OPINION

Towards a more comprehensive concept for prebiotics

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Abstract | The essential role of the gut microbiota for health has generated tremendous interest in modulating its composition and metabolic function. One of these strategies is prebiotics, which typically refer to selectively fermented nondigestible food ingredients or substances that specifically support the growth and/or activity of health-promoting bacteria that colonize the gastrointestinal tract. In this Perspective, we argue that advances in our understanding of diet–microbiome–host interactions challenge important aspects of the current concept of prebiotics, and especially the requirement for effects to be 'selective' or 'specific'. We propose to revise this concept in an effort to shift the focus towards ecological and functional features of the microbiota more likely to be relevant for host physiology. This revision would provide a more rational basis for the identification of prebiotic compounds, and a framework by which the therapeutic potential of modulating the gut microbiota could be more fully materialized.

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Introduction

The trillions of microorganisms that reside in the gastrointestinal tract of humans and other mammals (the gut microbiota)—most of which are bacteria, but archaea, fungi, and protozoa are also present—maintain a symbiotic relationship with their host species, playing a critical part in biological processes such as nutrient utilization, resistance against infections, maturation of the immune system and host metabolism.[1,2] Depending on the provision of adequate substrates, gut bacteria can generate metabolites (for example, bile acid derivatives, vitamins and organic acids such as branched-chain fatty acids and short-chain fatty acids [SCFAs]) that influence local and/or systemic host physiology. Despite these beneficial attributes, the gut microbiota is a contributing factor in several infectious, metabolic and immune-mediated pathologies, such as *C d , d ff c e* and *Ca bac e ,* infections,[3,4] IBD,[5,6] colon and liver cancers,[7,8] obesity and diabetes,[9–14] malnutrition,[15,16] cardiovascular disease,[17] autoimmune arthritis,[18,19] chronic kidney disease,[20] multiple sclerosis[21] and food allergies.[22] Animal models have provided evidence of a causative role of the gut microbiota in these diseases and have been successfully used to elucidate mechanisms by which these microorganisms influence disease outcomes.[23,24] The extent to which the gut microbiota is clinically relevant to human diseases is not as well established due to experimental limitations. Nevertheless, diseases with an established role of the microbiota in animal models are often associated with an alteration of gut microbiota composition in humans, which is referred to as dysbiosis.[25] Some dysbiotic patterns, such as a reduction in diversity, bloom of pathobionts and reduction of SCFA producers and/or bacteria with anti-inflammatory properties, occur in many diseases and might contribute to pathologies.[25] Although questions remain on cause and effect relationships, the information obtained from basic research creates a compelling case for the development of strategies that target the gut microbiota and, ideally, reverse dysbiotic patterns.[25–27]

The idea to change the human microbiota to improve health was proposed more than a century ago[28] and, today, it encompasses an entire spectrum of therapeutic tools, from transplanting an entire faecal microbiota to introducing single microorganisms or consortia of such organisms (probiotics).[26] Another important tool is the provision of growth substrates for resident microorganisms to induce compositional or metabolic changes, which incorporates the concept of prebiotics.[29] Strong rationales exist for increasing the supply of nondigestible substrates for bacterial fermentation to the gastrointestinal tract. First, the modern Western diet is much lower in nondigestible carbohydrates than all previous diets in human history, potentially contributing to increases in chronic lifestyle diseases.[30] Second, the metabolic end-products (for example, SCFAs) that result from bacterial fermentation in the gut have been shown to have beneficial physiological effects, with strong implications for health.[7,31–36]

The current prebiotic concept typically refers to nondigestible food ingredients or substances that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth and/or activity of health-promoting bacteria that colonize the large bowel. The definition has been discussed and refined several times since it was first introduced in 1995 by Gibson and Roberfroid[37] (Table 1). However, most definitions to date agree on the requirement that prebiotics have to be 'specific' or 'selective' for health-promoting taxonomic groups or beneficial metabolic activities.[29,38] According to Roberfroid *e a .*,[29] specificity was considered "the key condition that needs to be demonstrated, , in the complex human (animal) gut microbiota by applying the most relevant and validated methodology(ies) to quantify a wide variety of genera/species composing the gut microbiota". The bacteria considered health-promoting in the prebiotic literature are to a large degree restricted to the genera *B f d bac e ,* and *Lac bac ,* By contrast, bacterial groups such as *Bac e de* and Clostridia were often marked as detrimental because, among other reasons, they perform a proteolytic fermentation that results in toxic metabolites.[29,39] The concept of prebiotics triggered a vast amount of research and was instrumental for much of the progress in the field of gastrointestinal microbiology and, by showing that

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The authors declare no competing interests.

Table 1 | Evolution of the prebiotic concept

Year	Definition	Ingredients considered as prebiotics	What changed?	Reference
1995	Nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health	FOS	NA	Gibson & Roberfroid (1995)[37]
2003	Nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria	FOS tGOS Lactulose	Extension of the original definition to include other body sites and not just the colon Changed "improves host health" with "beneficial physiological effects"	Reid *et al.* (2003)[41] ISAPP inaugural meeting
2004	Selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host wellbeing and health	Inulin FOS tGOS Lactulose	Extension of the original definition to include the entire gastrointestinal tract First time that changes in "composition" were included, and the term "wellbeing"	Gibson *et al.* (2004)[105]
2007	Selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health	Inulin tGOS	Made no changes to the definition, but specifically stated that only two dietary oligosaccharides fulfil the criteria for prebiotic classification	Roberfroid (2007)[71]
2008	Nonviable food component that confers a health benefit on the host associated with modulation of the microbiota	Inulin FOS, GOS, SOS, XOS, IMO, lactulose, pyrodextrins, dietary fibres, resistant starches, other nondigestible oligosaccharides	Removes the selectivity criterion and the limitation to the gastrointestinal tract Replaces causality by association Does not require the prebiotic to be fermented or metabolized by the gut microbes, and therefore does not distinguish among substances that modulate gut microbiota composition solely through an inhibitory action As a consequence, antibiotics would be prebiotics according to this definition	FAO meeting (2008)[51]
2010	Dietary prebiotic: 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	Inulin FOS tGOS Lactulose Candidate prebiotics are listed	Specifically referred to dietary prebiotics (that target the gastrointestinal tract) Focus on health with no mention of "wellbeing" Continues to adhere to "selective fermentation" in disagreement to the FAO definition	Gibson *et al.* (2010)[52] 6th ISAPP meeting

Of note, Roberfroid *et al.*[29] published an extensive review on the topic in 2010. This article did not aim to propose a new definition of a prebiotic but rather to validate and expand the original idea of the prebiotic concept. Abbreviations: FAO, Food and Agriculture Organization of the United Nations; FOS, fructo-oligosaccharides; GOS, galacto-oligo-saccharides; IMO, isomalto-oligosaccharides, ISAPP, International Scientific Association of Probiotics and Prebiotics; NA, not applicable; SOS, soya-oligosaccharides, tGOS, transgalacto-oligo-saccharides; XOS, xylo-oligosaccharides.

changes in the gut microbiota can be associated with beneficial physiological effects, it greatly contributed to the appreciation of the gut microbiota as a therapeutic target in various pathophysiological contexts.[29,38]

Although the prebiotic concept is now 20 years old and heavily researched, several aspects remain insufficiently resolved. Little consensus exists on which compounds constitute prebiotics and which do not (Table 1). A particular issue is the overlap between the definitions of prebiotics and dietary fibre,[40,41] and scientists have begun to refer to the so-called prebiotic activities of dietary fibres[42–44] although it conflicts with the current definition of prebiotics as most dietary fibres do not lead to selective changes in the gut microbiota.[45] Although selectivity is the key qualifier for a prebiotic,[29] there is no clear understanding on how selective a prebiotic effect would have to be. In this context, it is important to recognize that no carbohydrate is likely to be fermented by only one or two bacterial groups in the gut, and none is fermented by all. So where should we draw the line? In the prebiotic literature, the effect is considered selective if putatively health-promoting microorganisms are specifically targeted, but this requirement comes with a whole new complication as there is little agreement on what constitutes the healthy fraction of the gut microbiota.[46]

Powered by novel technologies and major international initiatives, the research that followed the introduction of the prebiotic concept has transformed our understanding of the gut microbiota, including its characteristics, ecology, and its interactions with diet and health.[23,46–50] In this Perspective, we discuss how findings from this research and their implications now challenge important aspects of the prebiotic concept. We argue that in light of our current knowledge, the requirement of "specificity" and "selectively" is unconducive, if not obstructive, to progress in the field. The limitations of the current prebiotic concept and open questions that surround the concept are discussed. We then propose to refine and widen the concept in an effort to shift the focus towards targets within the microbiome more likely to be relevant for host physiology, and suggest viable areas of future research that would strengthen the concept.

The problem with specificity

Although almost all definitions of prebiotics require a specific effect towards health-promoting taxa, scientists have begun to challenge this requirement as it conflicts with our current understanding of gut microbiota ecology and its relation to health.[26,51] We have identified four key arguments that question the requirement of specificity: our current knowledge does not allow a reliable differentiation of beneficial and detrimental members within the gut microbiota; a diverse community of microorganisms is essential for intestinal homeostasis and host physiology; the key metabolic benefits assigned to prebiotics do not require a 'selective' fermentation; modern community-wide molecular approaches have revealed that even the established prebiotics are not as specific as previously assumed.

Reliable differentiation

In the prebiotic literature, bifidobacteria and lactobacilli (and sometimes *E. bac e* and *R eb a*) are considered beneficial,[29,37,52] whereas bacterial groups such as *Bac e de* and Clostridia are often branded detrimental.[29,37,39] However, these black-and-white considerations are problematic, as we have neither reached a consensus on which microorganisms constitute beneficial and detrimental members of the gut microbiota, nor is there a conviction that such a classification can even be made. In fact, strains belonging to bacterial genera that were previously pinpointed as detrimental in the prebiotic literature, such as Clostridia, have now been shown to be highly beneficial in models of colitis and allergy.[29,53] In addition, beneficial attributes are constantly discovered for many species, such as *A e a a c* and *Faeca bac e* .[54–56] In this context, it is important to consider that the net effect of a gut symbiont on the host and its pathogenic potential is also dependent on the specific circumstance (for example, host state, genotype, diet and lifestyle), meaning that microorganisms that are normally beneficial can become detrimental when conditions change.[27,57,58] We believe that the current prebiotic concept is therefore based on an outdated 'good versus evil' perspective.

Importance of diversity

Even if the identification of the healthy fraction of the gut microbiota was possible, it would probably require many species, and potentially entire collections of species, to achieve health and intestinal homeostasis. In community ecology, high levels of diversity are often considered important for the function of an ecosystem.[59] Thus, reduced diversity and microbial gene richness associated with human diseases (such as obesity, IBD or *C. d ff c e* infection) might constitute a contributing factor to these pathologies.[47,48,60] Accordingly, restoration of a diverse gut microbiota through faecal microbiata transplantation has been effective for the treatment of recurrent *C. d ff c e* infection and for increasing insulin sensitivity in individuals with metabolic syndrome.[61,62] The purposeful support of a few selected members within the ecosystem, as currently envisaged by the prebiotic concept, is therefore unlikely to achieve benefits for the host in many circumstances.

Key metabolic benefits

One key mechanism by which prebiotics are considered to exert health benefits is the production of SCFAs, which have antimicrobial activity and reduce intestinal pH (and thereby exclude pathogens), and have various beneficial physiological, metabolic and immunological effects.[37,63] Bifidobacteria and lactobacilli produce mainly lactate and acetate, both of which can contribute to the health effects of prebiotics.[64] However, these bacteria do not produce butyrate and propionate, two SCFAs that have been identified to exert highly beneficial local and systemic immunological and physiological effects.[7,31–36] Butyrate and propionate are produced, among others, by bacteria belonging to the *C d* clusters XIVa and IV, and to the Bacteroidetes phylum and Negativicutes class, respectively.[65,66] In addition, nondigestible carbohydrates such as resistant starches, pectins, arabinoxylosaccharides, and other dietary fibres, although broadly fermented, induce SCFA formation with benefits to the host.[32,43,67] Thus, the focus on bifidobacteria and lactobacilli seems unnecessarily narrow, and there is little rationale for the requirement of fermentation by 'selective' taxa, as broadly fermented carbohydrates and prebiotics confer similar physiological benefits, probably induced through equivalent mechanisms (that is, via SCFAs).[43,68,69]

Specificity of established prebiotics

When prebiotics were first defined,[37] selectivity was investigated mainly by selective culture techniques, and later by molecular methods that focused on a small number of bacterial groups. Findings obtained with these approaches suggested that prebiotics such as inulin, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) had highly selective effects on the human gut microbiota, increasing mainly population levels of bifidobacteria and lactobacilli whilst decreasing cell numbers of the genus *Bac e de* Clostridia and Fusobacteria.[70] However, even whilst relying on data from these targeted approaches, Roberfroid, in his revisit of the prebiotic concept published in 2007,[71] came to the conclusion that only two types of dietary oligosaccharides (inulin and trans-GOS) fulfil the criteria for classification as a prebiotic.

In the past decade, next-generation sequencing and microarray approaches emerged that enabled a community-wide analysis of the gut microbiota. These approaches revealed that even the most accepted prebiotics are not confined to a selective change in the composition and activity of the gut microbiota. For example, the administration of FOS and/or inulin has a broad effect on the gut microbial ecosystem,[55,72,73] and changes (both increases and decreases) the abundance of 102 taxa within the gut microbiota of genetically obese mice.[55] GOS seems to be more selectively bifidogenic.[52,74–76] Using a dynamic colon model and a ^{13}C-labelling technique, Maathuis and colleagues[76] showed that the primary members within the complex microbiota that were directly involved in GOS fermentation were *B f d bac e g* , *B. b f d* , *B. ca e a* , *Lac bac* , *ga e* and *L. a a* , although some other taxa, such as Enterobacteriaceae and *K eb e a*, also incorporated the ^{13}C label. Sequencing analysis of faecal samples from healthy volunteers consuming GOS revealed that the sole effects of GOS that reached statistical significance in the overall dataset were an increase in the abundance of bifidobacteria and *F. a* , and a decrease in abundance of *Bac e de* .[74] However, the authors also reported that many individualized GOS-induced shifts within diverse taxa were detected.[74] In mice fed a high-fat diet, administration of GOS led to a decrease of the Actinobacteria phylum (the phylum that encompasses bifidobacteria), and numerous other bacterial families and genera were also affected.[77] Finally, GOS administration to rats with renal injury increased the bacterial families Bifidobacteriaceae, Clostridiales Incertae Sedis XIV, and Porphyromonadaceae, among other changes.[78] Overall, studies indicate that shifts induced by current prebiotic carbohydrates are not as selective as previously assumed (probably due to functional redundancy among gut inhabitants and cross-feeding[27]), which means that the current prebiotic definition, if strictly adapted, would exclude virtually all carbohydrates.

Open questions on the concept

Various aspects of the prebiotic concept unrelated to specificity have been discussed in various publications, panel reports and included in previous definitions of the term (Table 1). A consensus, however, has not always been achieved, and previous definitions have often been inconsistent. Here we provide a summary of points that require future clarification. Although we recognize that further discussions are necessary and future research findings will have to be considered, we provide our opinion on these points.

Restriction to the gut?

Whether the prebiotic concept should be restricted to the gut or extended to other

body sites has been the subject of debate in the field. Other body sites harbour microbial populations that affect health, and therefore constitute potential therapeutic targets.[46,79–82] However, the prebiotic concept was originally devised as a nutritional concept restricting it to the gastrointestinal tract. Furthermore, compounds that are supposed to reach the large intestine require specific characteristics that do not apply to other body sites (for example, complete or partial resistance to digestion and absorption). We therefore think that there is a strong rationale to reserve the term "prebiotic" to nutritional strategies that target the gut microbiota specifically.

Is fermentation a requirement?

The importance of fermentation in the definition of prebiotics has been debated since the concept was introduced.[37] Given the physiological effects of metabolites that result from fermentation, especially SCFAs, there are strong arguments for its inclusion. However, the nonfermentable dietary fibre hydroxypropyl methylcellulose (HPMC) has been proposed as a potential prebiotic fibre because it modulates the composition of the gut microbiota of obese mice.[83] These changes might have resulted from a modulation of the intestinal nutrient environment through an increased excretion of faecal bile acids and fats, as well as increased faecal water content. However, whether the metabolic benefits of HPMC are mediated by the modulation of gut microbiota was not clearly established. Moreover, Cani and co-workers[84] have previously demonstrated using genetically obese mice that nonfermentable microcrystalline cellulose has little effect on metabolic parameters when compared with fermentable FOS. Therefore, no clear examples currently exist of candidates of prebiotics that improve host health through a modulation of the gut microbiota without being fermented. However, the term fermentation refers to a specific type of metabolism that uses organic carbon instead of oxygen as a terminal electron acceptor. As some nondigestible compounds are probably utilized by microorganisms in the gut using other types of metabolism, it might be useful to not restrict the prebiotic concept solely to "fermentation". Nevertheless, we do consider it essential that a compound be metabolized by microorganisms in the gut to be considered a prebiotic. This consideration is especially important to exclude antibiotics from the prebiotic concept, as they can induce health effects by affecting the gut microbiota without being metabolized. In this respect, an analytical characterization of the structural degradation of a prebiotic by the gut microbiota, and how this degradation is associated with physiological benefits, could become a viable experimental tool in prebiotic research, and could transform our understanding of how prebiotics work.

That prebiotic carbohydrates (such as GOS or FOS) might have beneficial effects that do not require fermentation, such as anti-adherence or direct immunomodulation,[85,86] is increasingly recognized, questioning the requirement of metabolization in the prebiotic definition. However, these effects occur without a contribution of the resident gut microbiota and therefore, in our opinion, are not so-called prebiotic effects *per se*. Restricting the prebiotic concept to compounds that exert their action through a modulation of the resident gut microbiota is important. Otherwise, any compound, drug or ingredient that is effective in the gut could be considered a prebiotic. Still, it should be emphasized that prebiotics might have additional biological activities not related to their effects on the gut microbiota and that do not require them to be metabolized, such as pathogen exclusion or direct immunomodulation.

Restriction to carbohydrates?

Although all current prebiotics are carbohy

effect is even more challenging, and has not been systematically performed in previous prebiotic research. As a minimum, evidence of a modulation of the gut microbiota and a beneficial physiological effect needs to be provided and correlations between both phenomena have to be established using appropriate statistical tests and models.[51] However, although this approach represents a realistic practical prerequisite for the substantiation of a prebiotic action, it has to be emphasized that it only establishes a correlative relationship, not a causal one (discussed later).

Our proposition for a definition

On the basis of the points discussed earlier, we propose the following definition for prebiotics: a prebiotic is a nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host.

This revision would shift the focus of the concept from subjective 'selective' targets towards ecological and functional characteristics of the microbiota more likely to be relevant for host physiology, such as ecosystem diversity, the support of broad consortia of microorganisms and production of SCFAs. The most notable immediate effect of this proposed definition would be the inclusion of all nondigestible carbohydrates that improve health through a modulation of the gut microbiota. This step would be well justified in light of the latest mouse studies indicating beneficial effects of fibre fermentation in the gut.[32,97]

In this context, it might be practical to not only define the term "prebiotic" but also the actual effect as the "prebiotic effect", for which we propose the following definition: a prebiotic effect is the beneficial physiological outcome that arises from the modulation

Future research on prebiotics

Historically, a large proportion of prebiotic research was focused on the determination of selectivity.[29] Additionally, health or physiological benefits were often investigated and, occasionally, correlations were established with changes in gut microbiota composition or metabolism. Little research to date has been devoted to establishing a causal role of the gut microbiota. We anticipate that removing the requirement of selectivity from the prebiotic concept will shift the focus from the characterization of the effects on gut microbiota composition towards research on the mechanisms by which health effects are achieved (Figure 1). The substantiation of health and physiological benefits in human and/or animal trials will remain important, but the field would greatly benefit from more mechanistic research focused on establishing the exact role of the gut microbiota. Although not necessary to obtain health claims, such research could provide mechanistic insights vital to the development of improved prebiotic strategies in the future, as well as elevate the scientific basis for the prebiotic concept.

Conclusive proof for causality in human trials is extremely difficult to attain, although novel statistical tools might become available in the future to determine causality. Although animal experiments have their own set of limitations and confounders, one approach that could be used to determine the causative role of the gut microbiota would be to compare physiological effects of putative prebiotics in conventionalized and germ-free animals.[101] However, although elegant, this approach is not appropriate to all pathological contexts, given that in the germ-free state some pathologies progress differently (or are even absent) and the immune system is not fully developed.[2,8,14] In these cases, the functional consequences of gut microbiota modulation by prebiotics could potentially be established by gut microbiota transfer using cohousing or crossfaunation.[23,102] The hypothesis is that if a beneficial physiological effect of a prebiotic is due to shifts in gut microbial composition or activity, then transfer of the gut microbiota should induce similar effects in recipient mice. For crossfaunation experiments, transfer of faecal microbiota from one set of mice to another should be done by gavage. For cohousing experiments, prebiotics could be administrated by gavage (to restrict prebiotic exposure to donor mice), and transfer would occur by coprophagy. These experiments could even be performed in mice colonized with a human microbiota to avoid host-related microbiota differences.[103] Such studies could become central when testing 'prebiotic candidacy' for a nondigestible compound that shows a physiological effect. If combined with the appropriate functional assays, these studies have the potential to provide unique insight into the mechanisms by which the gut microbiota confers the physiological benefits of prebiotic compounds. However, given the limitations of both human and animal experiments, prebiotic research will require insight from both human trials (in which correlations can be established) as well as from mechanistic studies in animals (to prove causation).

Conclusions

The concept of prebiotics, elaborated in the 1990s, was pioneering as it introduced the gut microbiota as an important factor in human and animal nutrition. The goal of this paper was to revisit the prebiotic concept and propose a revision (Box 1) that hopefully contributes to the ongoing debate in the field. This debate is crucial for scientific reasons but also for the agri-fo[redacted] sector, regulatory agenci43(o f)8.7(o)11.394 t2.3(

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