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EXPERT CONSENSUS DOCUMENT

The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics

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Abstract | In December 2016, a panel of experts in microbiology, nutrition and clinical research was convened by the International Scientific Association for Probiotics and Prebiotics to review the definition and scope of prebiotics. Consistent with the original embodiment of prebiotics, but aware of the latest scientific and clinical developments, the panel updated the definition of a prebiotic: a substrate that is selectively utilized by host microorganisms conferring a health benefit. This definition expands the concept of prebiotics to possibly include non-carbohydrate substances, applications to body sites other than the gastrointestinal tract, and diverse categories other than food. The requirement for selective microbiota-mediated mechanisms was retained. Beneficial health effects must be documented for a substance to be considered a prebiotic. The consensus definition applies also to prebiotics for use by animals, in which microbiota-focused strategies to maintain health and prevent disease is as relevant as for humans. Ultimately, the goal of this Consensus Statement is to engender appropriate use of the term 'prebiotic' by relevant stakeholders so that consistency and clarity can be achieved in research reports, product marketing and regulatory oversight of the category. To this end, we have reviewed several aspects of prebiotic science including its development, health benefits and legislation.

Improving human health through modulation of the microbiome is an evolving strategy that is part of a comprehensive, holistic approach to lifestyle wellness[1]. The rich, diverse microbial ecosystems inhabiting mucosal and cutaneous surfaces provide targets for approaches to maintain or improve health or to treat disease. The ability to shift the composition and metabolic signatures of these microbial populations is now possible, via dietary or non-dietary interventions[2,3].

Over 20 years ago, a class of compounds, termed prebiotics, were recognized for their ability to manipulate host microbiota to the benefit of the host[4]. At that time fructans (fructooligosaccharides (FOS) and inulin) and galactans (galactooligosaccharides or GOS) fit that category, with their effects acting through enrichment of *Lactobacillus* and/or *Bifidobacterium* spp. FOS and GOS currently dominate the prebiotic category as evidenced by numerous studies on their prebiotic effects.

Today, the prebiotic concept has expanded, in part, because of advances in tools for microbiome research (for example, high-throughput sequencing), which have improved our knowledge of the composition of the microbiota and enabled identification of additional substances influencing colonization. Concurrent with this progress is the realization that a broader range of beneficial microorganisms are affected by prebiotics and also that they might be effective at extraintestinal sites directly or indirectly[5]. Furthermore, the use of prebiotics has expanded to production and companion animals[6,7] and categories beyond food. Accordingly, researchers have advocated for reconsideration of the contemporary nature of prebiotics, which formed the aim of the consensus panel that was convened on 9 December 2016 in London, UK. The various aspects looked at in this review of evidence were: evolution of the term prebiotic; effects and selectivity; substrates that

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are prebiotics; metabolism of prebiotics; host benefits; companion animals; and guidance for producers, consumers and regulators. Herein, the term 'microbiota' refers to the collection of microorganisms in an ecosystem and 'microbiome' when genetic elements are also considered.

Methods

A panel of experts was organized by the board of directors of the International Scientific Association for Probiotics and Prebiotics (ISAPP), a non-profit collaboration of scientists dedicated to advancing scientific excellence in probiotics and prebiotics. ISAPP activities are determined by the board of directors, comprising global academic scientists. Through its Industry Advisory Committee, ISAPP incorporates industry scientists in its activities and raises funds to advance its mission. However, no input into this consensus panel process was provided by members of the Industry Advisory Committee. ISAPP functions as an independent, objective, science-based voice for the probiotic and prebiotic fields.

Panellists included experts involved with the original development of prebiotics and subsequent modifications of the definition. Specialties included microbiology, nutrition, biochemistry and clinical research in both humans and animals. To prepare, panellists developed a discussion outline and target questions. Several delivered brief presentations that addressed background and core issues. Discussion ensued for each issue until consensus was achieved. After the meeting, individual panellists wrote sections of the summary, which were compiled by G.R.G., M.E.S and G.R. into a draft report. This document was edited and agreed upon by all panel members, and finally by the ISAPP board of directors.

Evolution of the term prebiotic

In 1921, Rettger & Cheplin[8] described experiments with humans whose microbiota were enriched with lactobacilli following consumption of carbohydrates. The finding that the colon was dominated by anaerobes, many of which obtain energy by fermenting substrates from the diet[9,10], initiated research that played an important foundational part in many subsequent microbiome projects.

Although dietary oligosaccharides had long been used to impart health benefits, principally in Asia, the prebiotic concept was first defined in 1995 as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon" (REF. 4). The prebiotic concept was initiated to build on the probiotic concept, the most widely accepted definition of which was proposed in 2001 (REF. 11) and reaffirmed in 2014 (REF. 12). Prebiotics target human-associated and animal-associated microbiota with the goal of improving health. Whereas probiotics use live microorganisms, prebiotics are non-viable substrates that serve as nutrients for beneficial microorganisms harboured by the host, including administered probiotic strains and indigenous (resident) microorganisms. Thus, prebiotics differ from most dietary fibres such as pectins, cellulose and xylans, which encourage growth of a wide variety of gut microorganisms. Our meaning here is that a prebiotic should not be broadly metabolized, but elicit a metabolism biased towards health-promoting microorganisms within the indigenous ecosystem. The review by Simpson and Campbell[13] provides an overview of microbiota interactions and compares studies on fibre and prebiotics, concluding that prebiotics (particularly FOS and GOS) seem to promote increased abundance of bifidobacteria within the gut microbiota.

Most of the first prebiotics assessed in humans and used commercially were shown to stimulate *Lactobacillus* and *Bifidobacterium* specifically, but not pathogens such as certain members of the Clostridia class and *Escherichia coli*[14–16]. As these genera were commonly used as probiotics, this approach provided a commonality between probiotics and prebiotics. Thus, the prebiotic definition and the concept itself became imprinted in food, nutrition and microbiology fields[17]. In 2004, the definition of prebiotics was altered to "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (REF. 18). As per this definition, three criteria were required for a prebiotic: the ability to resist host digestion (for example gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption); that they are fermented by intestinal microorganisms; and that they selectively stimulate the growth and/or activity of intestinal bacteria associated with health and well-being. Thus, it was implicit that trials to demonstrate prebiotic effects should be performed in the target host. *In vitro* assessments designed to identify pathways or mechanisms would not confirm prebiotic status in the absence of studies providing evidence of health effects in the host.

However, as prebiotic concepts evolved, so too did their application to extraintestinal sites. The Food and Agricultural Organization (FAO) of the United Nations (UN) organized a Technical Meeting to update the definition of prebiotics in 2008. This panel proposed that prebiotics be redefined as "a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" (REF. 19). Here, selective fermentation was removed as a criterion, but in doing so the definition was criticized for not excluding antibiotics. Gibson et al.[20], 2 years later, defined the narrower category of 'dietary prebiotics' as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health".

In 2015, Bindels et al.[21] proposed that specificity requirements should be removed on the basis of reports showing that multiple taxa, rather than particular species, were enriched by prebiotics[22]. This proposal led to another definition of a prebiotic as "a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus, conferring a beneficial physiological effect on the host" (REF. 21). This definition limited prebiotics to interactions with the gut microbiota (excluding extraintestinal sites such as vagina and skin) and removed the requirement for selective fermentation. Selectivity with respect to microbial fermentation is viewed by this panel as key to the prebiotic concept. Importantly, however, this definition emphasized the functional effects of prebiotics on the microbiota.

Given the proposed definitions already described, as well as others, the need for a consensus definition was evident[23]. This need was amplified by views that the prebiotic concept required clarification on specificity, mechanisms of effect, health attributes and relevance, with some authors being critical of concepts already put forward and its approaches[24–26]. Thus, the current ISAPP consensus panel now proposes the following definition of a prebiotic: a substrate that is selectively utilized by host microorganisms conferring a health benefit (BOX 1). See BOX 2 for additional rationale used to adopt this new definition.

Prebiotic effect and selectivity

Prebiotics are not the only substances that can affect the microbiota[10] (FIG. 1). The criterion of selective utilization distinguishes prebiotics from many of these other substances[16]. Hopefully, the new definition will readily enable a developer to know whether a new substrate fits the prebiotic category.

In previous iterations of the term prebiotic, 'selectively' was interpreted as referring mostly to lactobacilli and bifidobacteria. Specific stimulation of bifidobacteria (bifidogenesis) was considered a prebiotic effect. Early research on gut microbial ecology was based on culture methods, which we now know were insufficient to reveal the complexity of prebiotic-induced microbial changes. Molecular-based methods, which have since identified a broader range of members of the gut microbial community, have enabled the appreciation that more bacterial genera might utilize some prebiotic substrates, by fermentation and other metabolic pathways. These microorganisms can vary depending upon the host and ecosystem under consideration. Hence, it is recognized today that prebiotic effects probably extend beyond bifidobacteria and lactobacilli, but to meet the selectivity criterion of a prebiotic, the range of microorganisms affected must be limited. To this end, in two human studies that used high-throughput sequencing, bifidobacteria responded to prebiotic use[22,27]. However, other groups such as *Faecalibacterium prausnitzii* also increased in abundance in one trial[22] and in another study *Anaerostipes* spp. were additionally elevated, whereas *Bilophila* spp. decreased[27]. Both studies used high-throughput sequencing to confirm selectivity of the prebiotic fermentation. Selectivity does not necessarily mean effects on just one microbial group; a selective effect could extend to several microbial groups, just not all. A prebiotic, in addition to having a selective effect on microorganisms, must also evoke a net health benefit. The guiding principles are that microorganisms affected and metabolites produced are considered to be beneficial and linked to a defined health aspect.

Envisaging every scenario is challenging. But, for example, is a product a prebiotic if its intake increases microbial production of butyrate? Short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate, and some other compounds, are recognized as having mechanistic links to health outcomes[28,29]. If the effect is a measurable benefit to host health, distinct from a control, it would constitute a 'prebiotic effect'. To verify that the product itself is prebiotic, experiments would have to demonstrate that the product is selectively utilized, in this case by showing that a defined range of butyrate-producing microorganisms grow because of the product. Alternatively, the product might stimulate growth of other members of the microbiota, releasing metabolites that in turn stimulate butyrate production by other microorganisms. This phenomenon could constitute a 'cross-feeding effect'. The net result is still selective in that propagation of

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Box 1 | Main conclusions of the consensus panel regarding prebiotics

- The definition of a prebiotic has been modified to 'a substrate that is selectively utilized by host microorganisms conferring a health benefit'
- Although most current prebiotics are administered orally, they can also be administered directly to other microbially colonized body sites, such as the vaginal tract and skin
- Health effects of prebiotics are evolving but currently include benefits to the gastrointestinal tract (for example, inhibition of pathogens, immune stimulation), cardiometabolism (for example, reduction in blood lipid levels, effects upon insulin resistance), mental health (for example, metabolites that influence brain function, energy and cognition) and bone (for example, mineral bioavailability), among others
- We acknowledge that definitive proof of causality is difficult to provide. However, a human or animal study showing a change in heath markers or symptoms after a specific influence on the microbial population (that is, a blinded placebo-controlled trial with appropriate exclusion and/or inclusion criteria) then it is reasonable to assume that the two are causally related
- Currently established prebiotics are carbohydrate-based, but other substances such as polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids might fit the updated definition assuming convincing weight of evidence in the target host
- The beneficial effect(s) of a prebiotic on health must be confirmed in the target animal for its intended use and mediated through the microbiota

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particular microorganisms led to this overall health effect. However, if pathogenic microorganisms are involved in butyrate generation and a negative consequence occurs for the host, then it cannot be termed a prebiotic. This distinction makes it important to determine both function and composition of the gut microbiota involved.

Similarly, prebiotics for use in the gut microbiota of humans should not form gas distension issues after ingestion; as such, their fermentation must be selective and preferably include genera that are not gas formers (such as *Clostridium*). This consideration points unequivocally towards the need for selective metabolism. Notably, neither bifidobacteria nor lactobacilli manufacture gas in their metabolism[16].

Moreover, it is implicit that such influences on host health be determined in mixed microbial ecosystems containing the full microbiota of interest (that is, *in vivo*). Making inferences on prebiotic effects from pure or co-culture experiments is inadequate. Similarly, any conclusion regarding prebiotic activity must be based on an assessment of the full microbial diversity, not simply increased abundance of gut bifidobacteria or lactobacilli, for example. The best techniques available need to applied, particularly as the microbiome field has benefited greatly from molecular-based technological advances. These techniques would include high-throughput sequencing, including metagenomics, which demonstrates quantifiable changes in the microbiota.

Similarly, metabonomic assessments, such as NMR or mass spectrometry, in appropriate biological materials can identify metabolic responses to prebiotics and help determine concomitant functionality of the microbiota.

Substrates that are prebiotics

A number of fermentable carbohydrates have been reported to convey a prebiotic effect, but the dietary prebiotics most extensively documented to have health benefits in humans are the non-digestible oligosaccharides fructans and galactans[30]. These oligosaccharides are preferentially metabolized by bifidobacteria[16]. A phenomenon explained by structure to function relationships; the linkage bonds in FOS and GOS can be readily degraded by degraded by β-fructanosidase β-galactosidase enzymes, respectively, which are prevalent in bifidobacteria. This genus also seems to preferentially metabolize the chain length size typical of oligosaccharides; that is, a degree of polymerization (DP) between 4 and 30 (REFS 31,32). Importantly, having the appropriate transport machinery to capture and deliver these substrates into the microbial cytoplasm is a key requirement and contributes to the selectivity of prebiotics in the target sites[33] and emphasizes their ability to do so in a competitive environment in mixed culture ecosystems such as the human gut.

Substrates that affect composition of the microbiota through mechanisms not involving selective utilization by host microorganisms are not prebiotics. These substrates would include antibiotics, minerals, vitamins and bacteriophages, which are not growth substrates, even though their intake might alter microbiota and metabolic composition.

Certain soluble fermentable fibres are candidate prebiotics[34], and some other types of dietary fibre can be prebiotic, provided that they are selectively utilized by the host microbiota and promote health. Categorizing fibres as prebiotics is complicated by the fact that a dietary fibre can be a prebiotic in one host but not another. For example, cellulose can be considered a prebiotic in ruminants but not in humans, as the latter's intestinal microbiota only poorly utilize β(1→4) linked D-glucose polysaccharides[35]. Furthermore, a substrate qualifying as a prebiotic might also depend on the target site. For example, xylitol can be considered as a prebiotic in the oral cavity, but has not been shown to be prebiotic elsewhere[16,18].

Among the first group of substances recognized for their ability to influence gastrointestinal health were the oligosaccharides present in human milk. Human milk oligosaccharides (HMOs) are particularly important for the development of the newborn baby's intestinal microbiota and metabolic and immunological systems, which have consequences for health later in life[36,37]. Consumption of mother's milk containing these HMOs clearly increases the proportion of HMO-consuming Bifidobacteriaceae and Bacteroidaceae[28]. *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) is the only *Bifidobacterium* spp. that has specifically evolved machinery to degrade the complete repertoire of HMOs. Other *Bifidobacterium* spp. predominant in adults, mainly *B. longum* subsp. *longum*, *B. adolescentis* and *B. lactis*, lack many of the enzymes necessary to directly utilize HMOs effectively[38,39].

Box 2 | Justification for the new definition of prebiotics

- It is a straightforward definition that avoids unnecessary technical jargon.

- It clarifies that prebiotic targets extend beyond stimulation of bifidobacteria and lactobacilli, and recognizes that health benefits can derive from effects on other beneficial taxa including (but not limited to) *Roseburia*, *Eubacterium* or *Faecalibacterium* spp.

- The term 'substrate' was chosen for its meaning of a substance on or from which an organism obtains its nourishment (for example, through fermentative breakdown of the substrate). This term aligns with the word 'utilized' and implies 'for growth through nourishment', therefore excluding viable microorganisms and antimicrobial agents as prebiotics.

- Prebiotics rely upon microbial metabolism. Non-microbial effects do not fit with our current classification. For the latter, these effects have tended to be researched in situations in which a resident microbiota is devoid or compromised. To confirm prebiotic traits, studies in the same species as the intended use are required.

- Prebiotics require selective utilization by live host microorganisms, not simply enzymes or bioactive chemicals, in a manner that sustains, improves or restores host health. Although many microorganisms might be able to breakdown a given substrate, it is the resultant health benefit to the host owing to selective utilization by microorganisms that enables it to be termed prebiotic. The actual mechanism of conferring benefit might also be mediated by microbial metabolic products. As such, both the microbiota changes and metabolites should be investigated, together with health outputs.

- It allows a prebiotic to invoke changes to any host microbial ecosystem, not just the gut. However, dietary prebiotics should still be non-digested by the host but utilized by the microbiota.

- Both prebiotic safety and use at appropriate dose are implicit in this definition. An appropriate dose must be sufficient to generate a prebiotic effect, but not too high to induce unwanted or adverse effects such as excessive gas formation or non-selective utilization. The 'adequate' dose will vary depending upon the microbial ecosystem and associated metabolic effects.

- Demonstration of health benefits in well-controlled studies in the target host is required.
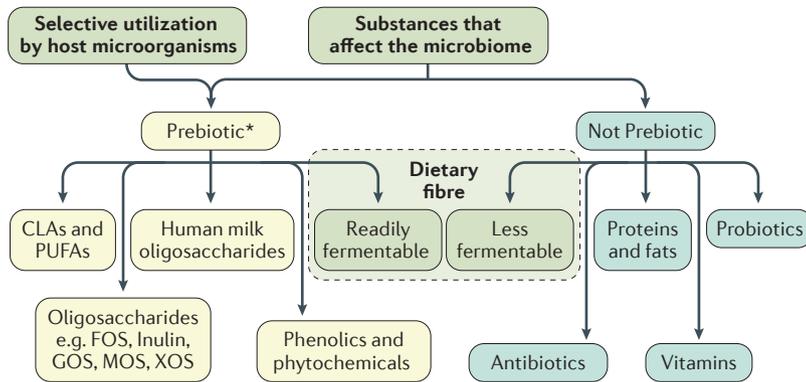

Figure 1 | **Distinguishing what is considered a prebiotic with the proposed definition.** Prebiotics must be selectively utilized and have adequate evidence of health benefit for the target host. Dietary prebiotics must not be degraded by the target host enzymes. *The figure shows candidate as well as accepted prebiotics in that levels of evidence currently vary, with FOS and GOS being the most researched prebiotics. CLA, conjugated linoleic acid; PUFA, polyunsaturated fatty acid; FOS, fructooligosaccharides; GOS, galactooligosaccharides; MOS, mannanoligosaccharide; XOS, xylooligosaccharide.

HMOs might indirectly affect composition of the intestinal microbiota by modulating immune responses and also have metabolism-independent mechanisms of action in the infant gut[40]. In particular, fucosylated and sialylated HMOs can prevent adhesion of pathogens to the intestinal epithelium through a competitive mechanism that ultimately protects the neonate from infection[41,42]. The main issues for this discussion are the following. Is there evidence that HMOs confer a health benefit in humans through the host's microbiota selectively utilizing them, and therefore fulfilling the prebiotic definition? And if compounds equivalent to HMO (or bovine milk oligosaccharides, BMOs) were to be produced by enzymatic synthesis, fermentation or extraction, could they still be considered as prebiotic?

The ability of HMOs, BMOs or synthesized compounds to act as a substrate for the selective growth of beneficial bacteria, such as *Bifidobacterium* spp., would be supportive evidence of a prebiotic nature[43]. To confirm their status as a prebiotic, a controlled human study showing selective growth of bifidobacteria resulting in a health benefit is also needed. However, the use of such compounds for *in vivo* studies is limited to only a few reports. In one study, a chemically synthesized compound, 2′-fucosyllactose (2′FL), equivalent to the naturally occurring 2′FL in HMO, was added to formula milk along with GOS. Although safe for infants, the 2′FL treatment provided no net difference in weight, length, head circumference and other measures compared with human milk over a 4-month period[44]. In another study by the same group, infants fed formula with 2′FL plus GOS had immune responses similar to breast-fed infants in that both groups had lower levels of inflammatory cytokines than infants fed formula plus GOS[45]. However, effects on the microbiota were not reported in this study. In a third study, 2′FL and another synthesized HMO, lacto-*N*-neotetraose, were administered to adults[46]. The treatments were well tolerated and led to an increase in abundance of *Bifidobacterium* spp. Collectively, these studies provide an incomplete assessment of the prebiotic properties of these synthesized

versions of HMOs. Although 2′FL is utilized by *B. infantis* as well as some strains of *B. longum* subsp. *longum* and *B. breve*[46,47], the ecological context (that is, infants versus adults) might dictate whether these HMOs are indeed prebiotic. Moreover, having structural equivalence to specific HMOs does not infer functional equivalence to the constellation of HMOs in milk[48]. Thus, for now, it is acceptable to state that some HMOs are candidate prebiotics.

Plant polyphenols constitute a class of compounds that can also meet the criteria of prebiotics, although far more studies in the target host are required. An estimated 90–95% of dietary polyphenols are not absorbed in the small intestine and, therefore, reach the colon[49] where they undergo extensive biotransformation by the colonic microbiota. Increasing evidence indicates that health benefits associated with polyphenol consumption depend on microbial utilization and the metabolites produced, rather than on parent compounds[50].

This evidence expands the prebiotic concept beyond non-digestible oligosaccharides such as FOS and GOS. However, evidence for these emerging prebiotics is scarce relative to the fructans and galactans[16] and more studies measuring health benefits are required to fulfil their prebiotic status.

Prebiotic utilization and host health

As selective utilization of a prebiotic by host microorganisms is key to its physiological effects, metabolic results of this utilization must, by deduction, be the main drivers. Some organic acids, for example, are principal end products of non-digestible carbohydrate or dietary fibre fermentation by host microorganisms. The main SCFAs (≥95%) generated mostly in the colon (humans) and caecum (rodents) as a result of several bacterial metabolic pathways are acetate (two carbon, C2), propionate (C3)) and *n*-butyrate (C4). These SCFAs are crucial for intestinal health and their activity can subsequently influence sites distant to the gut, with different SCFAs having varying functions. SCFAs can modulate certain aspects of metabolic activity including colonocyte function, gut homeostasis, energy gain, the immune system, blood lipids, appetite and renal physiology, as reviewed elsewhere[16,51,52].

In a study published in 2017, ^{13}C-labelling was used to show that colonic-administered acetate, propionate and butyrate were systemically available at 36%, 9% and 2%, respectively, with conversion of acetate into butyrate (24%) by the colonic microbiota[53]. Bifidobacteria, often stimulated by specific prebiotics, do not produce butyrate, so a probable scenario is that cross-feeding by other bacteria must have resulted in production of this SCFA. Much has been reported about the benefits of butyrate in the gut and beyond[54], leading to the potential of known butyrate producers such as *Faecalibacterium prausnitzii*, *Eubacterium rectale* or *Roseburia* spp. as possible probiotics and, therefore, new prebiotic targets. By contrast, in the vagina, butyrate formation is more equivocal as 2-hydroxyisovalerate and γ-hydroxybutyrate have been associated with bacterial vaginosis[55]. Rather, lactic acid production and an increase in IL-10 levels might be beneficial, indicating that prebiotics might be functional in the vaginal environment, because of their effects in

the gut[56]. Lactulose, which has potential benefits in the gut and vagina, can increase lactic acid levels and decrease β-glucuronidase activity, considered beneficial for the host[57]. Owing to the anatomical proximity of rectum to vulva, some microorganisms capable of utilizing prebiotics in the gut are also present in the vagina, including *Bifidobacterium* and *Lactobacillus* spp.[58–60].

Bile salt hydrolases are a family of enzymes produced exclusively by enteric microorganisms as a form of defence against their harsh, bile-rich environment. Bile acid transformation and/or metabolism in the gut is performed by a number of species, including *Lactobacillus,* with known beneficial effects on the host. Joyce & Gahan[61] demonstrated that elevated bile salt hydrolase activity could promote reduced weight gain in mice and influence host pathways involved in lipid metabolism, peripheral circadian rhythm, gut barrier function and immune homeostasis. One study[62] showed that enhanced bacterial deconjugation of taurine from primary bile acids occurred in the presence of prebiotic inulin, supporting the theory that faecal bile acid profiling might be a useful biomarker for the intake of prebiotics in mice and potentially also in humans.

The net result of prebiotic utilization within the gut could also extend to health benefits elsewhere in the body. For example, GOS stimulated growth of bifidobacteria in the mouse gut led to modulation of cortical IL-1β and 5-HT$_{2A}$ receptor expression and reduced anxiety levels[63], as well as enhancing brain barrier function in obese mice[64]. Similarly, utilization of prebiotics might also reduce blood ammonia levels and improve psychometric tests in patients with hepatic encephalopathy[65], presumably through the formation of relevant bacterial metabolites. Study findings suggest that prebiotics can reduce the development or severity of atopic dermatitis and eczema in children, presumably mediated by alterations to bacterial growth and interactions with the developing immune system, beginning in the gut[66,67]. The ability to increase water retention on the skin and reduce erythema formation is an emerging attribute of GOS ingestion, as reported in mouse studies[68]. On the skin, application of a prebiotic might stimulate changes in bacterial[69] or fungal[70] profiles perhaps by targeting epidermal growth factor receptor. The health consequences of this approach are currently unclear, but might include psoriasis, acne, dermatitis, eczema and wound development[66,67,71].

Studies in mice have shown that oligofructose (a fructan) reduced diet-induced obesity, diabetes, hepatic steatosis and inflammation by mechanisms linked with changes in specific gut microorganisms and metagenomics functions of bacteria[72]. A study in rats suggested that oligofructose consumption might normalize the metabolomic signature of insulin resistance in obese rats and reduce obesity in offspring[73]. The ability to enhance secretion of satiety hormones peptide YY and glucagon-like peptide-1 might be an associated attribute of prebiotic intervention and related SCFA production[74–76].

In the mouth, compounds such as algal lectins, cranberry juice and cocoa polyphenols have been used to reduce the abundance of cariogenic bacteria. However, these substrates do not function through being selectively utilized by beneficial host microorganisms in the mouth, so they are not prebiotics[77]. Short-chain GOS and long-chain FOS have been administered orally with *B. breve* and were found to increase peak expiratory flow and reduce systemic production of type 2 T-helper cytokines after allergen challenge in adults with allergic asthma[78]. The proposed mechanism, whereby microbial utilization of GOS and FOS, presumably in the intestine, could lead to immunological modulation that enabled the host to cope better with allergen exposure in the lungs, was not identified. In the nose and upper respiratory tract, bacterial species can be manipulated by prebiotics to influence health through immune reactions[79] or competition with aetiological agents of disease[80].

Conferring a health benefit

The ultimate goal of any intervention, including prebiotics, is to improve health and, therefore, reduce the risk or burden of disease. The most effective approaches are those that rely on prevention and recognize that early-life strategies that promote a resilient, diverse and healthy microbiota have greatest long-term potential to benefit health[81,82]. Evidence for the important relationship between the structure and function of the microbial community, prebiotic use and host health has accumulated rapidly over the past decade[20,23,30]. To satisfy the criterion of conferring a health benefit, controlled studies establishing direct links between the prebiotic and health are needed in the target host. The level of evidence should be commensurate with the strength of the health benefit claim. To date, numerous randomized controlled trials have shown health benefits of a variety of prebiotics across a range of populations, from healthy individuals to those with acute and chronic diseases. These and other human studies have been summarized elsewhere and are not discussed in detail here, but key examples are listed in TABLE 1 (REFS 16,65,67,83–120).

Importantly, the effects of any intervention will be affected by a variety of host and environmental factors[121]. Thus, the effects of prebiotics have the potential to vary widely on an individual basis. Microbial utilization of prebiotics can only occur if the appropriate bacteria are a component of the host's microbiota. This aspect might explain individual differences in responsiveness and in the outcomes of clinical trials. Host factors include variation in genetic predisposition to diseases (across multiple loci) as well as specific polymorphisms in microbial recognition pathways that can influence colonization and its biological effects[16]. A number of environmental factors, including mode of delivery and early feeding, antibiotics, disease status and adult diet, can influence the human microbiome and possibly the effects of prebiotic supplementation[122–125].

Application to benefit animals

Prebiotics have been studied and used for companion animals, livestock, poultry and aquaculture. The inherent differences among animal species with regards to living environment, anatomy and physiology, dietary composition and reliance on the gut microbiota for energy, must be considered when evaluating the effect of prebiotics on animal health[126].

Table 1 | **Health end points targeted in human trials of orally administered prebiotics**

Health end point	Prebiotic used	Refs
Metabolic health: overweight and obesity; type 2 diabetes mellitus; metabolic syndrome and dyslipidaemia; inflammation	Inulin, GOS, FOS	22,74,75,83–90
Satiety	FOS	75,76,90–92
Stimulation of neurochemical-producing bacteria in the gut	GOS	93,94
Improved absorption of calcium and other minerals, bone health	Inulin, FOS	95–99
Skin health, improved water retention and reduced erythema	GOS	100,101
Allergy	FOS, GOS	102–105
IBD	Inulin, lactulose	106
Urogenital health	GOS	107
Bowel habit and general gut health in infants	GOS, FOS,	108,109
Infections and vaccine response	FOS, GOS, polydextrose	110–114
Necrotizing enterocolitis in preterm infants	GOS, FOS	115
IBS	GOS	116
Traveller's diarrhoea	GOS	117
Constipation	Inulin	118,119
Immune function in elderly individuals	GOS	56,120

FOS, fructooligosaccharides; GOS, galactooligosaccharides.

TABLE 2 provides examples of the use of prebiotics in animals. Dogs and cats evolved as *Carnivora* eating diets high in protein and fat but low in fibre[126]. They are non-ruminants with short, simple gastrointestinal tracts that have little capacity to ferment non-digestible substances, which predominantly occurs in the colon[126]. Nevertheless, some health benefits have been achieved with prebiotic administration such as reduced infections, improved insulin sensitivity and better faecal consistency[127–131].

Prebiotics such as oligosaccharides of fructose, mannose and chitin protect piglets against high environmental stressors (such as antibiotics, etc.) and pathogen loads, including faecal *E. coli* shedding, and reduced infection-associated responses to *Salmonella enterica* serovar Typhimurium infection or porcine reproductive and respiratory syndrome virus[132–135].

Calves are born in a pre-ruminant state and function as non-ruminants until the rumen and other compartments of the stomach fully develop[136]. During the first few weeks of life, or longer in the case of veal calves maintained on low-roughage diets (that is, low in fibrous material), prebiotics can be used to increase growth, improve feed conversion ratio, reduce the incidence and severity of scours (diarrhoea) or reduce the incidence of respiratory diseases[136–139].

Poultry, which are used primarily for the production of meat or eggs, include landfowl (for example, chickens, turkeys and quail) and waterfowl (for example, duck or geese) species, respond to prebiotics despite most having a fairly short midgut and hindgut that includes a short, straight colon and twin caeca[140]. Dietary prebiotics, including inulin, yeast cell wall extracts, lactulose and GOS are usually fed at concentrations up to 0.2% (weight/volume) of diet[140–146].

Farmed aquatic species include finfish and shellfish. Although anatomy varies among carnivorous (for example, turbot), omnivorous (for example, catfish) and herbivorous (for example, sturgeon) species, all fish have a fairly simplistic and short gastrointestinal tract[147–149]. The short length and simple structure (lack of special adaptations) of the fish gut results in the rapid transit of digested material, limiting the time available for microbial or prebiotic activity. Effective prebiotic doses in aquatic host species are typically in the range of 1–3% (weight/volume) of diet[147–149].

Horses are large non-ruminant herbivores that rely heavily on microbial fermentation for energy, with more than half of their maintenance energy requirement coming from microbial fermentation occurring in their enlarged caecum and colon[126]. As their typical diet is high in roughage and feedstuffs that are consumed throughout the day, prebiotic interventions might help improve effectiveness of fermentation[150–152].

Guidance for stakeholders

Developing a consensus definition of prebiotic is useful for many stakeholders (FIG. 2), whose responsibilities are discussed here. Agreement on this definition will reduce misinformation and confusion among consumers and health-care providers, facilitate sensible regulatory approaches, and provide common terminology and scope for future prebiotic research.

Consumers. This consensus definition should enable consumers to understand the terms used on product labels. Proper use of the terms by all stakeholders will help avoid misleading messaging. Although consumers might not be expected to understand the mechanistic details for how prebiotics function to improve health, our proposed definition should be readily appreciated. Individuals can respond variably (due to their habitual diets, host microbiota, host genetics) to different

Table 2 | **Use of prebiotics in animals**

Animal species	Gastrointestinal tract anatomy	Prebiotic used	Outcomes	Refs
Dog	Short, simple gastrointestinal tract; hindgut (colonic) fermentation	• scFOS (obese dogs) • scFOS and MOS	• Improved insulin response • Reduced pathogen infection	127–129
Cat	Short, simple gastrointestinal; limited hindgut fermentation	Fructans and galactans	Increased levels of organic acids, increased bifidobacteria; modulation of glucose and amino acids metabolism	130,131
Piglets	Caecal and colonic fermentation	Soy polysaccharides, FOS, chito-oligosaccharides and MOS	Reduced pathogen load; improved growth	132–135
Pre-weaned calves	Pre-ruminant state	Cello-oligosaccharides, galactosyl-lactose, yeast cell wall extracts and MOS	Reduced pathogen load (gastrointestinal tract and lung); improved weight gain	136–139
Poultry	Short midgut; hindgut includes a short, straight colon and twin caeca	Inulin, yeast cell wall extracts, lactulose and GOS	Improved growth; reduced infection; improved bone density and egg quality	140–146
Farmed fish	Simple, short gastrointestinal tract	FOS, GOS and MOS	Improved survival rate; growth rate; pathogen resistance	147–149
Horses	Substantial hind-gut fermentation; large caecum, colon	• Yeast cell fermentation products and scFOS • scFOS (obese horses)	• Increased nutrient digestibility; reduced faecal pH levels and SCFA fluctuations in production • Improved insulin sensitivity	150–152

FOS, fructo-oligosaccharides; GOS, galactooligosaccharides; MOS, mannanoligosaccharides; SCFA, short-chain fatty acid; scFOS, short-chain fructooligosaccharides.

prebiotics. This aspect dovetails with the concept of individualized nutrition, which should be understood by consumers.

Media and publishers of scientific papers. The media (press, TV, web-based and others) should avoid use of headlines that misrepresent results. Presentation of association studies as if they contribute to an understanding of causality can be especially misleading. When discussing results of a single study, how that study fits into the totality of evidence for that topic should be reported, including null results. The media should use the term prebiotic consistent with this proposed definition.

Regulators. Regulators have primary responsibility for ensuring safety of marketed products and protecting consumers from fraudulent marketing. To accomplish these goals, they are bound by statutes and regulations adopted in their respective regions. Acceptance by regulators of the consensus definition of prebiotic would make it clear what can be expected of these substances from a scientific basis, and whether the term is being used appropriately. For example, most prebiotics for the gut require an oral dose of upwards of 3 g per day to elicit an effect[16]. Products containing doses lower than this level should not be called prebiotics, unless such a low dose has been proven to elicit selective effects upon the microbiota and concomitant health aspects. Incorporating a health benefit in the definition gives a tangible end point for producers and regulators alike to use in their assessment of whether a novel product fulfils the criteria.

Scientists. Scientists have the responsibility of considering all aspects of research on prebiotics (structural biochemistry, clinically relevant end points, effective dose, mechanisms of action, analytical methods) and consolidating findings such that a clear description of outcomes can be attained. Future prebiotic research should strive to confirm causality between an observed health benefit and microbiota-mediated mechanisms. This confirmation of causality has been challenging to achieve and some assumptions might be necessary, as is the case for most pharmaceutical interventions. To this end, well-controlled, placebo, blinded *in vivo* studies that exploit the latest multi-omic technologies are necessary. For example, in the case of a dietary prebiotic for humans, a full assessment of gut microbiota changes using robust molecular procedures that are fully and accurately quantifiable is required, such that selective substrate use can be ascertained. This analysis would be coupled with metabolic assessments of functionality (for example, metabonomics applied to blood, urine and faeces). In patients, symptomology should be determined, and in healthy or 'at-risk' populations reliable biomarkers of beneficial effects must be identified and measured. These biomarkers could include immunological changes, inflammatory mediators, serum lipid levels, genotoxicity, toxicity and cognitive function, among others, as appropriate to the study population. The study population must be reflective of the condition being researched, and an appropriate power calculation used to determine volunteer numbers. An effective prebiotic dose and duration must be established to compare effects. The test delivery vehicle (for example, foods such as cereal, bread or juices) should be considered such that prebiotic potential is not compromised. Exclusion and inclusion criteria are applied to control for fluctuations in diet and other major lifestyle changes. Following that, if the only discernible correlation is an improvement in health indices with selective microbiota changes (composition and function) then it could be assumed that two are inter-related and driven

by the prebiotic. When communicating results, scientists should be careful to present data in a manner that does not mislead readers.

Suppliers or manufacturers of prebiotics. Suppliers and manufacturers have the responsibility to accurately characterize the identity of their prebiotics and conduct research to evaluate health benefits and safety. They should be committed to high-quality, controlled, non-biased studies that assess effects on clinically relevant outcomes with associated peer-reviewed publication of the findings. They need to provide accurate technical information to end-product manufacturers.

End-product manufacturers. Producers of consumer products have a special responsibility to formulate and label prebiotic products in a manner that is true to the definition proposed herein, does not overstate the strength of evidence for health benefits and is consistent with dose and form used in efficacy studies. Producers can contribute by sponsoring research on health benefits of their final products. Advertising must be consistent with scientific definitions, not overstate the strength of evidence for health benefits and adhere to regulatory standards.

Health-care providers and standards or recommendation-setting organizations. By providing compelling data that prebiotics can improve health, it is hoped that clinical organizations will accept and use the new definition, review the data in totality and develop evidence-based recommendations. This approach will help health-care providers to make decisions about clinical use in the absence of formal recommendations (based upon their own risk–benefit analysis).

Further regulatory considerations

We anticipate that future prebiotic products will expand current applications, include products administered to many body sites and be developed as non-conventional (or novel) foods, pharmaceuticals or other categories. In this section further insights into regulatory considerations in two jurisdictions are provided as examples, but the way that prebiotics are regulated will differ in other countries.

European Union. In the European Union (EU), any health message carried by food requires assessment of the science by the European Food Safety Authority (EFSA) and authorization by the European Commission. Some prebiotic health claims have been approved, for example chicory inulin[153].

Inulin, FOS and GOS were used in the EU before 1997 and are considered safe food ingredients. However, prebiotic substances created after 1997 are considered novel and require safety clearance, a designation given, for example, to specific HMOs. To date, only one prebiotic, chicory inulin, has received an EU health claim: "Inulin improves bowel function" (REF. 153). This approval was based on demonstration of a cause–effect relationship between consumption of the non-fractionated mixture of monosaccharides (<10% of total carbohydrate), disaccharides, inulin-type fructans and inulin extracted from chicory with a mean DP ≥9, and maintenance of normal defecation by increasing stool frequency. Additional product approvals hopefully will be forthcoming, once relevant evidence is available, aided by the contents of this consensus document.

When prebiotics are considered to be novel foods, challenges arise to assessments as a food or individual ingredient. The EU considers HMOs added to a food as novel food ingredients, a legal construct determined by law[154]. A FOS or GOS with a markedly altered DP or with a different source or production method might be regarded as a novel food. An additional factor in the EU is the new consideration of safe history of use in countries outside the EU[154].

USA. Prebiotics is not yet a term recognized by the FDA. Prebiotics are regulated based on the category of product their intent and design dictates. Most prebiotics are sold as ingredients for foods (including infant formula) or are dietary ingredients in dietary supplements. The FDA issued an updated guidance to industry on the new dietary ingredient notification process in 2016 (REF. 155). Other regulatory categories that might apply to prebiotics are medical foods, drugs, cosmetics or devices developed for humans or animals. Changes to fibre labelling regulations in the USA in 2014 (in part owing to the different methods of analysis of fibre worldwide) will probably affect carbohydrate-based prebiotics[156].

In the past, various analytical methods determined fibre levels in foods. Prebiotics, detected as soluble fibre, could be listed as fibre on the nutrition facts label. Under the new regulations, this listing will not be allowed. Fibre has been redefined to be soluble and insoluble non-digestible carbohydrates (with three or more monomeric units) and lignin that are intrinsic and intact in plants, and certain isolated and synthetic non-digestible carbohydrates (with three or more monomeric units). Some prebiotics, such as inulin, fall under the latter category, but even so were not granted status as a fibre by the FDA. The new rules require that for a prebiotic to be listed as fibre, it must confer a beneficial physiological effect and this evidence must be submitted to the FDA either though the citizen
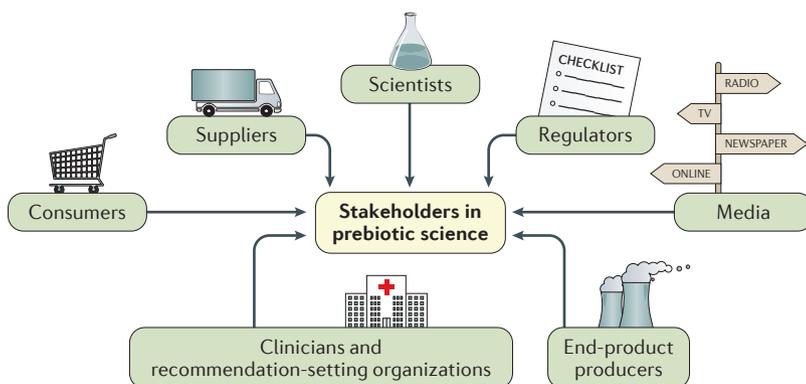

Figure 2 | **Stakeholders with an interest in prebiotic science.**

petition process or the health claims petition process for the FDA to authorize the health claim. The FDA has promised further guidance on this topic.

Conclusions

This paper describes conclusions of a consensus panel of experts regarding a definition of prebiotic and the rationale for that definition. It is hoped that this new definition and explanation will clarify what is required to call a substance a 'prebiotic'. Given that differences exist across animal species, prebiotic efficacy, safety and appropriate dosing should be demonstrated for the specific target host.

In conclusion, prebiotics have the potential to improve human and animal health and reduce risk of diseases mediated by microbiota aberrations. The field would greatly benefit from research focused on mechanisms of action, characterizing responders or non-responders, understanding how structure relates to function of prebiotic substances and correlating that function to health outputs. The use of prebiotics to improve health cannot be, and should not be, viewed in isolation, and will be part of a wider approach for healthy nutrition and lifestyle. The capacity exists for prebiotics to be used therapeutically in the management of disease and to preventively promote health.

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