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Prebiotics: why definitions matter

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The prebiotic concept was introduced twenty years ago, and despite several revisions to the original definition, the scientific community has continued to debate what it means to be a prebiotic. How prebiotics are defined is important not only for the scientific community, but also for regulatory agencies, the food industry, consumers and healthcare professionals. Recent developments in community-wide sequencing and glycomics have revealed that more complex interactions occur between putative prebiotic substrates and the gut microbiota than previously considered. A consensus among scientists on the most appropriate definition of a prebiotic is necessary to enable continued use of the term.

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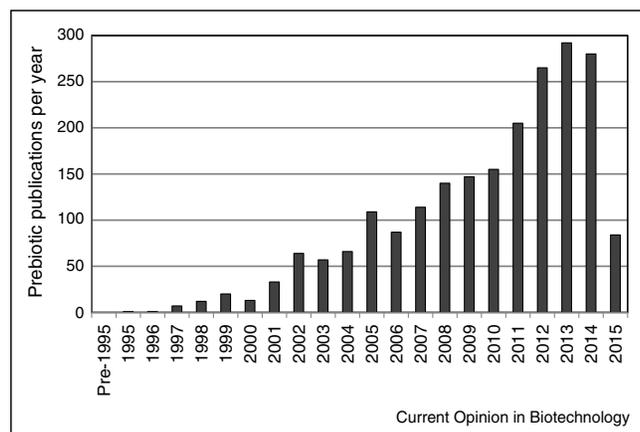
Introduction

The idea that certain nutrients (including carbohydrates) can modify the gut microbiota existed long before definitions for such nutrients had been proposed. Reports on the bifidogenic properties of inulin and oligofructose (produced from inulin), and fructooligosaccharides (FOS) synthetically produced from sucrose, as well as galactose-containing and xylose-containing oligosaccharides appeared in the 1980s and early 1990s [1–4]. Even earlier, in the 1950s, researchers described the presence of a so-called ‘bifidus factor’ in human milk, a component that enriched for bifidobacteria in infants [5]. Later, this factor was identified and shown to consist of complex oligosaccharides and glycans [6,7]. Oligosaccharides were also detected in bovine milk and milk products, but their physiological role was unclear [8]. Remarkably, there were even reports of human subjects consuming massive doses of lactose to enrich for beneficial lactic acid bacteria in the gut [9]. Fructans and other fermentable fibers likely comprised an important part of the diet of humans tens of thousands of years ago [10].

In 1995, Glenn Gibson and Marcel Roberfroid [11*] introduced the prebiotic concept. They defined a prebiotic as ‘a 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’. Although this original definition has been revised multiple times, the main features have mostly been retained [12–15].

The scientific community quickly adopted the new term. The original paper was cited, according to Web of Science, more than 2500 times. The prebiotic concept was well understood, widely accepted, and extensively used in the science community (Figure 1). It has had a profound effect on gut health research. However, the original and subsequent definitions (see below) have been criticized and misunderstood. First, ‘prebiotic’ had already been defined in the chemistry literature as building block structures that pre-date living organisms. Thus, there is extensive ‘origin of life’ literature on prebiotic chemicals unrelated to prebiotic food ingredients. Secondly, while consumers and health practitioners have a general understanding of probiotics, surveys have shown they are less knowledgeable about prebiotics and may even confuse the two terms [16,17].

Figure 1



Number of prebiotics publications cited in PubMed (search terms = prebiotic* AND bacteria NOT origins NOT inorganic), data for 2015 are through May.

In addition, while the original term was broadly defined, subsequent papers suggested that the fructans, oligofructose and inulin, FOS, lactulose, as well as the galactan, galactooligosaccharides (GOS) were the only established prebiotics at that time [18]. Early research focused mainly on these few substances. However, even by the early 2000s, development of next generation, rationally selected prebiotics was proposed [19]. Thus, resistant starch (RS), pectin and other fiber components, and milk oligosaccharides are now proposed as having prebiotic potential [20–22].

When first introduced, methods for identifying and quantifying members of the gut microbiota depended mostly on culture and probe-based techniques. The general results of these studies showed a specific enrichment of bifidobacteria (and in some experiments lactobacilli), consistent with the notion that prebiotics selectively stimulated these beneficial bacteria. Indeed, in 2000, Gibson and Fuller suggested that, given the state of scientific knowledge at that time, species of *Lactobacillus* and *Bifidobacterium* were the ‘preferred target organisms for prebiotics’ [23]. Physiological mechanisms by which these bacteria metabolize prebiotics have also been established [24].

In the past decade, however, community sequencing has revealed more complex outcomes of prebiotic administration, with quite different and sometimes unexpected members of the microbiota being enriched. In particular, several putatively beneficial autochthonous gut organisms, including *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, have been shown to respond to specific prebiotics [25*,26*,27–30]. The consumption of other molecules (e.g. human milk oligosaccharides, resistant

starch and various soluble and insoluble fibers) may also promote enrichment of other as yet unidentified members of the gut microbiota, either directly or via cross-feeding [31,32,33*,34*]. However, there is still a paucity of sequencing studies associated with a clinical intervention for many (potential) prebiotics.

In part to account for these new observations, Bird *et al.* and Bindels *et al.* proposed a broader definition for prebiotics. Bird *et al.* [20] further suggested that prebiotics should be viewed as ‘undigested dietary carbohydrates’ that are fermented by colonic bacteria yielding short chain fatty acids as end products. Importantly, given (i) the potential therapeutic benefits of a diverse gut microbiota, (ii) the broad impact of some well-established prebiotics on gut microbiota composition, and (iii) unresolved questions about the distinctions between beneficial and detrimental members, Bindels *et al.* proposed that selectivity and specificity were no longer relevant criteria (35). Bird *et al.* [20] and Martinez *et al.* [36] suggested that a broader range of food ingredients have prebiotic activity, including resistant starch and various dietary fibers. However, in the absence of evidence that the benefits of such substances are mediated through an impact on the microbiota prebiotics cannot be distinguished from other ingredients that reach the colon intact. Recently a diverse gut microbiota (measured via high microbial gene count) has been associated with health, but it is unknown if any one prebiotic or fiber ingredient could achieve such an effect [37,38]. Comprehensive *in vitro* studies have characterized the mechanistic strategies by which common gut bacteria degrade more complex fibers and other dietary polysaccharides, including yeast mannan, human milk oligosaccharides, xyloglucan and other complex xylans [39*,40*,41,42*,43,44]. However, many different microbiota species, each with a potentially different utilization strategy, likely compete for these substrates in each individual, making it challenging for researchers to determine specifically which pathways affect the gut microbiota and why certain microbial groups respond.

In this review, our goal is not to advocate for an existing definition nor is it our intent to propose a new definition for prebiotics. Rather, we address the fundamental question of why such a definition is important. The necessity for open discussion about this topic is reflected in that the very basis for defining prebiotics has recently been challenged [45,46]. They argue that these definitions are outdated, too narrow, too limiting, and too simplistic. Given the emerging evidence that many dietary ingredients also influence the microbiota, they suggest that it is better to use ‘pharmabiotics’ to describe substances having microbiota-influencing or therapeutic activities. This broad term may be useful, but it does not obviate the value of the ‘prebiotic’ term that is valued by generally healthy consumers.

Why definitions matter

Definitions for prebiotics and what specific outcomes define a prebiotic effect have been challenged almost since the concept was introduced only 20 years ago. Recently, Shanahan warned researchers to ‘resist becoming captive to restrictive language and outdated terms like prebiotics’ [45]. Whatever the merits or otherwise of this argument, ‘prebiotic’ is now very much ingrained in the lexicon. We contend that scientists, regulators, the food industry, and consumers and healthcare providers must find a way to use this term in a consistent and meaningful way.

For scientists

As noted above, there have been several revisions or clarifications to the original 1995 definition of prebiotics, including one proposed recently by Bindels *et al.* [35^{••}]. Importantly, once a revised definition is published in the scientific literature, it is usually widely adopted by research scientists, the medical community, and granting agencies. Nonetheless, for scientists, establishing the criteria for what constitutes a prebiotic has proven to be no easy matter and consensus has not yet been achieved. The 2008 FAO definition [14], for example, describes a prebiotic as a ‘nonviable food component that confers a health benefit on the host associated with modulation of the microbiota’. Yet, because this definition does not state that metabolism is necessary, substances such as bacteriocins or dietary compounds that have antimicrobial activity would qualify as prebiotics.

Despite the absence of a consensus definition, a key element of the original and all subsequent definitions is that prebiotics should improve host health or otherwise provide a beneficial physiological effect. Accordingly, Rastall and Gibson [22] suggested that while identification of new prebiotic target organisms are likely to emerge from microbiome analyses, identifying outcomes and health benefits that occur as a result of prebiotic consumption are vital. However, to distinguish prebiotics from other functional foods, it is critical that these benefits be mediated by modulation of the gut microbiota or via measurable changes in the community structure.

Accordingly, this clarification implies that prebiotic-provoked modulation of the microbiota is the actual effector of the health effect or benefit. However, the possibility that a health benefit and shifts in the microbiota could occur independently following prebiotic consumption also exists, but would be, at the present, difficult to establish. Indeed, ‘health benefit’ is, unfortunately, not well defined either, nor is there a clear mechanistic link between microbial activity and putative health benefits.

Although selectivity, the ability of a nutrient to enrich for specific members of the gut microbiota, was a key component of the original definition, this requirement has

recently been challenged [35^{••}]. In part, this is because the term, ‘selectivity’, itself is difficult to define or establish in the context of prebiotics. One dictionary definition is ‘the property of affecting some things and not others’. In the context of fermentations in the gut, what then is the ‘limit’ for the number of species or taxa that have to be enriched by a prebiotic to still be considered selective? Would any change in the microbiota composition (that would inherently involve increases in some species and not others) that confers a corresponding health benefit fulfill the ‘selectivity’ criterion?

At the current time there is evidently no consensus on these questions. Moreover, the use of metagenomics techniques to characterize the gut microbiota will inevitably expand the list of species responding to prebiotic consumption. It is also important to note that metabolism of complex dietary polysaccharides (including many prebiotics) involves a consortium of organisms, such that selectivity may not be applicable [35^{••},47,48]. Thus, for such polysaccharides, where selfish utilization or cross-feeding of degradation or fermentation products is possible, it may be necessary to obtain a more comprehensive understanding of how these materials influence the microbiota. Indeed, Scott *et al.* [49] noted that cross-feeding is an important contributor to the prebiotic effect and that the bacteria enriched by prebiotics extend beyond those initially identified. Consistent with these arguments, it has been shown that oligofructose from inulin increases the abundance of *A. muciniphila* even higher than *Bifidobacterium* spp. [25[•],26[•]]. However, *A. muciniphila* is unable to use oligofructose as an energy source [50]. Altogether, these examples emphasize the importance of cross-feeding as a key mechanism by which prebiotics influence the microbiota, in contrast to a direct impact on specific taxa. Finally, as Frese and Mills suggested recently [51], another way to assess the metabolic fate of prebiotics is by fecal glycomics — simply measuring what is excreted in feces and correlating those products with changes in microbiota composition and health.

Although reaching consensus on the prebiotic definition would not necessarily resolve the open questions noted above, doing so would still provide clarity to gut health researchers, as well as regulators, the food industry, health professionals and consumers.

For regulators

Neither the U.S. Food and Drug Administration (FDA) nor Europe’s European Food Safety Authority (EFSA) have established definitions for prebiotics [52,53]. In 2006, the FDA released a draft document that provided guidance to industry on when ‘complementary and alternative medicine (CAM) products’ were subject to FDA regulation [54]. The National Center for Complementary and Alternative Medicine (now known as the National

Center for Complementary and Integrative Health), a center within the National Institutes of Health, subsequently included prebiotics as part of a 'biologically-based' group of foods that also included botanicals, animal-derived extracts, vitamins, minerals, fatty acids, amino acids, proteins, whole diets, and functional foods. For this document, the 1995 definition of prebiotics was used, which underscores the argument that changes in the definition can take a long time until they find their way into the regulatory process. The FDA also permits food manufacturers to self-affirm GRAS (Generally Recognized as Safe) status for products ultimately labeled as prebiotics [55].

In Europe, EFSA uses the FAO prebiotic definition, which stipulates the need for a health benefit [14,53]. EFSA states that evidence of microbiota changes is not in itself a benefit, and further requires demonstration of a beneficial physiological effect or outcome of human intervention study [56]. Oligofructose and native chicory inulin were recently given positive opinions by EFSA [57,58] for 'reduction of post-prandial glycaemic responses' and 'maintenance of normal defecation', respectively. With respect to bowel habit as affected by inulin, fermentation leading to high stool bulk, softer stools and SCFA production (which are indirectly due to microbiota activity) were amongst the mechanisms cited.

A similar situation exists in Canada, where the use of the term, 'prebiotics' on labels or in advertising is considered as an 'implied health claim' (Canada Food Inspection Agency). Accordingly, this would mean that 'prebiotics' could be included on a food label only if it satisfied the health claim requirements. Thus, a statement specifying the 'measurable health benefit conferred by the prebiotic substance, as demonstrated in humans' would have to be included [59].

The regulatory environment in Japan is much different than in the U.S. and E.U. While prebiotics are not specifically defined, oligosaccharides, dietary fiber, and other polysaccharides are specified as 'foods to modify gastrointestinal conditions' and can be approved as 'foods for specific health uses' [60].

Collectively, the various ways by which regulatory agencies around the world deal with labeling prebiotic compounds reveals the need for a clear definition, at least in the USA and Europe.

For the food industry

The food industry is interested in prebiotics for their application and promise as functional ingredients in foods targeted toward health-conscious consumers. Benefits, ranging from maintaining and enhancing gut health, modulating the immune system, lowering glycemic response,

and reducing insulin resistance are of interest to consumers and therefore attractive targets for new functional foods. Some of these recently received positive opinions from EFSA [57,58]. However, if scientists do not agree on the scope and appropriate use of the term 'prebiotic'; regulatory agencies may establish their own definition, and this may have a negative impact on how prebiotics are labeled.

The term 'prebiotic' is a concept gaining acceptance by consumers, and frequent changes to the definition may be confusing. Therefore, any such change should be well-considered and meaningful. However, without an accepted and clear definition of a prebiotic by academic scientists, it is impossible to know if a substance is accurately labeled as a prebiotic, with implications for misleading consumers. It would be much preferred for scientists to provide the best definition for regulators, rather than have regulators determine this on their own. However, a global consensus effort is key to adopting a definition that is useful and scientifically sound.

For consumers and health professionals

The main source of health information for consumers is now the Internet [61]. In particular, Wikipedia has become a major online reference source for medical and health information, not just among consumers, but also public health professionals and scientists [62]. This site currently refers to prebiotics as '*chemicals* (emphasis added) that induce the growth or activity of microorganisms (e.g. bacteria and *fungi*) that contribute to the well-being of their host'. On another popular site, WebMD, prebiotics are described as 'food ingredients that help support growth of probiotic bacteria'. Elsewhere in this website (Vitamins and Supplements Guide), prebiotics are defined as 'non-digestible ingredients in foods that are used to spur the growth of probiotic bacteria in the body by providing a suitable environment in which the probiotics themselves can flourish'. While the latter definitions convey perhaps a general meaning, they inaccurately imply that probiotic bacteria are already present in the body (by definition, probiotics are an exogenously provided live microbial supplement). It also suggests that prebiotics directly influence the environment.

In contrast, the Mayo Clinic Health Letter (July 2014) provides a more accurate definition, describing prebiotics as 'nondigestible substances that act as food for the gut microbiota. Essentially, prebiotics stimulate growth or activity of certain healthy bacteria that live in your body.' This newsletter also notes that 'prebiotics are found in whole grains, bananas, onions, garlic, honey and artichokes'. However, a typical Western diet is estimated to contain only a few grams of naturally occurring prebiotics, and it is unclear if this is a sufficient dose to have any measurable effects [63,64].

Ultimately, however, what consumers and health care providers actually know about prebiotics is difficult to assess. According to recent surveys, prebiotics appear to be poorly understood by the general public. In one recent survey of 200 U.S. adults (inpatients at an urban hospital), only 11% were familiar with the term, 'prebiotics', and only 7% identified the correct definition among four other choices [17]. In a similar survey of 245 health care providers (including 100 physicians), only 22% of respondents were familiar with prebiotics [16]. There was also confusion about differences between probiotics and prebiotics. Interestingly, although most of the health care providers viewed prebiotics as beneficial to health, few actually recommended them to patients. It is likely that this poor understanding of the prebiotic concept by the general population hinders potential applications. It is also possible that this lack of clarity contributes to its low rate of recommendation by health care providers.

Conclusions

A major challenge for gut health scientists is how to best educate and inform other stakeholders about prebiotics. Having a definition that accurately describes prebiotics and is agreed upon by the scientific community is an essential first step. We can hardly expect regulators, consumers and healthcare providers to have a clear understanding of prebiotics if the scientific community does not. The prebiotic definition may evolve as science advances, and it is incumbent on the scientific community to interpret new findings and adjust the prebiotic concept as needed.

Metagenomic, metabolomic, glycomics, and other omic approaches and advances in analytical chemistry, as well as human clinical studies are likely to provide new insights into mechanisms by which prebiotics function *in vivo* [30]. Even if a consensus definition could be reached, it would need to be sufficiently flexible to accommodate the inevitable new discoveries in gut biology. Otherwise, the science on the effects of prebiotics on the microbiota and host health may simply outpace the definition. Ultimately, for scientists and non-scientists, and for industry and regulators, resolving the definition of prebiotics is necessary to enable continued use of the term.

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