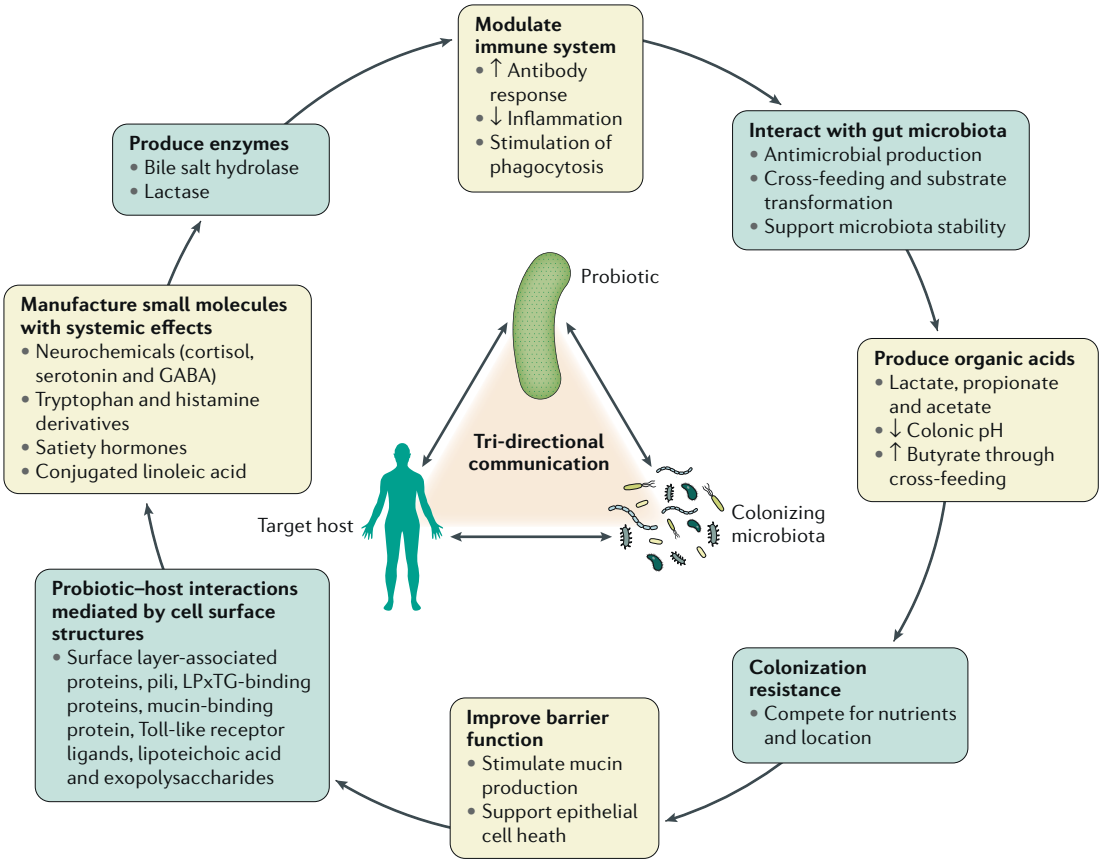

Fig. 1 | **Probiotic mechanisms of action.** Diverse mechanisms are likely to drive probiotic benefits to host health. In some cases, such as production of antimicrobial products and cross-feeding other resident microorganisms, these mechanisms are driven directly by interactions with the resident microbiota. In other cases, such as direct interaction with immune cells, their effects might be direct via interaction with host cells. Overall, clinical benefits delivered by probiotics could result from the combined action of several mechanisms. GABA, gamma-aminobutyric acid.

of *B. longum* and low levels of microbial carbohydrate utilization genes[32]. No clinical end points were tracked in this study, but the property of long-term persistence could contribute to physiological benefits. However, the results of many different clinical trials that did not include stratification of study participants by baseline microbiota suggest that probiotic function is not necessarily predicated on a specific microbiota baseline[33,34]. There may well be compositional patterns of microbiota that do not respond well to incoming probiotic strains, just as there are for certain drugs[35], but such profiles have not yet been fully defined.

Modulation of cell-mediated and humoral immune functions. Some probiotics have been shown to increase phagocytosis or natural killer cell activity and interact directly with dendritic cells (reviewed in REF.[36]). Some also demonstrate the ability to upregulate antibody secretion translating into improved defences against pathogens and augmenting vaccine responses[37–39]. Probiotic strains can increase levels of anti-inflammatory cytokines such as TNF with implications for abating colon cancer and colitis[10,36]. As discussed below, cell-surface architecture, such as fimbriae, capsule and surface structures expressed by certain probiotics, is a mechanistic driver for several of these activities.

Production of organic acids. Probiotic species belonging to the *Lactobacillus* and *Bifidobacterium* genera produce lactic and acetic acids as primary end products of carbohydrate metabolism. These organic acids when produced in situ can lower luminal pH and discourage growth of pathogens, as shown in various model systems[40–42]. *Lactobacillus* and *Bifidobacterium* do not produce butyrate but through cross-feeding other commensal microbiota (for example, *Faecalibacterium*), levels of butyrate and other SCFAs in the gut can increase, potentially influencing many aspects of physiology, including the cardiometabolic phenotype[43]. This phenotype can be derived from increased production of butyrate, correlating with improved insulin response, or abnormalities in propionate linked to type 2 diabetes mellitus[44]. Based upon analyses of weight, lifestyle, metabolic measurements and SCFA levels, the risk of an individual developing cardiometabolic diseases can be calculated[45].

Interaction with gut microbiota. Probiotic strains can interact with the gut microbiota through competition for nutrients, antagonism, cross-feeding and support of microbiota stability[46]. Many probiotic strains are antagonistic toward other microorganisms, in part due to saccharolytic metabolism, which produces organic

acids, but also by production of bacteriocins[47]. These antimicrobial compounds can be active against pathogens at many sites including the human urinary tract and the gut of humans and animals[48,49]. Bifidobacteria produce acetate and can cross-feed other members of the gut microbiota (reviewed in REF.[50]). Strains *B. longum* AH1206 and *B. bifidum* ATCC15696 have been shown to persist in the infant gut[32,51], although in the latter case the concomitant decrease in pathogen abundance was not tested for a link to bacteriocin production. The ability of certain probiotic strains to improve eradication of *Helicobacter pylori* might involve some inhibition of the pathogen, but there is stronger evidence that probiotics in this context reduce the adverse effects of antibiotics used in treatment[52].

Probiotic–host interactions. Interactions of probiotic strains with host tissues are mediated by cell surface macromolecules, including proteins (surface layer associated proteins, mucin-binding proteins, pili, and LPxTG-binding proteins) and non-protein components (lipoteichoic acid, peptidoglycan, exopolysaccharides)[53]. These structures have been shown to affect binding to intestinal and vaginal cells, mucin, and immune or dendritic cells, resulting in increased transit times and improved barrier integrity (reviewed in REF.[53]). An example of the different surface structures can be seen in the genome comparison of *Lactobacillus rhamnosus* GG that uses pili to interact with the intestine and *L. rhamnosus* GR-1 with a unique cluster of exopolysaccharides that aid vaginal activity[54].

Improvement in barrier function. Primarily through studies in cell lines, several probiotic *Lactobacillus* and *Bifidobacterium* strains have been shown to increase expression of tight junction proteins (reviewed in REF.[55]). A study using human intestinal epithelial enteroids and colonoids showed that pretreatment with *L. rhamnosus* GG counters damage to tight junction zonula occludens 1 and occludin caused by IFNγ[56]. Another way in which probiotic strains might improve barrier function is through upregulating expression of mucus-secretion genes, thereby reducing pathogen binding to epithelial cells[57,58]. Downregulating inflammation is also regarded as a factor that improves barrier function[53]. Of note, although some probiotic strains have the capacity to improve barrier function, this process does not always occur in every cohort for reasons not yet fully understood[59].

Manufacture of small molecules with local and non-local effects. Small molecules produced by certain probiotic strains with different effects on the host and its microbiota have been described[58]. Perhaps one of the more intriguing findings is the production of neurochemicals such as oxytocin, gamma-aminobutyric acid, serotonin, tryptamine, noradrenaline, dopamine and acetylcholine (reviewed in REFS[60–62]) that are known to affect brain function. In a rat model of stress, *L. helveticus* NS8 feeding resulted in lower plasma corticosterone and adrenocorticotropic hormone levels and restored hippocampal serotonin and noradrenaline levels[63].

Production of enzymes. Microbial enzymes such as β-galactosidase[64] and bile salt hydrolase[65], which are produced and delivered by some probiotic strains, improve lactose digestion and blood lipid profiles in humans, respectively. In the case of *Streptococcus thermophilus* in yogurt, which facilitates lactose digestion, its predisposition to be permeabilized by bile when entering the small intestine promotes the delivery of microbial β-galactosidase to the small intestine to break down lactose into digestible glucose and galactose[64]. This results in clinical benefit to individuals who are lactose intolerant. Indeed, the European Food Safety Authority considered evidence of this effect sufficient to authorize a health claim that *S. thermophilus* and *L. bulgaricus* as components of yogurt can alleviate symptoms of lactose maldigestion[66].

Admittedly, cause-and-effect evidence of mechanisms in human hosts remains to be gathered, but technological advances in genome sequencing and microbiome analyses, and surgical advances that enable real-time sampling in vivo, should help acquire elucidating data over the next few years.

Prebiotics

If we are to understand how prebiotics work, and more importantly exploit them to manipulate the microbiota to propagate health, then we need to keep in mind that microorganisms live in complex functional ecosystems. Within these ecosystems, bacteria have a multitude of roles, including the conversion of incoming dietary carbohydrates, proteins and some fats into metabolites that can have either positive or negative effects upon host health[67–70]. Current prebiotics are predominantly carbohydrate-based, but other substances such as polyphenols and polyunsaturated fatty acids might exert prebiotic effects[6]. An example of polyphenols is water-insoluble cocoa fraction, which has been shown in a gut model to substantially increase bifidobacteria, lactobacilli and butyrate production[71].

Low-molecular-weight carbohydrates are very efficiently metabolized by microorganisms such as bifidobacteria, which possess a range of cell-associated and extracellular glycosidases and specific transport systems enabling them to rapidly assimilate low-molecular-weight sugars[72,73]. Other microorganisms, such as members of the *Bacteroides* genus, are adept at breaking down high molecular weight polysaccharides[74,75]. Some bacteria might be regarded as keystone species in having the ability to initiate breakdown of particular substrates[76]; for example, *Ruminococcus* spp. can facilitate the degradation of resistant starch[77]. Liberated low-molecular-weight dextrins are then metabolized by the microbial community. The pathway from a polysaccharide to a SCFA is therefore a complex and indirect network of metabolism. Acetate and lactate, the main metabolic end products of bifidobacteria and lactic acid bacteria, are utilized by other microorganisms to produce, for example, propionate[78] and butyrate[50,79]. Probable ecological networks involved in the metabolism of carbohydrates have been elucidated[74,80,81], although the extent to which they operate in the gut is not clear at the present time.

A further complication in studies of the ecosystem response to carbohydrates is that it is heavily influenced by the microorganisms that are already present. It has become clear that individual microbiomes that are *Prevotella*-dominant can ferment carbohydrates more rapidly than can *Bacteroides*-dominant microbiomes[82]. Furthermore, when these distinct fecal inocula, dominated by *Prevotella* or *Bacteroides*, were incubated with prebiotic fructo-oligosaccharides (FOS) or with two different arabinoxylans, the profile of SCFA produced was distinctly different and correlated with the microbiome[83]. Cultures using *Prevotella*-dominant inocula produced substantially higher ratios of propionate to acetate and butyrate than the *Bacteroides*-dominant microbiotas. A similar influence of starting microbiome composition on carbohydrate fermentation has been seen using isomalto-oligosaccharides as a carbon source in an in vitro batch fermentation model with human microbiota[84].

Microbiome studies based on 16S ribosomal DNA sequencing have given rise to an increased awareness of the richness of the gut microbial ecosystem[85] and, in some cases, have revealed associations between certain microorganisms or microbiome profiles and disease states. These include IBD[86], type 2 diabetes mellitus[87–89], IBS[90,91] and obesity[92,93]. These profiles have frequently been termed 'dysbioses', although it is not currently possible to define such a state as 'normobiosis' or a 'normal' microbiota. Such associations tend to be merely the starting point for investigation into the role of specific microorganisms in disease. Sequencing studies do not provide an understanding of the functional interactions between members of the gut microbiota, and it is imperative that this functional ecology is studied in more detail. It is becoming clear that although there might be a huge diversity of individual taxa in the gut microbiomes of individuals, there is a high level of functional redundancy, and specific ecological functions are provided by a range of bacteria across different individuals[94,95].

Given that we have an imperfect understanding of the functional ecology of the gut microbiota, uncovering the mechanisms of action of prebiotics presents a challenge. Despite this issue, we can postulate probable mechanisms by which a prebiotic can lead to health benefits. These pathways are presented in FIG. 2 and discussed here. All of these postulated mechanisms have support from research carried out through in vitro or animal models, although in many cases, establishing that they actually occur within human gut microbiota is difficult.

Defence against pathogens. Although mechanistically challenging to establish in humans in vivo, pathogen defence can be investigated in vitro using model systems[96,97]. As noted for probiotics, production of organic acids through prebiotic administration and propagation of beneficial bacteria will result in a reduction in luminal pH, inhibiting growth of pathogens. Establishment of a stable population of commensal microorganisms will reduce nutrient availability for invading microorganisms, inhibiting colonization. In studies of elderly individuals, 10 weeks of daily galacto-oligosaccharide (GOS) consumption induced increases in immune function, notably enhanced phagocytic activity and activity of natural killer cells[98,99].

Immune modulation. Although the exact mechanisms are unclear, there is evidence that prebiotic intervention can reduce type 2 T helper responses and therefore affect allergy. The most supportive data come from studies in infants. GOS and long-chain FOS in infant formula administered in a double-blind, randomized, placebo-controlled trial in 259 infants was associated with a reduction in incidence of atopic dermatitis, wheezing and urticaria to less than 50% of the incidence in non-prebiotic formula-fed infants[100,101]. In a prospective, double-blind, placebo-controlled fashion, not as yet replicated, healthy term infants at risk of atopy fed prebiotic-supplemented hypoallergenic formula for 6 months had a greater than fivefold reduction in prevalence of allergies 5 years after feeding[102].

Increased mineral absorption. Since most absorption of minerals takes place by active transport mechanisms in the small intestine[103], scavenging calcium could make a substantial positive contribution to health. As already discussed, fermentation of prebiotics leads to production of SCFA, which reduces luminal pH. This drop in pH can increase calcium solubility, thereby providing a greater driving force for passive uptake. A problem with proving this process is that many calcium salts in supplements and food have pH-dependent solubility and limited availability, and depending on the starting pH, the solubility of calcium can actually increase with increasing pH[104].

Studies have shown that consumption by young adolescents of a mixture of FOS and inulin[105,106] or GOS[107] can result in marked increases in absorption and calcium mineralized into bone. Such early intervention could reduce the incidence of osteoporosis later in life. This hypothesis is supported by data from animal models[108], but long-term studies in humans are lacking.

Improved bowel function. Improvements in bowel function have often been ascribed to simple fecal bulking by consumption of dietary fibre. However, animal studies have shown that SCFAs produced by fermentation of prebiotics can regulate gut hormones that in turn modulate the local motor responses of the gut[109,110]. The humectant water-binding capacity of prebiotic carbohydrates also has the effect of softening stools, making passage easier[111,112].

There are surprisingly few studies on the effect of prebiotics on bowel function, although they have consistently led to improvements in stool consistency and defecation frequency in randomized trials[113,114].

Metabolic effects. As discussed earlier, prebiotic intervention results in the elaboration of SCFAs that can act to improve barrier function in the gut, and prebiotic intervention with GOS has been shown to improve barrier function in vivo[115]. Impaired barrier function can allow translocation of inflammatory mediators such as bacterial lipopolysaccharide (LPS) from the gut into the

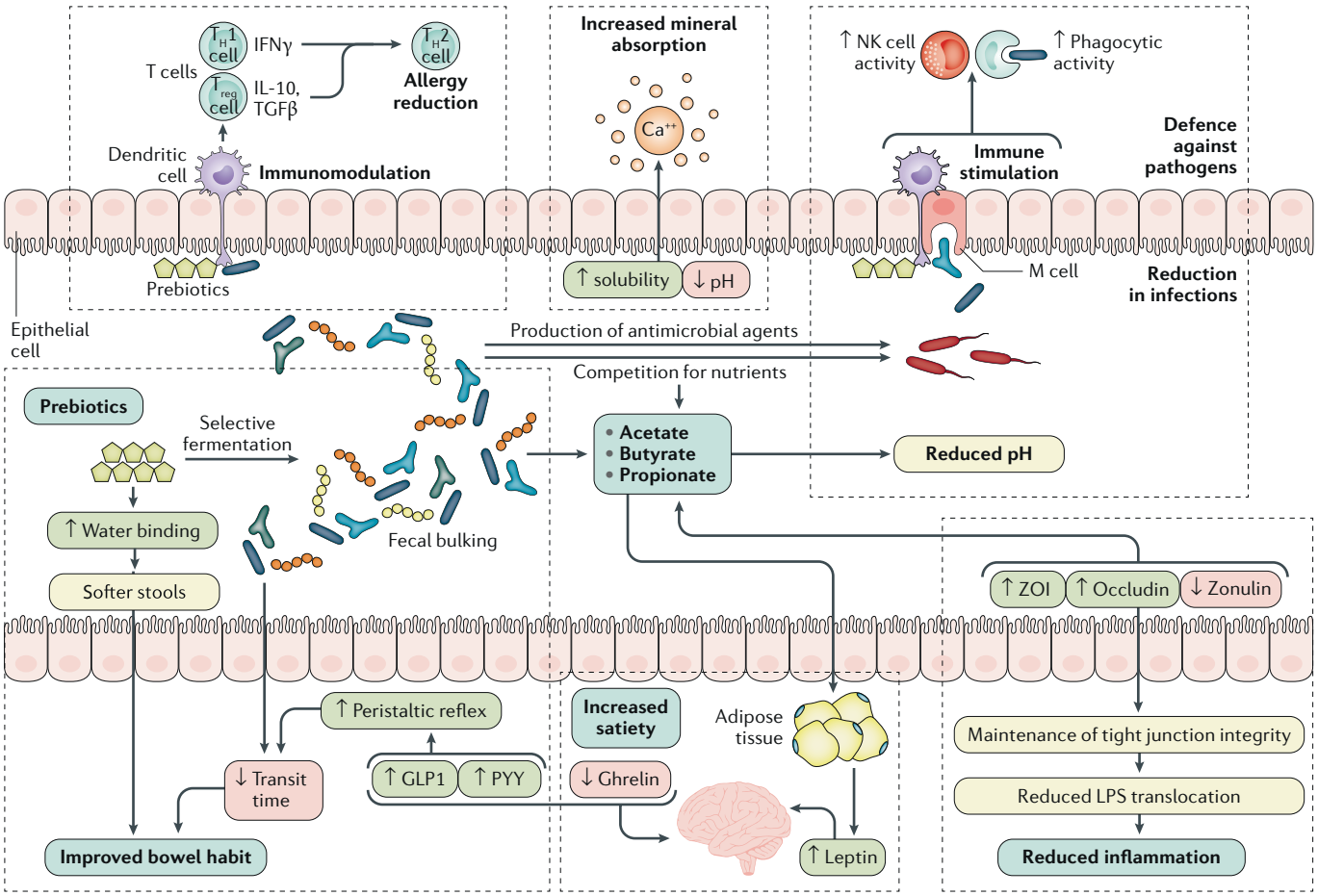

Fig. 2 | **Identified mechanisms of action of prebiotics.** The premise is that prebiotics enter the gut and are selectively utilized. This step increases bacterial growth and functionality of specific genera or species. As a result of either or both of these effects, health benefits can accrue. Fecal bulking and improved bowel habits occur due to microbial growth. Immune regulation can be influenced by increased biomass and cell wall components of the bacteria. Metabolic products include organic acids, which lower intestinal pH and have concomitant effects upon microbial pathogens and mineral absorption. Metabolic products can also influence epithelial integrity and hormonal regulation. Bacteria that respond to prebiotic intake can influence the microbiota composition through elaboration of antimicrobial agents (for example, peptides) and competitive interactions, possibly reducing infections and bacteria containing lipopolysaccharide (LPS). GLP1, glucagon like peptide1; M cell, microfold cell; NK cell, natural killer cell; PYY, peptide YY; TGFβ, transforming growth factor-β; T_H1 cell, type 1 T helper cell; T_H2 cell, type 2 T helper cell; T_{reg} cell, regulatory T cell; ZO1, zonula occludens 1.

systemic circulation, which has been termed metabolic endotoxaemia[116] and has been suggested to be a causative factor in diabetes and obesity, although the evidence for this is from studies in mice[117,118].

The metabolic effects of prebiotics have been the subject of several meta-analyses[119–122] and although the results among studies vary, the general consensus is that prebiotic intervention has a positive effect on glucose homeostasis, inflammation and blood lipid profile in humans. Although interventions with GOS[123] and inulin[124] have been shown to improve inflammatory markers in individuals with obesity, these were relatively short-term studies over a few months, and the effect on metabolic health over a long period of consumption is yet to be established.

The hypothesis underlying much research on prebiotics and barrier function and inflammation is that fermentation products such as SCFA probably mediate the beneficial effects through mechanisms discussed earlier. However, it has been shown that, at least in vitro, GOS can directly stimulate the expression of tight junction proteins in intestinal epithelial cell lines and decrease transepithelial flux[125,126]. However, given that GOS is fermented in the gut, the extent to which such mechanisms act in vivo is unclear at present. It is possible that the effect of inulin in improving glycaemic response could be due to direct inhibition of the intestinal isomaltase–sucrase enzyme complex, but so far the evidence is only from mouse studies[127]. Identification of direct mechanisms from metabolic studies in humans is, however, extremely difficult.

Effect on satiety. SCFAs produced by fermentation in the gut can interact with specific fatty acid receptors, FFAR2 and FFAR3, and regulate lipolysis and release of the incretin glucagon-like peptide-1 (REFS[128,129]). These receptors are found on many tissues and could be a key mechanistic link between prebiotic fermentation

and systemic health benefits. SCFAs can regulate appetite via several mechanisms[130], with studies showing that the interaction between SCFA and colonic L cells results in production of anorexigenic hormones such as PYY and GLP-1. Other examples are SCFAs surviving metabolism by colonic epithelial cells and reaching the liver via the hepatic portal vein where propionate stimulates gluconeogenesis that acts as a satiety signal[131]. SCFAs entering the circulation could also interact with FFAR2 and FFAR3 located on adipose tissue, resulting in leptin stimulation. According to a study in mice, acetate, the principal SCFA formed by prebiotic fermentation, can cross the blood–brain barrier and enter the hypothalamus, promoting anorectic signals[132].

Translation to the clinic

Many clinical gastrointestinal indications could benefit from probiotic and prebiotic interventions. In the case of prebiotics, a link between the clinical benefit and microbiota function should be established. For probiotics, a clinical indication is needed. For both, robust product information is required[133] (BOX 1).

There are clinical indications for the use of certain probiotic strains supported by robust evidence. In paediatric and/or adult populations, the following have been suggested as indications for the use of probiotics: necrotizing enterocolitis[134], antibiotic-associated diarrhoea and *H. pylori* infection[135–137], defecation frequency[138,139], infantile colic[140], mild to moderate ulcerative colitis[141], IBS[142], acute diarrhoea[143], prevention of *Clostridium difficile*-associated diarrhoea[144] and neonatal sepsis[28]. A meta-analysis published in 2019 provided evidence that probiotic use has the potential to decrease antibiotic utilization in children[145]. Some clinical guidelines have been issued for probiotic use in children[146,147]. There is evidence that probiotics act systemically from the gut to reduce the incidence and duration of upper respiratory tract infections[148,149]. No official recommendations have been made for the use of probiotics in adults. Additional research clarifying the most effective strains and doses is needed for many clinical targets so far researched[150–152]. Although many clinical indications are promising, data are still emerging for end points including brain, metabolic and cardiovascular effects.

Generally, the strength of evidence for prebiotic interventions lags behind that for probiotics. Perhaps the strongest support for prebiotic use comes from prebiotic infant formulae. Such products are now routinely supplemented with mixtures of GOS and fructans[153,154] and this blend of prebiotics in a 9:1 ratio has been shown to reduce respiratory tract infections to levels found in breast-fed infants[101,155,156]. There is less evidence that prebiotics can reduce infections in adults, although in one placebo-controlled, randomized, double-blind study in 159 healthy volunteers, GOS reduced the incidence of diarrhoea[157].

Much of the research focus on prebiotics has been in the realm of functional food (improves well-being through benefit beyond its nutrient content) applications. The one example of a prebiotic food application recognized by European regulatory authorities is improvement in bowel function in healthy adults resulting from consumption of 12 g of chicory inulin per day[158,159].

Prebiotic foods designed to increase satiety and reduce energy intake are a promising approach to augmenting compliance with weight-loss diets. Oligofructose-enriched inulin in overweight children has been shown to increase satiety, and reduce energy intake, BMI and body fat mass over 16 weeks (body weight decrease of 3.1% and percent body fat decrease of 2.4% compared with children given placebo (who showed increases of 0.5% and 0.05%, respectively)[160,161]. Oligofructose ingested daily by 29 adults for 12 weeks in a granola bar formulation reduced lean mass by 0.3 kg (standard deviation 1.2 kg) and waist circumference by 2.2 cm (standard deviation 3.6 cm), with a concomitant decrease in intake and an increase in satiety[162]. However, not all studies have indicated benefits. One study of 97 overweight or obese children given oligofructose for 12 weeks did not show a statistically significant change in BMI-for-age *z*-score versus placebo[163]. This study did not measure the effect of the prebiotic on the gut microbiota and its function, which would have provided mechanistic insights to better understand the null study results and enabled better design of future interventions.

The replacement of glycaemic carbohydrates in food products with non-glycaemic carbohydrates to reduce post-prandial glycaemic responses has already received a positive European Food Safety Authority opinion[164]. Prebiotic carbohydrates might be expected to bring additional benefits in terms of increasing satiety in such a replacement strategy. Promising results were observed from a double-blind, randomized, controlled crossover trial of 40–42 healthy adults who consumed a yogurt drink containing oligofructose. The intervention improved post-prandial glucose responses[165].

There is now some evidence that the stool microbiota profiles of patients with inflammatory conditions, such as IBD, differ from those of heathy individuals[166], but it is not clear at the present time why. It is unclear whether these differences are caused by the underlying medical condition, are a consequence of the disease pathology, or are due to confounding factors such as medications or altered dietary habits. Probiotic or

Box 1 | **Overcoming barriers to translation to the clinic**

- More high-quality, adequately powered, randomized, controlled trials that test well-defined probiotic (strain or strain combinations, dose and delivery matrix) and prebiotic interventions on substantive clinical outcomes
- Better tracking of safety data during the conduct of short-term and long-term clinical trials
- Improved availability of high-quality, properly labelled and effective commercial products[133]
- Application by clinicians of available efficacy data in an evidence-based manner; this approach comprises assessment of the totality of data (positive and null) through unbiased systematic review processes for specific probiotic and prebiotic interventions
- Better understanding of the characteristics of the host (including diet, baseline microbiota, medications and disease) that improve response to probiotics or prebiotics
- Clinicians need clarification about probiotic and prebiotic products: are they safe, who will benefit (how and to what extent), and can the product labels be trusted?

prebiotic interventions hold promise for the mitigation of the disease or its symptoms through microbiota modulation. An understanding of the microbiome composition and function in the donor and recipient will help us understand the extent to which clinical success depends on these factors[167]. Indeed, some clinical trials have noted the importance of baseline microbiota composition among responders[168–170]. Microbiota patterns can be influenced by lifestyle, living conditions, diet, medications and stool consistency, among other transient variables. Advanced age is also thought to be a factor, but one study of Chinese individuals has shown that healthy centenarians have similar microbiota to healthy young people[171], suggesting that factors other than age are more important drivers of microbiota composition. Furthermore, research methodologies and data management may lead to spurious interpretations of microbiota assessments, which has the potential to mislead[172]. Although clinical benefits have been observed with probiotic and prebiotic interventions, the onus is on researchers to clarify the role of the microbiome in these successes to optimize short- and long-term outcomes[173–177].

Careful phenotypic and genotypic descriptions of study participants could also be important to the success of clinical trials targeting the microbiome. Host genetic studies could help, for example, in the microbiome-mediated disease of IBD, in which 163 loci have been identified as meeting genome-wide significance thresholds[178]. However, in the majority of patients with IBD, the disease is not the result of a single host gene defect[179], complicating the development of clinical interventions based on host genetics. Another complication is that identified genes are risk factors, not causal determinants, for a disease, and therefore clinical strategies based on host genomics have not been forthcoming.

IBD comprises two main forms, Crohn's disease and ulcerative colitis. In Crohn's disease, there seem to be distinct molecular subclasses of genomic associations, further complicating development of effective management strategies[180]. This aspect might in part explain why the use of probiotic strains in the management of Crohn's disease has generally failed to be effective[181,182]. It is not known why mild to moderate ulcerative colitis is somewhat improved by probiotic intervention[183] but Crohn's disease is not. The future success of microbiota manipulation to mitigate serious inflammatory conditions will require an understanding of the interactions between the microbiome and the human genetic risk factors, and will necessitate moving beyond microbial genomic sequencing to transcriptomic, metabolomic and proteomic investigations.

The promise of treating or curing disease with microbiota manipulation continues to be explored using probiotic species different from those traditionally employed[184]. Many probiotics in current use are from the genera *Lactobacillus* and *Bifidobacterium*. Although many of them were derived from the feces or intestinal mucosa of healthy individuals, researchers today are considering the utility of many newly explored human resident microorganisms, such as *Akkermansia, Eubacterium, Propionibacterium, Faecalibacterium* and *Roseburia*. This research will require going beyond laboratory animal experiments that have proliferated in the literature[62,185].

Fecal microbial transplantation (FMT), which has been a reasonably successful treatment for recurrent *C. difficile* infection[186–188], has shown mixed success in the treatment of other conditions[189–191]. Although FMT is not a probiotic application since it is not suitably defined microbiologically to meet the probiotic definition[2], the approach is based on the concept that microorganisms derived from healthy donor feces can restore proper function to a dysbiotic microbial ecosystem. It is noteworthy that there have only been a few blinded, randomized controlled trials on FMT for treatment of recurring *C. difficile infection*, but these have been relatively small studies and we have little information on the long-term changes that such a broad, poorly defined and nonspecific treatment might induce in individuals. A well-defined reproducible probiotic intervention is more suitable for rigorous research investigation and could be safer long-term than FMT, as suggested by several researchers attempting to assemble a defined consortium of microorganisms for such purposes[192]. Whether these defined consortia, typically comprising many human commensal microbial species, can reach the same levels of cure as FMT remains to be seen.

The potential impact of gut microbiota manipulation on clinical medicine is promising. However, in the excitement over potential, stakeholders often forget that association does not mean causation. For example, blinded reviews of 34 oesophageal biopsy samples found that these microbiomes could be classified into two types. Type 1 is dominated by the genus *Streptococcus* and is phenotypically normal, but type 2, which demonstrates a greater proportion of Gram-negative anaerobes and/or microaerophiles, correlates with oesophagitis and Barrett's oesophagus[150]. Like many other microbiome findings, this finding does not prove causation and there are numerous potential reasons why these associations might exist, including diet, drugs and lifestyle. One hypothesis might be that administering a safe, select *Streptococcus* could reduce oesophagitis and Barrett's oesophagus, but this theory has not been tested. Microbiome differences do not necessarily mean that microbiota modification will lead to improved health.

The future

The gut microbiota might be central to the cause of many disorders and its modulation could hold the key to new effective therapies. So, what are the roles of probiotics and prebiotics? In a general sense, both interventions serve to increase the community of beneficial microorganisms and products of their growth and metabolism in the host. In this context, effects relayed systemically might exert influences in, for example, the cardiovascular system, urogenital tract, skin and brain[193].

The field is poised for conceptual advances. Target microorganisms will expand beyond the typical *Bifidobacterium* spp. and *Lactobacillus* spp. (as mentioned earlier) to include other genera and perhaps more yeast species[194–197]. These microorganisms might be new probiotic candidates or further targets for prebiotic

utilization. Improved precision, accuracy and repeatability of measures of microbial composition, which lead to genuine and not misleading interpretations, are needed in this field[172]. Improved assessments will lead to an expanded range of probiotic and prebiotic products. For example, propionate and butyrate are both considered to be beneficial gut microbial metabolites, but neither is produced by bifidobacteria or lactobacilli[198,199]. Therefore, an opportunity exists to define microorganisms with metabolic capabilities beyond those afforded by traditional probiotics. Another development could be anti-adhesive molecules and carbohydrates that attenuate microbial virulence. These factors would be adjuncts to current prebiotic approaches in that they are not selectively utilized substrates.

To have robust proof that gut microbiome alterations can reduce disease incidence or mitigate disease, more well-designed randomized controlled trials are needed. By randomly assigning individuals to intervention groups, most biases are reduced and the chances of useful results are improved. Owing to the easy availability and relatively low cost of high-throughput sequencing technology, microbiome analysis is becoming widespread and differences among disease states increasingly well publicized. The expertise and databases required for metabolomic analysis is also on an upward trend. This advance will be vital to optimize clinical translation, as a much greater awareness of the functional ecology of the gut is needed together with improved clarity of how this ecosystem influences systemic health. Microbiota and host transcriptomic studies are also important, but they are expensive and time-consuming, and require substantial bioinformatic support. Ultimately, the application of probiotic and prebiotic regimens has the potential to improve human health and contribute greatly to how patients are managed and/or disease risk is reduced.

Conclusions

Although certain commonalities allow us to group substances under the 'probiotic' or 'prebiotic' umbrellas, benefits to human health are tied to specific products, not the categories en masse. To the extent that a clinical outcome is associated with a specific mechanism of action, then it could be hypothesized that a similar strain or prebiotic expressing that mechanism might also be beneficial. However, it is important not to overgeneralize conclusions about specific entities. In general, whether an intervention is effective or ineffective, it must be recognized that the results are tied to specific formulations, doses, clinical end points and target populations. It is incumbent upon responsible scientists to consider the totality of available information on specific interventions as a basis for overall conclusions on effectiveness. Furthermore, clinicians should scrutinize both positive and null studies for bias, as only in eliminating bias in research will we move the field toward truth, thereby realizing the potential of probiotics and prebiotics.

The body of research suggests that these interventions can not only improve symptomology, but also have a meaningful effect on reducing pathology and even saving lives. The prevention of sepsis and necrotizing enterocolitis in infants provides compelling examples. These findings demonstrate effective translation of human microbiome research. Such clinical impact has changed practices in many health-care environments; however, many constituencies have yet to embrace the concept through critically considering the strengths and weaknesses of existing data.

In developing countries, probiotics that are widely available in developed countries are either not accessible or affordable to most people. However, a programme has introduced inexpensive sachets containing a probiotic *L. rhamnosus* (GR-1 or Yoba 2012) plus *S. thermophilus* C106 that allow locals to produce different forms of fermented foods (yoghurt, millet, cereals, juices) that not only influence health but also empower poverty-stricken communities to improve social well-being[4]. With over 260,000 consumers being reached each week in East Africa, the potential is enormous to use these beneficial microorganisms and local food sources to impact communities (G.R. et al., unpublished observations).

Diseases and poor health often result from the interplay of microbiological and biological ecosystems along with societal issues including pollution, food shortages and poor medical care[200,201]. We encourage more research and translational efforts on probiotics and prebiotics to serve the people of developing countries, who might stand to benefit most from these interventions.

Published online 11 July 2019

1. Food and Agriculture Organization of the United Nations & World Health Organization. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *FAO* http://www.fao.org/3/a-a0512e.pdf (2001).
2. Hill, C. et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **11**, 506–514 (2014).
This Consensus Statement examines the definition, evolution, uses, types and health attributes of probiotics.
3. Rook, G., Backhed, F., Levin, B. R., McFall-Ngai, M. J. & McLean, A. R. Evolution, human-microbe interactions, and life history plasticity. *Lancet* **390**, 521–530 (2017).
This article discusses how some microorganisms have co-evolved with humans and have crucial roles in host physiology and metabolism, whereas others are intrusive.
4. Reid, G. et al. Expanding the reach of probiotics through social enterprises. *Benef. Microbes* **9**, 707–715 (2018).
5. Gibson, G. R. & Roberfroid, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* **125**, 1401–1412 (1995).
6. Gibson, G. R. et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 491–502 (2017).
This Consensus Statement examines the definition, evolution, uses, types and health attributes of prebiotics.
7. Collins, S. L. et al. Promising prebiotic candidate established by evaluation of lactitol, lactulose, raffinose, and oligofructose for maintenance of a *Lactobacillus*-dominated vaginal microbiota. *Appl. Environ. Microbiol.* **84**, e02200-17 (2018).
8. Rodriguez, J. M. et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb. Ecol. Health Dis.* **26**, 26050 (2015).
9. Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **14**, 20–32 (2016).
10. Rowland, I. et al. Gut microbiota functions: metabolism of nutrients and other food components. *Eur. J. Nutr.* **57**, 1–24 (2018).
11. Thursby, E. & Juge, N. Introduction to the human gut microbiota. *Biochem. J.* **474**, 1823–1836 (2017).
This article provides current understanding of the development and composition of the human gut microbiota, and its effects on gut integrity and host health.
12. Fava, F. et al. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. *Int. J. Obes. (Lond.)* **37**, 216–223 (2013).
13. Dicks, L. M. T., Geldenhuys, J., Mikkelsen, L. S., Brandsborg, E. & Marcotte, H. Our gut microbiota: a long walk to homeostasis. *Benef. Microbes* **9**, 3–20 (2018).
14. Dethlefsen, L. & Relman, D. A. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation.

Proc. Natl Acad. Sci. U. S. A. **108** (Suppl. 1), 4554–4561 (2011).
15. Gagliardi, A. et al. Rebuilding the gut microbiota ecosystem. *Int. J. Environ. Res. Public Health* **15**, E1679 (2018).
16. Hatton, G. B., Madla, C. M., Rabbie, S. C. & Basit, A. W. All disease begins in the gut: influence of gastrointestinal disorders and surgery on oral drug performance. *Int. J. Pharm.* **548**, 408–422 (2018).
17. John, G. K. et al. Dietary alteration of the gut microbiome and its impact on weight and fat mass: a systematic review and meta-analysis. *Genes (Basel)* **9**, (E167 (2018).
18. Yoshida, N., Yamashita, T. & Hirata, K. I. Gut microbiome and cardiovascular diseases. *Diseases* **6**, E56 (2018).
19. Hansen, L. B. S. et al. A low-gluten diet induces changes in the intestinal microbiome of healthy Danish adults. *Nat. Commun.* **9**, 4630 (2018).
20. Parker, R. B. Probiotics, the other half of the antibiotic story. *Anim. Nutr. Health* **29**, 4–8 (1974).
21. Havenaar, R. & Huis In't Veld, J. M. J. in *Lactic Acid Bacteria in Health and Disease* Vol. 1 (ed. Wood, B. J. B.) 151–170 (Elsevier Applied Science Publishers, 1992).
22. Ng, S. C. et al. Immunosuppressive effects via human intestinal dendritic cells of probiotic bacteria and steroids in the treatment of acute ulcerative colitis. *Inflamm. Bowel Dis.* **16**, 1286–1298 (2010).
23. Mujagic, Z. et al. The effects of *Lactobacillus plantarum* on small intestinal barrier function and mucosal gene transcription; a randomized double-blind placebo controlled trial. *Sci. Rep.* **7**, 40128 (2017).
24. Del Piano, M. et al. The use of probiotics in healthy volunteers with evacuation disorders and hard stools: a double-blind, randomized, placebo-controlled study. *J. Clin. Gastroenterol.* **44** (Suppl. 1), S30–S34 (2010).
25. Reid, G., Gadir, A. A. & Dhir, R. Probiotics: reiterating what they are and what they are not. *Front. Microbiol.* **10**, 424 (2019).
26. Cabre, E. & Gassull, M. A. Probiotics for preventing relapse or recurrence in Crohn's disease involving the ileum: are there reasons for failure? *J. Crohns Colitis* **1**, 47–52 (2007).
27. Kelly, J. R. et al. Lost in translation? The potential psychobiotic *Lactobacillus rhamnosus* (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain Behav. Immun.* **61**, 50–59 (2017).
28. Panigrahi, P. et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. *Nature* **548**, 407–412 (2017).
29. Costeloe, K. et al. *Bifidobacterium breve* BBG-001 in very preterm infants: a randomised controlled phase 3 trial. *Lancet* **387**, 649–660 (2016).
30. Sorbara, M. T. & Pamer, E. G. Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them. *Mucosal Immunol.* **34**, 1608 (2018).
31. Chiu, L. et al. Protective microbiota: from localized to long-reaching co-immunity. *Front. Immunol.* **8**, 1678 (2017).
32. Maldonado-Gomez, M. X. et al. Stable engraftment of *Bifidobacterium longum* AH1206 in the human gut depends on individualized features of the resident microbiome. *Cell Host Microbe* **20**, 515–526 (2016).
33. Murphy, R. et al. Eczema-protective probiotic alters infant gut microbiome functional capacity but not composition: sub-sample analysis from a RCT. *Benef. Microbes* **10**, 5–17 (2019).
34. Korpela, K. et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome* **6**, 182 (2018).
35. Clarke, G. et al. Gut reactions: breaking down xenobiotic-microbiome interactions. *Pharmacol. Rev.* **71**, 198–224 (2019).
36. Klaenhammer, T. R., Kleerebezem, M., Kopp, M. V. & Rescigno, M. The impact of probiotics and prebiotics on the immune system. *Nat. Rev. Immunol.* **12**, 728–734 (2012).
Here, four experts discuss probiotics, prebiotics and immunity, then provide their thoughts on the future application as a disease therapy.
37. Przemska-Kosicka, A. et al. Effect of a synbiotic on the response to seasonal influenza vaccination is strongly influenced by degree of immunosenescence. *Immun. Ageing* **13**, 6 (2016).
38. Vitetta, L., Saltzman, E. T., Thomsen, M., Nikov, T. & Hall, S. Adjuvant probiotics and the intestinal microbiome: enhancing vaccines and immunotherapy outcomes. *Vaccines (Basel)* 5, (E50 (2017).
39. Childs, C. E. et al. Xylo-oligosaccharides alone or in synbiotic combination with *Bifidobacterium animalis* subsp. *lactis* induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. *Br. J. Nutr.* **111**, 1945–1956 (2014).
40. Flint, H. J., Duncan, S. H., Scott, K. P. & Louis, P. Links between diet, gut microbiota composition and gut metabolism. *Proc. Nutr. Soc.* **74**, 13–22 (2015).
41. Aoudia, N. et al. Biofilms of *Lactobacillus plantarum* and *Lactobacillus fermentum*: effect on stress responses, antagonistic effects on pathogen growth and immunomodulatory properties. *Food Microbiol.* **53**, 51–59 (2016).
42. Rios-Covian, D. et al. Intestinal short chain fatty acids and their link with diet and human health. *Front. Microbiol.* **7**, 185 (2016).
43. Canfora, E. E., Jocken, J. W. & Blaak, E. E. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat. Rev. Endocrinol.* **11**, 577–591 (2015).
44. Sanna, S. et al. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat. Genet.* **51**, 600–605 (2019).
45. Stefan, N., Fritsche, A., Schick, F. & Haring, H. U. Phenotypes of prediabetes and stratification of cardiometabolic risk. *Lancet Diabetes Endocrinol.* **4**, 789–798 (2016).
46. van Baarlen, P., Wells, J. M. & Kleerebezem, M. Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. *Trends Immunol.* **34**, 208–215 (2013).
47. Hegarty, J. W., Guinane, C. M., Ross, R. P., Hill, C. & Cotter, P. D. Bacteriocin production: a relatively unharnessed probiotic trait? *F1000Res.* **5**, 2587 (2016).
48. Mokoena, M. P. Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. *Molecules* **22**, E1255 (2017).
49. Bali, V., Panesar, P. S., Bera, M. B. & Kennedy, J. F. Bacteriocins: recent trends and potential applications. *Crit. Rev. Food Sci. Nutr.* **56**, 817–834 (2016).
50. Riviere, A., Selak, M., Lantin, D., Leroy, F. & De Vuyst, L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front. Microbiol.* **7**, 979 (2016).
51. Abdulkadir, B. et al. Routine use of probiotics in preterm infants: longitudinal impact on the microbiome and metabolome. *Neonatology* **109**, (239–247 (2016).
52. Fang, H. R., Zhang, G. Q., Cheng, J. Y. & Li, Z. Y. Efficacy of *Lactobacillus*-supplemented triple therapy for *Helicobacter pylori* infection in children: a meta-analysis of randomized controlled trials. *Eur. J. Pediatr.* **178**, 7–16 (2019).
53. Sanders, M. E., Benson, A., Lebeer, S., Merenstein, D. J. & Klaenhammer, T. R. Shared mechanisms among probiotic taxa: implications for general probiotic claims. *Curr. Opin. Biotechnol.* **49**, 207–216 (2018).
54. Petrova, M. I. et al. Comparative genomic and phenotypic analysis of the vaginal probiotic *Lactobacillus rhamnosus* GR-1. *Front. Microbiol.* **9**, 1278 (2018).
55. La Fata, G., Weber, P. & Mohajeri, M. H. Probiotics and the gut immune system: indirect regulation. Probiot. *Antimicrob. Proteins* **10**, 11–21 (2018).
56. Han, X. et al. *Lactobacillus rhamnosus* GG prevents epithelial barrier dysfunction induced by interferon-gamma and fecal supernatants from irritable bowel syndrome patients in human intestinal enteroids and colonoids. *Gut Microbes* **10**, 59–76 (2019).
57. Mack, D. R., Michail, S., Wei, S., McDougall, L. & Hollingsworth, M. A. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *Am. J. Physiol.* **276**, G941–G950 (1999).
58. Yan, F. et al. A *Lactobacillus rhamnosus* GG-derived soluble protein, p40, stimulates ligand release from intestinal epithelial cells to transactivate epidermal growth factor receptor. *J. Biol. Chem.* **288**, 30742–30751 (2013).
59. Stadlbauer, V. et al. *Lactobacillus casei* Shirota supplementation does not restore gut microbiota composition and gut barrier in metabolic syndrome: a randomized pilot study. *PLOS ONE* **10**, e0141399 (2015).
60. Kim, N., Yun, M., Oh, Y. J. & Choi, H. J. Mind-altering with the gut: modulation of the gut-brain axis with probiotics. *J. Microbiol.* **56**, 172–182 (2018).
61. Janik, R. et al. Magnetic resonance spectroscopy reveals oral *Lactobacillus* promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *Neuroimage* **125**, 988–995 (2016).
62. Reid, G. Disentangling what we know about microbes and mental health. *Front. Endocrinol.* **10**, 81 (2019).
63. Liang, S. et al. Administration of *Lactobacillus helveticus* NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. *Neuroscience* **310**, 561–577 (2015).
64. Kotz, C. M., Furne, J. K., Savaiano, D. A. & Levitt, M. D. Factors affecting the ability of a high beta-galactosidase yogurt to enhance lactose absorption. *J. Dairy Sci.* **77**, 3538–3544 (1994).
65. Costabile, A. et al. An in vivo assessment of the cholesterol-lowering efficacy of *Lactobacillus plantarum* ECGC 13110402 in normal to mildly hypercholesterolaemic adults. *PLOS ONE* **12**, e0187964 (2017).
66. European Food Safety Authority Panel on Dietetic Products. Scientific opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion (ID 1143, 2976) pursuant to article 13(1) of regulation (EC) No 1924/2006. *EFSA J.* **8**, 1763 (2010).
67. Li, D., Wang, P., Wang, P., Hu, X. & Chen, F. The gut microbiota: a treasure for human health. *Biotechnol. Adv.* **34**, 1210–1224 (2016).
68. Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A. & Kimura, I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients* **7**, 2839–2849 (2015).
69. Verbeke, K. A. et al. Towards microbial fermentation metabolites as markers for health benefits of prebiotics. *Nutr. Res. Rev.* **28**, 42–66 (2015).
70. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
71. Fogliano, V. et al. In vitro bioaccessibility and gut biotransformation of polyphenols present in the water-insoluble cocoa fraction. *Mol. Nutr. Food Res.* **55** (Suppl. 1), S44–S55 (2011).
72. Falony, G. et al. In vitro kinetic analysis of fermentation of prebiotic inulin-type fructans by *Bifidobacterium* species reveals four different phenotypes. *Appl. Environ. Microbiol.* **75**, 454–461 (2009).
73. Riviere, A., Selak, M., Geirnaert, A., Van den Abbeele, P. & De Vuyst, L. Complementary mechanisms for degradation of inulin-type fructans and arabinoxylan oligosaccharides among bifidobacterial strains suggest bacterial cooperation. *Appl. Environ. Microbiol.* **84**, e02893-17 (2018).
74. Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P. & Forano, E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* **3**, 289–306 (2012).
75. Hamaker, B. R. & Tuncil, Y. E. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J. Mol. Biol.* **426**, 3838–3850 (2014).
76. Ze, X., Le Mougen, F., Duncan, S. H., Louis, P. & Flint, H. J. Some are more equal than others: the role of "keystone" species in the degradation of recalcitrant substrates. *Gut Microbes* **4**, 236–240 (2013).
77. Ze, X., Duncan, S. H., Louis, P. & Flint, H. J. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* **6**, 1535–1543 (2012).
78. Hosseini, E., Grootaert, C., Verstraete, W. & Van de Wiele, T. Propionate as a health-promoting microbial metabolite in the human gut. *Nutr. Rev.* **69**, 245–258 (2011).
79. Louis, P. & Flint, H. J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* **294**, 1–8 (2009).
80. Falony, G., Calmeyn, T., Leroy, F. & De Vuyst, L. Coculture fermentations of *Bifidobacterium* species and *Bacteroides thetaiotaomicron* reveal a mechanistic insight into the prebiotic effect of inulin-type fructans. *Appl. Environ. Microbiol.* **75**, 2312–2319 (2009).
81. Scott, K. P., Martin, J. C., Duncan, S. H. & Flint, H. J. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. *FEMS Microbiol. Ecol.* **87**, 30–40 (2014).
82. Flint, H. J., Duncan, S. H. & Louis, P. The impact of nutrition on intestinal bacterial communities. *Curr. Opin. Microbiol.* **38**, 59–65 (2017).
83. Chen, T. et al. Fiber-utilizing capacity varies in *Prevotella*- versus *Bacteroides*-dominated gut microbiota. *Sci. Rep.* **7**, 2594 (2017).

84. Wu, Q. et al. Fermentation properties of isomaltooligosaccharides are affected by human fecal enterotypes. *Anaerobe* **48**, 206–214 (2017).
85. Qin, J. et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
86. Frank, D. N. et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl Acad. Sci. U. S. A.* **104**, 13780–13785 (2007).
87. Larsen, N. et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLOS ONE* **5**, e9085 (2010).
88. Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).
This study provides a definition and description of the minimal gut metagenome and bacterial genome in terms of functions.
89. Karlsson, F. H. et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498**, 99–103 (2013).
90. Carroll, I. M., Chang, Y. H., Park, J., Sartor, R. B. & Ringel, Y. Luminal and mucosal-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Gut Pathog.* **2**, 19 (2010).
91. Krogius-Kurikka, L. et al. Microbial community analysis reveals high level phylogenetic alterations in the overall gastrointestinal microbiota of diarrhoea-predominant irritable bowel syndrome sufferers. *BMC Gastroenterol.* **9**, 95 (2009).
92. Turnbaugh, P. J. et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).
93. Zhang, H. et al. Human gut microbiota in obesity and after gastric bypass. *Proc. Natl Acad. Sci. U. S. A.* **106**, 2365–2370 (2009).
94. Ha, C. W., Lam, Y. Y. & Holmes, A. J. Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. *World J. Gastroenterol.* **20**, 16498–16517 (2014).
95. Moya, A. & Ferrer, M. Functional redundancy-induced stability of gut microbiota subjected to disturbance. *Trends Microbiol.* **24**, 402–413 (2016).
96. Fooks, L. J. & Gibson, G. R. In vitro investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. *FEMS Microbiol. Ecol.* **39**, 67–75 (2002).
97. Tzortzis, G., Baillon, M. L., Gibson, G. R. & Rastall, R. A. Modulation of anti-pathogenic activity in canine-derived *Lactobacillus* species by carbohydrate growth substrate. *J. Appl. Microbiol.* **96**, 552–559 (2004).
98. Vulevic, J., Drakoularakou, A., Yaqoob, P., Tzortzis, G. & Gibson, G. R. Modulation of the fecal microflora profile and immune function by a novel *trans*-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. *Am. J. Clin. Nutr.* **88**, 1438–1446 (2008).
99. Vulevic, J. et al. Influence of galacto-oligosaccharide mixture (B-GOS) on gut microbiota, immune parameters and metabonomics in elderly persons. *Br. J. Nutr.* **114**, 586–595 (2015).
100. Moro, G. et al. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch. Dis. Child.* **91**, 814–819 (2006).
101. Ivakhnenko, O. S. & Nyankovskyy, S. L. Effect of the specific infant formula mixture of oligosaccharides on local immunity and development of allergic and infectious disease in young children: randomized study. *Pediatr. Pol.* **88**, 398–404 (2013).
102. Arslanoglu, S. et al. Early neutral prebiotic oligosaccharide supplementation reduces the incidence of some allergic manifestations in the first 5 years of life. *J. Biol. Regul. Homeost. Agents* **26**, 49–59 (2012).
103. Diaz de Barboza, G. & Guizzardi, S. & Tolosa de Talamoni, N. Molecular aspects of intestinal calcium absorption. *World J. Gastroenterol.* **21**, 7142–7154 (2015).
104. Goss, S. L., Lemons, K. A., Kerstetter, J. E. & Bogner, R. H. Determination of calcium salt solubility with changes in pH and P(CO(2)), simulating varying gastrointestinal environments. *J. Pharm. Pharmacol.* **59**, 1485–1492 (2007).
105. Abrams, S. A., Griffin, I. J., Hawthorne, K. M. & Ellis, K. J. Effect of prebiotic supplementation and calcium intake on body mass index. *J. Pediatr.* **151**, 293–298 (2007).
106. Abrams, S. A., Griffin, I. J. & Hawthorne, K. M. Young adolescents who respond to an inulin-type fructan substantially increase total absorbed calcium and daily calcium accretion to the skeleton. *J. Nutr.* **137**, 2524S–2526S (2007).
107. Whisner, C. M. et al. Galacto-oligosaccharides increase calcium absorption and gut bifidobacteria in young girls: a double-blind cross-over trial. *Br. J. Nutr.* **110**, 1292–1303 (2013).
108. Chonan, O., Matsumoto, K. & Watanuki, M. Effect of galactooligosaccharides on calcium absorption and preventing bone loss in ovariectomized rats. *Biosci. Biotechnol. Biochem.* **59**, 236–239 (1995).
109. Kanauchi, O., Andoh, A. & Mitsuyama, K. Effects of the modulation of microbiota on the gastrointestinal immune system and bowel function. *J. Agric. Food Chem.* **61**, 9977–9983 (2013).
110. Hurst, N. R., Kendig, D. M., Murthy, K. S. & Grider, J. R. The short chain fatty acids, butyrate and propionate, have differential effects on the motility of the guinea pig colon. *Neurogastroenterol. Motil.* **26**, 1586–1596 (2014).
111. Lamsal, B. P. Production, health aspects and potential food uses of dairy prebiotic galactooligosaccharides. *J. Sci. Food Agric.* **92**, 2020–2028 (2012).
112. Hager, A.-S. et al. Influence of the soluble fibres inulin and oat β-glucan on quality of dough and bread. *Eur. Food Res. Technol.* **232**, 405–413 (2011).
113. Collado Yurrita, L., San Mauro Martin, I., Ciudad-Cabanas, M. J., Calle-Puron, M. E. & Hernandez Cabria, M. Effectiveness of inulin intake on indicators of chronic constipation; a meta-analysis of controlled randomized clinical trials. *Nutr. Hosp.* **30**, 244–252 (2014).
114. Buddington, R. K., Kapadia, C., Neumer, F. & Theis, S. Oligofructose provides laxation for irregularity associated with low fiber intake. *Nutrients* **9**, E1372 (2017).
115. Krumbeck, J. A. et al. Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome* **6**, 121 (2018).
116. Cani, P. D. et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772 (2007).
117. Cani, P. D. et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* **57**, 1470–1481 (2008).
118. Cani, P. D. et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091–1103 (2009).
119. Kellow, N. J., Coughlan, M. T. & Reid, C. M. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br. J. Nutr.* **111**, 1147–1161 (2014).
120. Beserra, B. T. et al. A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity. *Clin. Nutr.* **34**, 845–858 (2015).
121. Liu, F., Prabhakar, M., Ju, J., Long, H. & Zhou, H. W. Effect of inulin-type fructans on blood lipid profile and glucose level: a systematic review and meta-analysis of randomized controlled trials. *Eur. J. Clin. Nutr.* **71**, 9–20 (2017).
122. Guo, Z. et al. Effects of inulin on the plasma lipid profile of normolipidemic and hyperlipidemic subjects: a meta-analysis of randomized controlled trials. *Clin. Lipidol* **7**, 215–222 (2012).
123. Vulevic, J., Juric, A., Tzortzis, G. & Gibson, G. R. A mixture of *trans*-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. *J. Nutr.* **143**, 324–331 (2013).
124. Dewulf, E. M. et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* **62**, 1112–1121 (2013).
125. Bhatia, S. et al. Galacto-oligosaccharides may directly enhance intestinal barrier function through the modulation of goblet cells. *Mol. Nutr. Food Res.* **59**, 566–573 (2015).
126. Akbari, P. et al. Characterizing microbiota-independent effects of oligosaccharides on intestinal epithelial cells: insight into the role of structure and size: structure-activity relationships of non-digestible oligosaccharides. *Eur. J. Nutr.* **56**, 1919–1930 (2017).
127. Neyrinck, A. M. et al. Intestinal sucrase as a novel target contributing to the regulation of glycemia by prebiotics. *PLOS ONE* **11**, e0160488 (2016).
128. Stoddart, L. A., Smith, N. J. & Milligan, G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: pharmacology and pathophysiological functions. *Pharmacol. Rev.* **60**, 405–417 (2008).
129. Bolognini, D. et al. Chemogenetics defines receptor-mediated functions of short chain free fatty acids. *Nat. Chem. Biol.* **15**, 489–498 (2019).
130. Chambers, E. S., Morrison, D. J. & Frost, G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? *Proc. Nutr. Soc.* **74**, 328–336 (2015).
131. Mithieux, G. Metabolic effects of portal vein sensing. *Diabetes Obes. Metab.* **16** (Suppl. 1), 56–60 (2014).
132. Frost, G. et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.* **5**, 3611 (2014).
133. Jackson, S. A. et al. Improving end-user trust in the quality of commercial probiotic products. *Front. Microbiol.* **10**, 739 (2019).
134. AlFaleh, K. & Anabrees, J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst. Rev.* **4**, CD005496 (2014).
135. Vanderhoof, J. A. et al. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. *J. Pediatr.* **135**, 564–568 (1999).
136. Szajewska, H., Albrecht, P. & Topczewska-Cabanek, A. Randomized, double-blind, placebo-controlled trial: effect of *Lactobacillus* GG supplementation on *Helicobacter pylori* eradication rates and side effects during treatment in children. *J. Pediatr. Gastroenterol. Nutr.* **48**, 431–436 (2009).
137. Goldenberg, J. Z. et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst. Rev.* **4**, CD004827 (2015).
138. Eskesen, D. et al. Effect of the probiotic strain *Bifidobacterium animalis* subsp. *lactis*, BB-12®, on defecation frequency in healthy subjects with low defecation frequency and abdominal discomfort: a randomised, double-blind, placebo-controlled, parallel-group trial. *Br. J. Nutr.* **114**, 1638–1646 (2015).
139. Yang, Y. X. et al. Effect of a fermented milk containing *Bifidobacterium lactis* DN-173010 on Chinese constipated women. *World J. Gastroenterol.* **14**, 6237–6243 (2008).
140. Sung, V. et al. *Lactobacillus reuteri* to treat infant colic: a meta-analysis. *Pediatrics* **141**, e20171811 (2018).
141. Mardini, H. E. & Grigorian, A. Y. Probiotic mix VSL#3 is effective adjunctive therapy for mild to moderately active ulcerative colitis: a meta-analysis. *Inflamm. Bowel Dis.* **20**, 1562–1567 (2014).
142. Whorwell, P. J. et al. Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. *Am. J. Gastroenterol.* **101**, 1581–1590 (2006).
143. Szajewska, H. et al. Systematic review with meta-analysis: *Lactobacillus rhamnosus* GG for treating acute gastroenteritis in children - a 2019 update. *Aliment. Pharmacol. Ther.* **49**, 1376–1384 (2019).
144. Goldenberg, J. Z. et al. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. *Cochrane Database Syst. Rev.* **12**, CD006095 (2017).
145. King, S. et al. Does probiotic consumption reduce antibiotic utilization for common acute infections? A systematic review and meta-analysis. *Eur. J. Public Health* **29**, 494–499 (2019).
146. Cruchet, S. et al. The use of probiotics in pediatric gastroenterology: a review of the literature and recommendations by Latin-American experts. *Paediatr. Drugs* **17**, 199–216 (2015).
147. Cameron, D. et al. Probiotics for gastrointestinal disorders: proposed recommendations for children of the Asia-Pacific region. *World J. Gastroenterol.* **23**, 7952–7964 (2017).
148. Hao, Q., Dong, B. R. & Wu, T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst. Rev.* **9**, CD006895 (2015).
149. King, S., Glanville, J., Sanders, M. E., Fitzgerald, A. & Varley, D. Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis. *Br. J. Nutr.* **112**, 41–54 (2014).
150. Yang, L. et al. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology* **137**, 588–597 (2009).
151. Szymanski, H. & Szajewska, H. Lack of efficacy of *Lactobacillus reuteri* DSM 17938 for the treatment of acute gastroenteritis: a randomized controlled trial. *Pediatr. Infect. Dis. J.* https://doi.org/10.1097/INF.0000000000002355 (2019).

152. van den Akker, C. H. P. et al. Probiotics for preterm infants: a strain-specific systematic review and network meta-analysis. *J. Pediatr. Gastroenterol. Nutr.* **67**, 103–122 (2018).
153. Arslanoglu, S., Moro, G. E. & Boehm, G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J. Nutr.* **137**, 2420–2424 (2007).
154. Arslanoglu, S. et al. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *J. Nutr.* **138**, 1091–1095 (2008).
155. Boehm, G. et al. Prebiotics in infant formulas. *J. Clin. Gastroenterol.* **38**, S76–S79 (2004).
156. Shahramian, I. et al. The effects of prebiotic supplementation on weight gain, diarrhoea, constipation, fever and respiratory tract infections in the first year of life. *J. Paediatr. Child Health* **54**, 875–880 (2018).
157. Drakoularakou, A., Tzortzis, G., Rastall, R. A. & Gibson, G. R. A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers' diarrhoea. *Eur. J. Clin. Nutr.* **64**, 146–152 (2010).
158. Micka, A., Siepelmeyer, A., Holz, A., Theis, S. & Schon, C. Effect of consumption of chicory inulin on bowel function in healthy subjects with constipation: a randomized, double-blind, placebo-controlled trial. *Int. J. Food Sci. Nutr.* **68**, 82–89 (2017).
159. European Food Safety Authority Panel on Dietetic Products. Scientific opinion on the substantiation of a health claim related to "native chicory inulin" and maintenance of normal defecation by increasing stool frequency pursuant to article 13.5 of regulation (EC) No 1924/2006. *EFSA J.* **13**, 3951 (2015).
160. Hume, M. P., Nicolucci, A. C. & Reimer, R. A. Prebiotic supplementation improves appetite control in children with overweight and obesity: a randomized controlled trial. *Am. J. Clin. Nutr.* **105**, 790–799 (2017).
161. Nicolucci, A. C. et al. Prebiotics reduce body fat and alter intestinal microbiota in children who are overweight or with obesity. *Gastroenterology* **153**, 711–722 (2017).
162. Pol, K., de Graaf, C., Meyer, D. & Mars, M. The efficacy of daily snack replacement with oligofructose-enriched granola bars in overweight and obese adults: a 12-week randomised controlled trial. *Br. J. Nutr.* **119**, 1076–1086 (2018).
163. Liber, A. & Szajewska, H. Effect of oligofructose supplementation on body weight in overweight and obese children: a randomised, double-blind, placebo-controlled trial. *Br. J. Nutr.* **112**, 2068–2074 (2014).
164. European Food Safety Authority Panel on Dietetic Products. Scientific opinion on the substantiation of a health claim related to non-digestible carbohydrates and a reduction of post-prandial glycaemic responses pursuant to article 13(5) of regulation (EC) No 1924/2006. *EFSA J.* **12**, 3513 (2015).
165. Lightowler, H., Thondre, S., Holz, A. & Theis, S. Replacement of glycaemic carbohydrates by inulin-type fructans from chicory (oligofructose, inulin) reduces the postprandial blood glucose and insulin response to foods: report of two double-blind, randomized, controlled trials. *Eur. J. Nutr.* **57**, 1259–1268 (2018).
166. Olbjorn, C. et al. Fecal microbiota profiles in treatment-naive pediatric inflammatory bowel disease - associations with disease phenotype, treatment, and outcome. *Clin. Exp. Gastroenterol.* **12**, 37–49 (2019).
167. Backhed, F. et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe* **12**, 611–622 (2012).
168. Dey, M. Toward a personalized approach in prebiotics research. *Nutrients* **9**, 92 (2017).
169. Healey, G. et al. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind, placebo-controlled, cross-over, human intervention study. *Br. J. Nutr.* **119**, 176–189 (2018).
170. Tandon, D. et al. A prospective randomized, double-blind, placebo-controlled, dose-response relationship study to investigate efficacy of fructo-oligosaccharides (FOS) on human gut microflora. *Sci. Rep.* **9**, 5473 (2019).
171. Bian, G. et al. The gut microbiota of healthy aged Chinese is similar to that of the healthy young. *mSphere* **2**, e00327–17 (2017).
172. Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V. & Egozcue, J. J. Microbiome datasets are compositional: and this is not optional. *Front. Microbiol.* **8**, 2224 (2017).
173. Yatsunenko, T. et al. Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
This study considers the gut microbiome in evaluating human development, nutritional needs, physiological variations and the effects of westernization.
174. Marques, T. M. et al. Programming infant gut microbiota: influence of dietary and environmental factors. *Curr. Opin. Biotechnol.* **21**, 149–156 (2010).
175. Claesson, M. J. et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature* **488**, 178–184 (2012).
176. Clarke, S. F. et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* **63**, 1913–1920 (2014).
177. Engen, P. A., Green, S. J., Voigt, R. M., Forsyth, C. B. & Keshavarzian, A. The gastrointestinal microbiome: alcohol effects on the composition of intestinal microbiota. *Alcohol Res.* **37**, 223–236 (2015).
178. Jostins, L. et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* **491**, 119–124 (2012).
179. Picoraro, J. A. & LeLeiko, N. S. Omes of inflammatory bowel disease: a primer for clinicians. *J. Pediatr. Gastroenterol. Nutr.* **66**, 374–377 (2018).
180. Weiser, M. et al. Molecular classification of Crohn's disease reveals two clinically relevant subtypes. *Gut* **67**, 36–42 (2018).
181. Bourreille, A. et al. *Saccharomyces boulardii* does not prevent relapse of Crohn's disease. *Clin. Gastroenterol. Hepatol.* **11**, 982–987 (2013).
182. Van Gossum, A. et al. Multicenter randomized-controlled clinical trial of probiotics (*Lactobacillus johnsonii*, LA1) on early endoscopic recurrence of Crohn's disease after ileo-caecal resection. *Inflamm. Bowel Dis.* **13**, 135–142 (2007).
183. Tursi, A. et al. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. *Am. J. Gastroenterol.* **105**, 2218–2227 (2010).
184. O'Toole, P. W., Marchesi, J. R. & Hill, C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat. Microbiol.* **2**, 17057 (2017).
185. Ridaura, V. K. et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* **341**, 1241214 (2013).
186. van Nood, E. et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N. Engl. J. Med.* **368**, 407–415 (2013).
187. Lee, C. H. et al. Frozen versus fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent *Clostridium difficile* infection: a randomized clinical trial. *JAMA* **315**, 142–149 (2016).
188. Kelly, C. R. et al. Effect of fecal microbiota transplantation on recurrence in multiply recurrent *Clostridium difficile* infection: a randomized trial. *Ann. Intern. Med.* **165**, 609–616 (2016).
189. Halkjaer, S. I. et al. Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. *Gut* **67**, 2107–2115 (2018).
190. Delaune, V. et al. Fecal microbiota transplantation: a promising strategy in preventing the progression of non-alcoholic steatohepatitis and improving the anti-cancer immune response. *Expert Opin. Biol. Ther.* **18**, 1061–1071 (2018).
191. Moayyedi, P. et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* **149**, 102–109 (2015).
192. Gupta, S., Allen-Vercoe, E. & Petrof, E. O. Fecal microbiota transplantation: in perspective. *Therap. Adv. Gastroenterol.* **9**, 229–239 (2016).
193. Reid, G. et al. How do probiotics and prebiotics function at distant sites? *Benef. Microbes* **8**, 521–533 (2017).
An ISAPP working group article that looks at how microbiome programming in early life influences the gut microbiota communication with distant sites, such as airways, heart and brain, and influences metabolism.
194. Hiippala, K. et al. The potential of gut commensals in reinforcing intestinal barrier function and alleviating inflammation. *Nutrients* **10**, E988 (2018).
195. Crusell, M. K. W. et al. Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. *Microbiome* **6**, 89 (2018).
196. Cousin, F. J. et al. The probiotic *Propionibacterium freudenreichii* as a new adjuvant for TRAIL-based therapy in colorectal cancer. *Oncotarget* **7**, 7161–7178 (2016).
197. Zullo, B. A. & Ciafardini, G. Evaluation of physiological properties of yeast strains isolated from olive oil and their in vitro probiotic trait. *Food Microbiol.* **78**, 179–187 (2019).
198. Gonzalez-Rodriguez, I. et al. Catabolism of glucose and lactose in *Bifidobacterium animalis* subsp. *lactis*, studied by 13C nuclear magnetic resonance. *Appl. Environ. Microbiol.* **79**, 7628–7638 (2013).
199. Kostinek, M. et al. Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. *Int. J. Food Microbiol.* **114**, 342–351 (2007).
200. Hancock, T., Capon, A., Dooris, M. & Patrick, R. One planet regions: planetary health at the local level. *Lancet Planet. Health* **1**, e92–e93 (2017).
201. Garchitorena, A. et al. Disease ecology, health and the environment: a framework to account for ecological and socio-economic drivers in the control of neglected tropical diseases. *Philos. Trans. R. Soc. B.* **372**, 20160128 (2017).

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
M.E.S. declares personal fees related to probiotics from the following entities: California Dairy Research Foundation, Clorox, Danone, Danone USA, Dutch Mill, General Mills, JHeimbach, Kelley Drye & Warren, Kellogg, Kerry, Medscape, Nestle, New Chapter, Pepsico, Pfizer, Pharmavite, Probi, Procter & Gamble, Trouw Nutrition, Visalia Dairy Company, Williams Mullen, Winclove Probiotics and Yakult. D.J.M. declares personal fees for consulting for Bayer and Pharmavite. G.R. declares that he helped develop and commercialize probiotic strains GR-1 and RC-14, but has had no financial interest in them for over 10 years. He is Chief Scientific Officer for Seed, a company producing probiotic products. Over the past 3 years, he has consulted on probiotics with Acerus Pharmaceuticals, Altmann, Chr. Hansen, Danone, KGK Science, Kimberly-Clark, Metagenics and Seed. G.R.G. and R.A.R. declare no competing interests.

Publisher's note

Reviewer information
Nature Reviews Gastroenterology & Hepatology thanks H. Szajewska, and the other, anonymous, reviewer(s), for their contribution to the peer review of this work.