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REVIEW

The prebiotic concept and human health: a changing landscape with riboflavin as a novel prebiotic candidate?

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Emerging evidence suggests that the gut microbiota has a critical role in both the maintenance of human health and the pathogenesis of many diseases. Modifying the colonic microbiota using functional foods has attracted significant research effort and product development. The pioneering concept of prebiotics, as introduced by Gibson and Roberfroid in the 1990s, emphasized the importance of diet in the modulation of the gut microbiota and its relationships to human health. Increasing knowledge of the intestinal microbiota now suggests a more comprehensive definition. This paper briefly reviews the basics of the prebiotic concept with a discussion of recent attempts to refine the concept to open the door for novel prebiotic food ingredients, such as polyphenols, minerals and vitamins.

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INTRODUCTION

Emerging evidence suggests that the gut microbiota has a critical role in both the maintenance of human health and the pathogenesis of many diseases.[1,2] Recent data, both from animal models and human studies, have revealed close relationships between the gut microbiota and host physiology. In addition, microbial perturbations have been reported to be associated with a number of diseases, including inflammatory bowel disease (IBD),[3] diabetes[4] and obesity.[5] The recognition that the gut microbiota influences signaling pathways that participate in the communication between the gut and the brain has led to the suggestion of the concept of a 'microbiota–gut–brain axis', a topic that has been reviewed extensively recently.[6] The routes of communication between the gut microbiota and the brain are not fully elucidated but presumably include neural, endocrine and immune signaling pathways.[7]

The concept that the gut microbiota could be modulated to improve human health was proposed >100 years ago[8] and today includes a spectrum of therapeutic measures ranging from the transplantation of entire fecal microbiota to the administration of isolated microorganisms, that is, probiotics. The unparalleled effectiveness of fecal microbiota transplantation in the treatment of *Clostridium difficile* infection is one proof of principle that modification of the gut microbiota can be an effective therapeutic strategy for the treatment of a human disease.[9] The importance of diet in modulating the composition of the gut microbiota has been demonstrated in several studies and incorporates the concept of prebiotics.[10–14] For example, David *et al.*[11] recently reported that short-term consumption of diets composed entirely of animal or plant products rapidly changes microbial community structures, overwhelming interindividual differences in microbial gene expression. Cani *et al.*[12,13] and Kellow *et al.*[14] demonstrated in animals and humans that prebiotic modulation of the gut microbiota affects endogenous secretion of gastrointestinal hormones, regulating gut barrier function, satiety and glucose metabolism. The potential to modulate the gut microbiota by nutritional intervention, therefore, represents an important area of research for both academia and the food industry. This paper briefly reviews the basics of the prebiotic concept with a discussion of recent attempts to refine the concept to open the door for novel prebiotic foods.

THE PREBIOTIC CONCEPT

Japanese researchers demonstrated already in the 1970/80s that in man non-digestible oligosaccharides (especially fructooligoscc-charides) given orally were selectively fermented.[15,16] This was confirmed by Gibson and Roberfroid,[10] who first introduced the concept of prebiotics in 1995 ('A prebiotic is 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 in the colon, and thus improves host health' (Table 1)). Since then, the definition has been discussed and further refined several times to accommodate emerging knowledge,[17–23] and although the main features have mostly been retained, some of the criteria that need to be fulfilled for a food ingredient to qualify as prebiotic are still a matter of debate. Traditionally, complex carbohydrates, that is, resistant starch and non-starch polysaccharides, such as cellulose, hemicellulose, lignin, pectin and oligosaccharides, have been the major focus of prebiotic research,[24,25] because they are resistant to gastric acidity and hydrolysis by mammalian enzymes, escape digestion and reach the large intestine as primary substrates for microbial fermentation. In addition, simple sugars, disaccharides and sugar alcohols (when reaching the colon owing to overfeeding or malabsorption) as well as human milk oligosaccharides in infants can be substrates for colonic microbial fermentation.[26] Proteins, amino acids and certain lipids can also escape digestion and become substrates for the gut microbiota.[19,27] However, these compounds have not been heavily investigated as prebiotics, in part, because of the production of potentially harmful

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Table 1. Some developing criteria for classifying a food ingredient as prebiotic

Definition	*Food ingredient qualified as prebiotic*
Gibson and Roberfroid (1995)[10] '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 in the colon, and thus improves host health'	Fructooligosaccharides (FOS)
Gibson et al. (2004)[17] 'A 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, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose
FAO Technical Meeting on Prebiotics, Rome (2008)[21] 'Nonviable food component that confers a health benefit on the host associated with modulation of the microbiota'	Inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose, xylooligosaccharides (XOS), resistant starch (RS), human milk oligosaccharides (HMOs), beta-glucan, other dietary fibers and non-digestible oligosaccharides Non-carbohydrate compounds, including polyphenols, minerals and vitamins?
Bindels et al. (2015)[20] 'A non-digestible compound that, through its metabolism by micro-organisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host'	Inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose, xylooligosaccharides (XOS), resistant starch (RS), human milk oligosaccharides (HMOs), beta-glucan, other dietary fibers and non-digestible oligosaccharides Non-carbohydrate compounds, including polyphenols, minerals and vitamins?

metabolites, including amines, ammonia and phenolic compounds, during anaerobic proteolysis.[19,27]

Colonic fermentation is a highly complex process of nutritional interactions between different microbial species[25,28] that each has its own specialized ecological niche and metabolic activities. In many cases, metabolic end products are excreted by certain species and serve as the substrate for others, for example, gut microorganisms that are unable to degrade complex carbohydrate structures directly grow by 'cross-feeding' on fragments or metabolites produced by others. Complex polysaccharides are primarily fermented by fibrolytic species that include genera such as *Bacteroides*, *Bifidobacterium* and genera of *Clostridium* group IV and XIVa (including *Faecalibacterium* and *Roseburia*), whereas glycolytic bacteria including genera such as *Lactobacillus*, *Escherichia* or *Enterococcus* grow on more simple sugars.[17,25,29] The bulk of prebiotic research has focused on bifidobacteria and lactobacilli because they are reported to have direct health-promoting properties, such as the inhibition of exogenous pathogens or the prevention of antibiotic-associated diarrhea.[10,30–34] In addition, these bacteria are generally regarded as safe and can easily be cultured from stool, whereas other gut anaerobes are hard to culture and are of unknown or insufficiently assessed safety.[35,36]

Short-chain fatty acids (SCFAs), the principal end products of carbohydrate fermentation, are considered the key metabolites underpinning the physiological benefits of prebiotics. Butyrate is the primary energy source for colonocytes; propionate is thought to regulate liver cholesterol synthesis and acetate controls hepatic lipogenesis and is used to generate ATP in muscle tissue.[37] Moreover, SCFAs reduce intestinal pH, thus antagonizing pathogens, and possess antimicrobial activity.[10,17,19,37] These and other functions are speculated to translate into a number of clinical benefits, including reductions in *C. difficile* infections,[38] improvements in symptoms of IBD and inflammatory bowel syndrome,[39,40] reductions in traveler's diarrhea[41] and repression of allergic symptoms.[42] More current research, in addition, suggests a link between SCFAs and various parameters associated with metabolic syndrome, such as the control of food intake and body weight, as well as glucose homeostasis, either directly via central hypothalamic pathways or indirectly via the secretion of gut hormones such as glucagon-like peptide-1 and peptide YY.[13,43–47]

As mentioned above, which food ingredients qualify as a 'true' prebiotic is still a matter of debate. According to some papers that suggest a 'selective stimulation' (that is, Lactobacillus and Bifidobacterium as the 'preferred target organisms for prebiotics), only a small number of non-digestible carbohydrates meet the criteria for a prebiotic, including inulin and fructooligosaccharides, galactooligosaccharides and lactulose.[17,18,48,49] Other papers, in contrast, have proposed a broader definition that does not restrict prebiotics to being 'selective', opening the door for many more food ingredients to be considered (Table 1).[20,21,50] They suggest that a diverse community of microorganisms, rather than isolated strains, is essential for intestinal homeostasis and optimal host health and argue that the current understanding of diet–microbiota–host interactions does not allow, in fact, reliable differentiation of beneficial and detrimental members within the gut microbiota. Moreover, on the basis of the many cross-feeding interactions between colonic bacteria, it is argued that it is unlikely that a beneficial physiological effect can be isolated to only a limited number of species. Several recent findings support the hypothesis of the importance of a diverse microbial community: (i) restoration of a diverse gut microbiota through fecal transplantation is effective in the treatment of *C. difficile* infections; (ii) in patients with metabolic syndrome, transfer of intestinal microbiota from lean donors increases insulin sensitivity;[51] (iii) dietary fibers that are fermented broadly by a variety of microorganisms exert similar health benefits as the accepted selective prebiotics;[52–54] (iv) other bacteria in addition to bifidobacteria and lactobacilli are constantly discovered that may confer benefits to human health, including *Faecalibacterium*, *Eubacterium*, *Roseburia* and *Akkermansia*;[55–57] (v) bacteria that have been regarded previously as potentially harmful, such as some species of *Clostridium*,[10,48] appear to have beneficial characteristics under certain conditions such as colitis;[58] and (vi) studies employing novel sequencing techniques have revealed that even the currently accepted prebiotics are less selective than previously thought and include changes in many other microbial groups than bifidobacteria and lactobacilli.[57,59,60]

Whether 'fermentation' is an absolute requirement or whether it should be extended to any type of metabolism is also a matter of debate. An obvious argument for fermentation as a requirement is that SCFAs are metabolites that only result from fermentation processes. However, certain food ingredients may be utilized by microorganisms in the gut with beneficial effects for host health using other types of metabolism. Both the Food and Agriculture Organization of the United Nations (FAO)[20] and the definition by Bindels *et al*.[20] do not include microbial fermentation as a criteria. What requires consideration, however, is that, although Bindels *et al*.[20] suggest that a prebiotic confers beneficial physiological effects on the host though metabolism by microorganisms, the FAO definition[21] does not state that metabolism is necessary, thus also allowing dietary compounds that have antimicrobial activity to qualify as prebiotic. With regard to the requirement of a 'carbohydrate structure' for a prebiotic, none of the previous definitions have stated that prebiotics should be restricted to carbohydrates. Nevertheless, all current prebiotics are carbohydrates, although numerous non-carbohydrates, including micronutrients, are metabolized by the gut microbiota and may exert beneficial physiological effects.[61,62]

So far, the European Food Safety Agency (EFSA) does not consider a modulation of the gut microbiota (that is, changes in gut microbial composition or production of SCFA) as physiological benefit *per se* but states that it further requires human intervention studies to established a clear link between prebiotic use and a relevant clinical outcome.[63] As a result, the European regulatory bodies currently do not allow the word 'prebiotic' to appear on products because the definition of 'prebiotic' contains an inherent health claim. It is not surprising that this has complicated advances in prebiotic food research significantly in recent years. More recently, non-digestible native chicory inulin was given a positive opinion for the 'maintenance of normal defecation' and increases in bacterial cell mass and fermentation of inulin to SCFAs were cited among the mechanisms involved.[64] In addition, a new EFSA guideline document was published in 2016[65] that provides more clarity on the criteria to be considered in order to improve the likelihood of a positive regulatory opinion. Together with the efforts to reach consensus on a prebiotic definition, this should stimulate academic and industrial research for novel prebiotic foods and food ingredients that can be used to better substantiate the health benefits required for market approval.

NOVEL PREBIOTIC FOOD INGREDIENTS

Besides the acceptance of all non-digestible carbohydrates as 'true' prebiotics, several non-carbohydrate structures such as polyphenols,[66,67] minerals[68] or vitamins[69,70] that can exert beneficial effects through the modulation of the gut microbiota may qualify as prebiotics. For example, in a recent study in healthy male volunteers, administration of red wine resveratrol beneficially modulated the gut microbiota by increasing the relative abundance of *Bacteroides*, *Bifidobacterium* and other beneficial microorganisms.[67] Because an estimated 95% of dietary polyphenols may actually reach the colon,[71] it is likely that these compounds affect physiological processes to protect against, for example, chronic western diet-associated diseases. Also calcium supplementation was reported recently to modulate the gut microbiota in a prebiotic manner in dietary obese mice.[68] An effect of riboflavin (vitamin B2) on the growth of *Faecalibacterium prausnitzii* has been suggested by Khan *et al*.[70] and Clifford,[71] which we will discuss in more detail in the following paragraph.

Riboflavin as novel prebiotic ingredient?

Oxygen stress is one of the main stressors for strict anaerobic bacteria, such as *F. prausnitzii* or *Roseburia*.[72] Oxygen and even more aggressive reactive oxygen species such as free oxygen radicals rapidly oxidize their sensitive enzyme systems, as these microorganisms have no direct enzymatic means to protect themselves (for example, catalases and peroxidases), as is the case in facultative anaerobic and aerobic bacteria such as *Escherichia coli* and *Salmonella*.[73–76] It has been suggested, therefore, that oxygen tension and related redox conditions are important in determining the microbial composition and thus the health and function of the gut microbiota.[77,78] Oxygen tension and redox conditions may be particularly important at the mucus layer, where a steep oxygen gradient exists from the epithelium (where oxygen is delivered by the blood circulation) to the anaerobic lumen (where oxygen is consumed by microbial respiration).[79–81]

We hypothesized recently that compounds such as vitamins or other antioxidants that have redox-mediating functions may possess powerful prebiotic effects. When they are in an oxidized state, they may accept electrons from microbial metabolism, whereas in a reduced state they may donate electrons to reduce oxygen to water or reduce other electron acceptors that eventually lower the redox potential (Figure 1). Indeed, using an *in vitro* model mimicking the gut mucosa with its steep oxygen gradient, we found that *F. prausnitzii* can survive in moderately oxygenized environments by exploiting extracellular antioxidants such as riboflavin and cysteine (usually abundantly present in the gut) to transfer electrons to oxygen to lower the redox potential.[69,70] The growth of *F. prausnitzii* may be stimulated by the extracellular electron transport because it can transfer the electron and protons from NADH, generated in the glycolysis of glucose, to riboflavin through this process. Riboflavin will reduce other redox mediators such as cysteine and finally oxygen to water (Figure 1). In this way, using riboflavin as electron acceptor, the reducing equivalents of glycolysis can be coupled to the proton motive force and ATP generation, and the intracellular redox mediators do not have to be regenerated by fermentation, which seems to be a special form of respiration by these strict anaerobes.

The use of riboflavin by *F. prausnitzii* is a potential new function of this vitamin. Riboflavin, or vitamin B2, is a water-soluble vitamin that is readily taken up in the small intestine. It is sufficiently present in a healthy diet, with a recommended dietary allowance of 1.3 mg.[77,78] However, whether dietary riboflavin in this concentration also reaches the colon is questionable. Absorption takes place

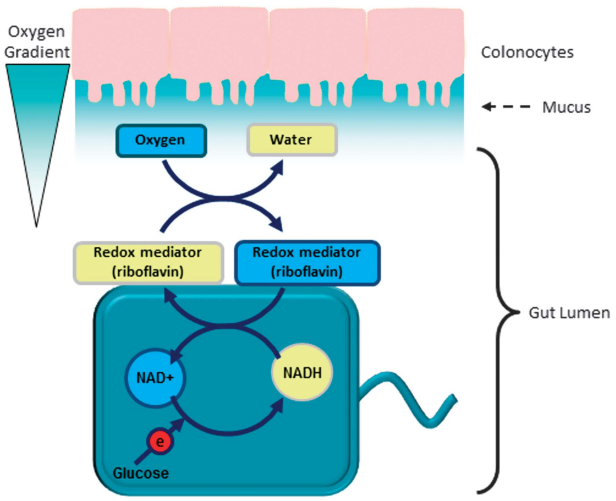

Figure 1. Schematic representation of the function of vitamins as redox mediators carrying reduction equivalents that are liberated during the glycolysis of carbohydrates via NADH to oxygen. Glucose is an example of a carbohydrate electron donor. The electrons reduce the mediators and finally oxidize electron acceptors from the epithelium, such as oxygen or nitrate. The flagellated box represents a bacterium, such as *F. prausnitzii.* In the presence of redox mediators, the anaerobic bacteria can reduce the oxygenated environment using their metabolism and thus reduce oxidative stress.[69,70]

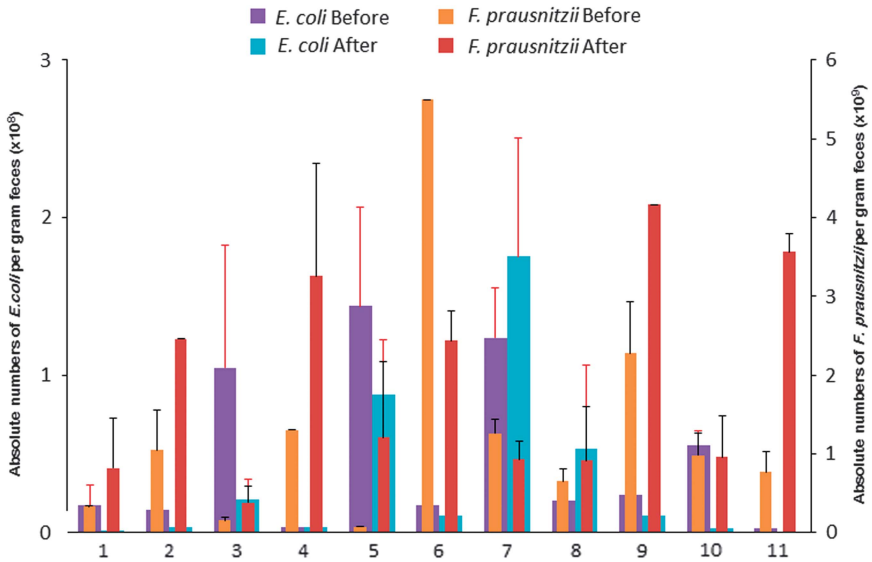

Figure 2. Counterbalance between the increased numbers of *F. prausnitzii* and decreased numbers of *E. coli* upon intervention with 100 mg/day of riboflavin in 11 volunteers. Two samples were taken before intervention, 1-week apart and a sample after 1- and 2-week intervention. The numbers of bacteria are counted by fluorescence *in situ* hybridization with a *F. prausnitzii*-specific probe Fprau645 as described previously.[97] The numbers are calculated back per gram of wet feces, and the two samples before and the two after were averaged and s.d. depicted in the error bars. Numbers of *E. coli* are depicted on the left axis and those of *F. prausnitzii* are depicted at the right axis. A counterbalance was detected in volunteers 1, 2, 3, 5, 9 and 11 (6 out of 11) but did not reach significance yet in Wilcoxon signed-rank test.

predominantly in the proximal small intestine through an active, carrier-mediated, saturable transport process that is reported to be linear up to approximately 30 mg riboflavin in a meal.[82,83] Because there is little additional absorption of riboflavin in amounts greater than this,[84] it can be assumed that riboflavin reaches the colon when administered in amounts >30 mg. Riboflavin is also produced locally by several anaerobic and facultative bacteria abundantly present in the gut, although, surprisingly, *F. prausnitzii* is not able to produce its own riboflavin.[85] The actual concentrations of available riboflavin in the gut are hard to determine and are currently unknown. We recently performed a pilot experiment to test whether the concentration of riboflavin is limiting for *F. prausnitzii* to grow optimally. A small group of adult volunteers were supplemented with 100 mg riboflavin for 14 days. Because riboflavin has been used in doses up to 400 mg per day up to 3 months in several studies in both adults and children,[86–88] this dose was considered to be safe. Indeed, we found that the number of *F. prausnitzii* per gram of feces increased during supplementation, and the number dropped again, although not to the baseline levels, after a 1-week washout period.[89] In addition to this increase, we also noticed an increase in another group of anaerobes, namely *Roseburia* species, and a decrease in *E. coli* (Figure 2), indicating an improvement in the anaerobic conditions and redox state in the gut.

The effect of riboflavin on *F. prausnitzii* may be of clinical interest because, in contrast to bifidobacteria and lactobacilli, *F. prausnitzii* is a bacterium that directly produces butyrate.[90] In addition, *F. prausnitzii* has been shown to possess anti-inflammatory properties and improve gut barrier function, not only through the production of butyrate[91] but also by producing specific anti-inflammatory peptides.[56,92] Interestingly, IBD and especially Crohn's disease are characterized by low levels of *F. prausnitzii* and an increased number of *E. coli* and other *Enterobacteriaceae*. This ratio *of F. prausnitzii/E. coli* may be indicative of oxidative stress during gut inflammation.[93] Neutrophils and other immune cells may cause an oxidative burst of reactive oxygen species that raise the redox potential to an oxidative state.[94–96] An improvement in the ratio of *F. prausnitzii/E. coli* in favor of *F. prausnitzii* may, therefore, indicate an improvement in redox conditions, which may be beneficial to restore dysbiosis during a remission period of IBD. Whether the use of riboflavin is a therapeutic or a supportive agent during the treatment of IBD, and especially Crohn's disease, or in healthy subjects is currently unknown but warrants urgent investigation.

CONCLUSION

The large intestine was long considered an organ that only absorbs water and electrolytes and processes undigested foods to facilitate their excretion. In the past decades, however, the colon and its microbial ecosystem has been recognized as affecting host physiology and health, and modifying the colonic microbiota by functional foods has attracted significant research and product development. The concept of prebiotics, as introduced by Gibson and Roberfroid[10] in the 1990s, was pioneering in emphasizing the importance of diet in the modulation of the gut microbiota and its relationship to human health. Increasing knowledge of the intestinal microbiota may now open the door for novel prebiotic foods, such as vitamins, polyphenols or minerals. For example, riboflavin, although it does not provide a direct substrate for microbial fermentation, may beneficially modulate the composition of the gut microbiota by being metabolized and changing the gastrointestinal redox state.

CONFLICT OF INTEREST

RES and PW are employees of DSM Nutritional Products, Basel, Switzerland. The other authors declare no conflict of interest.

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REFERENCES

1 Owyang C, Wu GD. The gut microbiome in health and disease. *Gastroenterology* 2014; **146**: 1433–1436.
2 Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G *et al.* The gut microbiota and host health: a new clinical frontier. *Gut* 2015; **65**: 330–339.

3 Frank St DN, Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; **104**: 13780–13785.
4 de Goffau MC, Luopajarvi K, Knip M, Ilonen J, Ruohtula T, Harkonen T *et al.* Fecal microbiota composition differs between children with beta-cell autoimmunity and those without. *Diabetes* 2013; **62**: 1238–1244.
5 Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022–1023.
6 Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 2012; **13**: 701–712.
7 Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. *Front Physiol* 2011; **2**: 94.
8 Metchnikoff E. The prolongation of life: optimistic studies. In: Chalmers Mitchell P (ed). GP Putnam's Sons: New York, London, 1908.
9 van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM *et al.* Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* 2013; **368**: 407–415.
10 Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995; **125**: 1401–1412.
11 David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; **505**: 559–563.
12 Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O *et al.* Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009; **58**: 1091–1103.
13 Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D *et al.* Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am J Clin Nutr* 2009; **90**: 1236–1243.
14 Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr* 2014; **111**: 1147–1161.
15 Yazawa K, Imai K, Tamura Z. Oligosaccharides and polysaccharides specifically utilizable by bifidobacteria. *Chem Pharm Bull (Tokyo)* 1978; **26**: 3306–3311.
16 Mitsuoka T, Hidaka H, Eida T. Effect of fructo-oligosaccharides on intestinal microflora. *Nahrung* 1987; **31**: 427–436.
17 Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* 2004; **17**: 259–275.
18 Roberfroid M. Prebiotics: the concept revisited. *J Nutr* 2007; **137**(Suppl 2): 830S–837S.
19 Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A *et al.* Dietary prebiotics: current status and new definition. *Food Sci Technol Bull (Funct Foods)* 2010; 1–19.
20 Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 303–310.
21 Pineiro M, Asp NG, Reid G, Macfarlane S, Morelli L, Brunser O *et al.* FAO Technical meeting on prebiotics. *J Clin Gastroenterol* 2008; **42**: S156–S159.
22 Hutkins RW, Krumbeck JA, Bindels LB, Cani PD, Fahey Jr G, Goh YJ *et al.* Prebiotics: why definitions matter. *Curr Opin Biotechnol* 2016; **37**: 1–7.
23 Reid G, Sanders ME, Gaskins HR, Gibson GR, Mercenier A, Rastall R *et al.* New scientific paradigms for probiotics and prebiotics. *J Clin Gastroenterol* 2003; **37**: 105–118.
24 De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010; **107**: 14691–14696.
25 Chassard C, Lacroix C. Carbohydrates and the human gut microbiota. *Curr Opin Clin Nutr Metab Care* 2013; **16**: 453–460.
26 Marcobal A, Sonnenburg JL. Human milk oligosaccharide consumption by intestinal microbiota. *Clin Microbiol Infect* 2012; **18**: 12–15.
27 Nyangale EP, Mottram DS, Gibson GR. Gut microbial activity, implications for health and disease: the potential role of metabolite analysis. *J Proteome Res* 2012; **11**: 5573–5585.
28 Riviere A, Gagnon M, Weckx S, Roy D, De Vuyst L. Mutual cross-feeding interactions between *Bifidobacterium longum* NCC2705 and *Eubacterium rectale* ATCC 33656 explain the bifidogenic and butyrogenic effects of arabinoxylan-oligosaccharides. *Appl Environ Microbiol* 2015; **81**: 7767–7781.
29 Salyers AA. Energy sources of major intestinal fermentative anaerobes. *Am J Clin Nutr* 1979; **32**: 158–163.
30 Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C *et al.* Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ* 2007; **335**: 80.
31 Bouhnik Y, Flourie B, D'Agay-Abensour L, Pochart P, Gramet G, Durand M *et al.* Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *J Nutr* 1997; **127**: 444–448.
32 Jaskari J, Kontula P, Siitonen A, Jousimies-Somer H, Mattila-Sandholm T, Poutanen K. Oat beta-glucan and xylan hydrolysates as selective substrates for Bifidobacterium and Lactobacillus strains. *Appl Microbiol Biotechnol* 1998; **49**: 175–181.
33 Kleessen B, Sykura B, Zunft HJ, Blaut M. Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. *Am J Clin Nutr* 1997; **65**: 1397–1402
34 Rastall RA, Gibson GR. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Curr Opin Biotechnol* 2015; **32**: 42–46.
35 Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M *et al.* Safety of probiotics that contain lactobacilli or bifidobacteria. *Clin Infect Dis* 2003; **36**: 775–780.
36 Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M *et al.* Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635–1638.
37 Verbeke KA, Boobis AR, Chiodini A, Edwards CA, Franck A, Kleerebezem M *et al.* Towards microbial fermentation metabolites as markers for health benefits of prebiotics. *Nutr Res Rev* 2015; **28**: 42–66.
38 Lewis S, Burmeister S, Brazier J. Effect of the prebiotic oligofructose on relapse of *Clostridium difficile*-associated diarrhea: a randomized, controlled study. *Clin Gastroenterol Hepatol* 2005; **3**: 442–448.
39 Whelan K. Probiotics and prebiotics in the management of irritable bowel syndrome: a review of recent clinical trials and systematic reviews. *Curr Opin Clin Nutr Metab Care* 2011; **14**: 581–587.
40 Leenen CH, Dieleman LA. Inulin and oligofructose in chronic inflammatory bowel disease. *J Nutr* 2007; **137**(11 Suppl): 2572S–2575S.
41 Drakoularakou A, Tzortzis G, Rastall RA, Gibson GR. A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers' diarrhoea. *Eur J Clin Nutr* 2010; **64**: 146–152.
42 Osborn DA, Sinn JK. Prebiotics in infants for prevention of allergy. *Cochrane Database Syst Rev* 2013; **3**: CD006474.
43 Verhoef SP, Meyer D, Westerterp KR. Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. *Br J Nutr* 2011; **106**: 1757–1762.
44 Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr* 2009; **89**: 1751–1759.
45 Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L *et al.* The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* 2014; **5**: 3611.
46 Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015; **11**: 577–591.
47 De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A *et al.* Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 2014; **156**: 84–96.
48 Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I *et al.* Prebiotic effects: metabolic and health benefits. *Br J Nutr* 2010; **104**: S1–S63.
49 Gibson GR, Fuller R. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. *J Nutr* 2000; **130**: 391S–395S.
50 Bird AR, Conlon MA, Christophersen CT, Topping DL. Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics. *Benef Microbes* 2010; **1**: 423–431.
51 Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF *et al.* Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; **143**: 913–916 e917.
52 Slavin J. Fiber and prebiotics: mechanisms and health benefits. *Nutrients* 2013; **5**: 1417–1435.
53 Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol* 2014; **426**: 3838–3850.
54 Birt DF, Boylston T, Hendrich S, Jane JL, Hollis J, Li L *et al.* Resistant starch: promise for improving human health. *Adv Nutr* 2013; **4**: 587–601.
55 Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ *et al. Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008; **105**: 16731–16736.
56 Quevrain E, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J *et al.* Identification of an anti-inflammatory protein from *Faecalibacterium*

prausnitzii, a commensal bacterium deficient in Crohn's disease. *Gut* 2016; **65**: 415–425.
57 Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM *et al.* Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* 2011; **60**: 2775–2786.
58 Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H *et al.* Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 2013; **500**: 232–236.
59 Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM *et al.* Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013; **62**: 1112–1121.
60 Serino M, Luche E, Gres S, Baylac A, Berge M, Cenac C *et al.* Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. *Gut* 2012; **61**: 543–553.
61 Anhe FF, Roy D, Pilon G, Dudonne S, Matamoros S, Varin TV *et al.* A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of mice. *Gut* 2015; **64**: 872–883.
62 Neyrinck AM, Van Hee VF, Bindels LB, De Backer F, Cani PD, Delzenne NM. Polyphenol-rich extract of pomegranate peel alleviates tissue inflammation and hypercholesterolaemia in high-fat diet-induced obese mice: potential implication of the gut microbiota. *Br J Nutr* 2013; **109**: 802–809.
63 Salminen S, van Loveren H. Probiotics and prebiotics: health claim substantiation. *Microb Ecol Health Dis* 2012; **18**: 23.
64 EFSA Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion on the substantiation of a health claim related to 'native chicory inulin' and maintenance of normal defecation by increasing stool frequency pursuant to Article 13.5 of Regulation (EC) No 1924/2006. *EFSA J* 2015; **13**: 3951.
65 EFSA Panel on Dietetic Products, Nutrition and Allergies. Guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms. *EFSA J* 2016; **14**: 23.
66 Duenas M, Munoz-Gonzalez I, Cueva C, Jimenez-Giron A, Sanchez-Patan F, Santos-Buelga C *et al.* A survey of modulation of gut microbiota by dietary polyphenols. *Biomed Res Int* 2015; **2015**: 850902.
67 Queipo-Ortuno MI, Boto-Ordonez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R *et al.* Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am J Clin Nutr* 2012; **95**: 1323–1334.
68 Chaplin A, Parra P, Laraichi S, Serra F, Palou A. Calcium supplementation modulates gut microbiota in a prebiotic manner in dietary obese mice. *Mol Nutr Food Res* 2016; **60**: 468–480.
69 Khan MT, Browne WR, van Dijl JM, Harmsen HJ. How can *Faecalibacterium prausnitzii* employ riboflavin for extracellular electron transfer? *Antioxid Redox Signal* 2012; **17**: 1433–1440.
70 Khan MT, Duncan SH, Stams AJ, van Dijl JM, Flint HJ, Harmsen HJ. The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic-anoxic interphases. *ISME J* 2012; **6**: 1578–1585.
71 Clifford MN. Diet-derived phenols in plasma and tissues and their implications for health. *Planta Med* 2004; **70**: 1103–1114.
72 Rigottier-Gois L. Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis. *ISME J* 2013; **7**: 1256–1261.
73 Carmel-Harel O, Storz G. Roles of the glutathione- and thioredoxin-dependent reduction systems in the *Escherichia coli* and *Saccharomyces cerevisiae* responses to oxidative stress. *Annu Rev Microbiol* 2000; **54**: 439–461.
74 Jones SA, Gibson T, Maltby RC, Chowdhury FZ, Stewart V, Cohen PS *et al.* Anaerobic respiration of *Escherichia coli* in the mouse intestine. *Infect Immun* 2011; **79**: 4218–4226.
75 Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM *et al.* Host-derived nitrate boosts growth of *E. coli* in the inflamed gut. *Science* 2013; **339**: 708–711.
76 Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW *et al.* Gut inflammation provides a respiratory electron acceptor for Salmonella. *Nature* 2010; **467**: 426–429.
77 Neish AS, Jones RM. Redox signaling mediates symbiosis between the gut microbiota and the intestine. *Gut Microbes* 2014; **5**: 250–253.
78 Schroeder BO, Wu Z, Nuding S, Groscurth S, Marcinowski M, Beisner J *et al.* Reduction of disulphide bonds unmasks potent antimicrobial activity of human beta-defensin 1. *Nature* 2011; **469**: 419–423.
79 Espey MG. Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. *Free Radic Biol Med* 2013; **55**: 130–140.
80 Albenberg L, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A *et al.* Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* 2014; **147**: 1055–1063 e1058.
81 Van den Abbeele P, Van de Wiele T, Verstraete W, Possemiers S. The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. *FEMS Microbiol Rev* 2011; **35**: 681–704.
82 Jusko WJ, Levy G. Absorption, metabolism, and excretion of riboflavin-5'-phosphate in man. *J Pharm Sci* 1967; **56**: 58–62.
83 McCormick DB. Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol Rev* 1989; **69**: 1170–1198.
84 Zempleni J, Galloway JR, McCormick DB. Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* 1996; **63**: 54–66.
85 Heinken A, Khan MT, Paglia G, Rodionov DA, Harmsen HJ, Thiele I. Functional metabolic map of *Faecalibacterium prausnitzii*, a beneficial human gut microbe. *J Bacteriol* 2014; **196**: 3289–3302.
86 Condo M, Posar A, Arbizzani A, Parmeggiani A. Riboflavin prophylaxis in pediatric and adolescent migraine. *J Headache Pain* 2009; **10**: 361–365.
87 Schoenen J, Jacquy J, Lenaerts M. Effectiveness of high-dose riboflavin in migraine prophylaxis. A randomized controlled trial. *Neurology* 1998; **50**: 466–470.
88 Boehnke C, Reuter U, Flach U, Schuh-Hofer S, Einhaupl KM, Arnold G. High-dose riboflavin treatment is efficacious in migraine prophylaxis: an open study in a tertiary care centre. *Eur J Neurol* 2004; **11**: 475–477.
89 Sadaghian Sadabad M. Interaction between the gut and its microbiota in inflammatory bowel disease. PhD thesis, University of Groningen, Groningen, The Netherlands, 2015, ISBN 9789462990340.
90 Lopez-Siles M, Khan TM, Duncan SH, Harmsen HJ, Garcia-Gil LJ, Flint HJ. Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids, and host-derived substrates for growth. *Appl Environ Microbiol* 2012; **78**: 420–428.
91 Ploger S, Stumpff F, Penner GB, Schulzke JD, Gabel G, Martens H *et al.* Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Ann NY Acad Sci* 2012; **1258**: 52–59.
92 Rossi O, Khan MT, Schwarzer M, Hudcovic T, Srutkova D, Duncan SH *et al. Faecalibacterium prausnitzii* strain HTF-F and its extracellular polymeric matrix attenuate clinical parameters in DSS-induced colitis. *PLoS One* 2015; **10**: e0123013.
93 Willing B, Halfvarson J, Dicksved J, Rosenquist M, Jarnerot G, Engstrand L *et al.* Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 653–660.
94 Winter SE, Baumler AJ. A breathtaking feat: to compete with the gut microbiota, Salmonella drives its host to provide a respiratory electron acceptor. *Gut Microbes* 2011; **2**: 58–60.
95 Gill N, Ferreira RB, Antunes LC, Willing BP, Sekirov I, Al-Zahrani F *et al.* Neutrophil elastase alters the murine gut microbiota resulting in enhanced Salmonella colonization. *PLoS One* 2012; **7**: e49646.
96 Vong L, Pinnell LJ, Maattanen P, Yeung CW, Lurz E, Sherman PM. Selective enrichment of commensal gut bacteria protects against *Citrobacter rodentium*-induced colitis. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G181–G192.
97 Benus RF, van der Werf TS, Welling GW, Judd PA, Taylor MA, Harmsen HJ *et al.* Association between *Faecalibacterium prausnitzii* and dietary fibre in colonic fermentation in healthy human subjects. *Br J Nutr* 2010; **104**: 693–700.