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**FODMAPS, PREBIOTICS AND GUT HEALTH.
THE FODMAP HYPOTHESIS REVISITED**

Short title: FODMAPs, prebiotics and gut health

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SUMMARY

Background: Restriction of dietary FODMAP intake can alleviate symptoms in patients with irritable bowel syndrome. Because many FODMAPs have prebiotic actions, there is concern that their dietary restriction leads to dysbiosis with health consequences, and their intake is being encouraged by addition to foods and via supplements.

Aims: To examine the hazards and benefits of high and low FODMAP intake.

Methods: Current literature was reviewed and alternative hypotheses formulated.

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Results: Low FODMAP intake reduces abundance of faecal Bifidobacteria without known adverse outcomes and has no effect on diversity, but the reduction in bacterial density may potentially be beneficial to gut health. Supplementary prebiotics can markedly elevate the intake of FODMAPs over levels consumed in the background diet. While this increases the abundance of Bifidobacteria, it adversely affects gut health in animal studies by inducing colonic mucosal barrier dysfunction, mucosal inflammation and visceral hypersensitivity. Rapid colonic fermentation is central to the identified mechanisms that include injury from high luminal concentrations of short-chain fatty acids and low pH, and inflammatory effects of increased endotoxin load and glycation of macromolecules. Whether these observations translate into humans requires further study. Opposing hypotheses are presented whereby excessive intake of FODMAPs might have health benefits via prebiotic effects, but might also be injurious and contribute to the apparent increase in functional intestinal disorders.

Conclusions: Reduced FODMAP intake have few deleterious effects on gut microbiota. Consequences (both positive and negative) of excessive carbohydrate fermentation in the human intestines from elevated FODMAP intake require more attention.

INTRODUCTION

In 2005, a collective group of dietary short-chain carbohydrates that are slowly absorbed in the small intestine or non-digestible due to inactivity or lack of hydrolases were described and termed FODMAPs (Fermentable Oligosaccharides, Monosaccharides, Disaccharides and Polyols) [1]. A hypothesis was presented for their involvement in the pathogenesis of Crohn's disease. However, their ability to induce abdominal symptoms when consumed at usual dietary amounts in patients with irritable bowel syndrome (IBS) and the symptomatic alleviation that reliably occurs when they are reduced in the diet somewhat diverted attention away from potential harmful effects of FODMAPs on intestinal integrity [2,3]. Furthermore, the notion that at least the oligosaccharide components of FODMAPs are prebiotic for colonic microbiota raised concerns that strict restriction of their intake might be potentially harmful to health, in line with prebiotic concepts [2]. Hence, FODMAPs migrated from potentially inflammation-inducing to promoting of health. Recent studies in rats, mice and humans have once again indicated that FODMAPs might indeed be harmful to the colon. This is of particular relevance to prebiotic FODMAPs since these are being liberally added to the food supply, including baby formulas [4], particularly as fructans (inulin, chicory root extract, fructo-oligosaccharide), with the view that they will promote health by favourable effects on gut microbial communities.

Hence, the aim of this review is to revisit the FODMAP hypothesis and address the issue of whether too many FODMAPs might be harmful.

FODMAPS VERSUS PREBIOTICS

FODMAPs are defined by their molecular size, absorptive characteristics in the small intestine and fermentability [2,3]. Prebiotics are defined by their effects on microbiota and health, and are substrates that are selectively utilised by host microorganisms conferring a health benefit [5]. Many dietary carbohydrates that have prebiotic actions are members of the FODMAP group of carbohydrates. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), polyols and possibly fructose selectively stimulate the growth of specific types of bacteria such as *Bifidobacteria*, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, which are believed to be health-promoting [6,7]. That dietary FODMAPs have prebiotic effects was clearly demonstrated in a feeding intervention of a diet containing a higher FODMAP oligosaccharide content (5.5 g/d) compared with that of the low FODMAP arm (1.57 g/d) or the habitual dietary intake (3.9 g/d) in patients with IBS and in healthy subjects [8]. Similarly observations were made in a small cohort with quiescent Crohn's disease [9]. Bifidogenic effects occur at relatively small doses of inulin and FOS (5 g/d) [10,11] and dose-dependent effects (inulin: 5 vs 8 g/d; synthetic GOS: 3.5 vs 7 g/d) have been observed [11]. However, whether larger doses, such as 15-20 g/d used in some studies, achieve enhanced selective actions is not known, but do lead to increased fermentation and risk of symptoms related to it [11]. If prebiotic effects do plateau, the efficacy of supplements of, for example, FOS/inulin may be blunted if the background dietary intake of FOS/inulin is already moderately high. Prebiotic effects appear to be greater when the baseline relative abundance of the *Bifidobacteria* is low [10], but whether this baseline low abundance was related to habitual dietary intake of prebiotic oligosaccharides was not assessed. Indeed, background diet has not been taken into account in nearly all human prebiotic interventions (in contrast to animal interventions where this is controlled). This confounder might explain the failure of most studies to show clear health benefits of prebiotic supplementation. For example, in a well-powered study in patients with Crohn's disease in whom background diet was not assessed, 15 g/d of FOS had no health benefits and did not selectively stimulate *Bifidobacteria*, but was associated with increased fermentative symptoms such as bloating [12]. The inefficacy of supplemental FOS might reflect adequate background dietary prebiotic intake in the patients via foods containing fructans (e.g., wheat and onions), such that no additional prebiotic effect on microbial communities was able to be achieved and that non-specific fermentation of the

FOS led to increased symptoms. This issue may potentially be overcome by the use of synthetic oligosaccharide prebiotics if they have different bacterial specificities to dietary prebiotics, but such a concept has not been tested.

The general acceptance of the health benefits of prebiotics is based upon metabolic and immunological end-points, but these have seldom been backed up by quality data on health-related outcomes in clinical trials in most areas of study, as shown in systematic reviews in several areas of health promotion [11,13-21]. The difficulty in showing benefits might, as discussed above, relate to the lack of consideration to background diet in the design of trials, or it might relate to the fact that the concept is overblown and benefits may be very modest indeed. Whichever is true, the current practice within the food industry to add prebiotics to multiple foods, and the likely high intake of food-related prebiotics in some subgroups of the population (such as vegetarians and vegans) might not be promoting health as thought. Of even more importance is that the passion to show benefits of prebiotics might contribute to a cognitive bias in not recognising or accepting adverse effects of high doses on health, effects that might not relate directly to effects on microbial community structure. For example, marked tiredness in patients with IBS induced by a 'high FODMAP diet' in a controlled short-term feeding study [22] might be viewed differently if the dietary intervention was described (correctly) as a 'high prebiotic diet'.

WHAT REPRESENTS HIGH AND LOW DAILY INTAKE OF FODMAPS?

Human studies

In order to understand the implications of interventional studies where the intake of FODMAPs was altered, concepts of 'low' and 'high' are needed. *Normal FODMAP intake* was estimated by the CNAQ in a study of 72 healthy Australian adults, utilising the Monash University database of FODMAP content of foods [23]. Intake of non-digestible oligosaccharides, principally fructans and galacto-oligosaccharides (GOS), is about 3-6 g/d and of the slowly-absorbed polyols about 2-3 g/d. The intake of fructose in excess of glucose (when fructose is slowly absorbed) may be 10-14 g/d, but this is less easy to interpret as the rapidity of its absorption is variable amongst individuals and dependent on the concomitant intake of other foods containing glucose. Intakes of lactose and sucrose are only meaningful as FODMAPs when the activities of the brush border hydrolases, lactase and sucrase-isomaltase respectively, are known in the individual.

Low FODMAP intake is easy to define as direct measurements of whole diets containing no high FODMAP foods have been reported from studies and might, for example, contain <3 g/d oligosaccharides and <1.0 g/d polyols [24,25]. *Moderate-to-high FODMAP intake* might comprise 6-8 g/d of oligosaccharides and 3.5-4.5 g/d of polyols [25]. Actual individual intake varies greatly due to the choice of foods; for example, higher intake of GOS occurs in UK populations compared with those in Australia, due presumably to variations in legume consumption [24,25]. Certainly, where legumes are used as the main protein source and, therefore, the major component of the dish, as seen in vegetarian and vegan diets, oligosaccharide content could easily exceed 2.0 g per sitting. It would be difficult to achieve >10-12 g oligosaccharides from food choice alone, provided the food is not supplemented with fructans (usually inulin).

The importance of these 'ball-park' figures is to put into perspective the daily intake observed in studies where oligosaccharide supplements are used. Fructans have been supplemented at 5-15 g/d and GOS at 3.5-7 g/d. When added to those from the habitual (background) diet, oligosaccharide intake reaches levels not readily achieved by the consumption of food, as illustrated in Figure 1. Many processed foods are spiked with inulin or other oligosaccharides, mainly for their putative prebiotic effects, although the actual amount consumed per standard portion by this means is not readily calculated. However, it is highly likely that the intake of oligosaccharides from 'fortified' foods does reach levels much in excess of those from whole food diets. Such factors have to be kept in mind when interpreting data from dietary studies.

Animal studies

The advantages of studying the effects of dietary manipulation in experimental animals (usually rodents) is that the diet can be strictly controlled and that tolerance to dietary change is less limiting. However, the different anatomy, physiology, biochemistry and microbiology of animal (especially rodent) intestine to those of the human gut, which have been well described [26-28] present considerable interpretative and translational challenges. Furthermore, the diets used in animal studies, both experimental and comparator, are not easy to extrapolate to humans as they are not often tolerated or are not possible to translate into the clinical setting. The oligosaccharide intake as a proportion by weight of a human high FODMAP diet would not exceed 1%. In a sample of rodent studies, the experimental diet has been supplemented with 4-10% FOS [29-32], 5% inulin [33] and 10% multiple FODMAPs (fructose, FOS and lactose) [34]. This would equate to approximately 0.5-1.5 g of FODMAP

per day. Claims that these doses are equivalent to a moderate increase in fructan intake in humans [33] are not easy to justify. The comparator diet is often standard chow or chow that is devoid of non-digestible carbohydrates. Moreover, the chow is seldom assessed for FODMAP content, which actually varies considerably across commercially available chows [35]. With such uncertainty, the differences in effects on the colon and its microenvironment achieved by the intervention compared with control might reflect the impact of unrealistical and unachievable intake of fructans or other FODMAPs, abnormally low intake in the control animals, or both. These issues have to be considered in the interpretation of published data. The purposes then of studying animals is to address concepts, to gain insight into what might be possible in the human, and then to design studies to support or refute observations made.

OTHER INTERPRETATIVE LIMITATIONS

Altering FODMAP content of the diet will also affect other aspects of the diet due to differences in the foods chosen. FODMAPs co-exist with other non-digestible carbohydrates in many foods, especially oligosaccharide with wheat and legumes, which are important source of non-starch polysaccharides (NSP). How and where FODMAPs are fermented in the large bowel is dictated by dose and by also the concomitant presence of more slowly fermented, or non-fermentable, carbohydrates. In several rodent studies, fermentation of FODMAPs appears to continue through the length of the colon as outlined above, probably indicating that the dose was very large and implying that the conditions created are unlikely to mimic the effects in the human. Concomitant slowly fermented, or non-fermentable, NSP intake will slow fermentation of rapidly fermented substrates and push fermentation around the colon [36]. This situation was observed in one human volunteer study where supplemental FODMAPs (FOS) increased faecal lactate concentrations as evidence of local fermentation [37]. Those subjects consumed a very high fibre diet. The important corollary of this is that lowering NSP intake together with reducing FODMAP intake is likely to exhaust substrates for carbohydrate fermentation in the more proximal colon and promote protein fermentation in the distal colon [38]. In studies where the fibre intake was not corrected in conjunction with lowering FODMAP intake, this scenario indeed occurred [39,40]. In those studies. The enhance protein fermentation was more likely to be due to reduced fibre intake than the lowering of FODMAPs per se.

FODMAPs are not the only highly and rapidly fermentable dietary load in the diet. Longer-chain inulin is added to many foods and being promoted as a prebiotic. Despite inulin having a chain length that can vary from 2-60 fructose units [41], it is a rapidly-fermented fructan and

is generally considered a FODMAP, although there is no precise definition for the maximal chain length of a carbohydrate that is still considered a FODMAP. However, other non-digestible carbohydrates (including resistant starch and other rapidly fermentable NSP) are also being added to the food supply for their putative prebiotic effects and for improving organoleptic properties (i.e., the taste, smell, appearance and texture) of food. Evidence is presented below to indicate fermentation of such longer-chain carbohydrates might cause effects similar to those of FODMAPs [36]. When issues with excessive fermentation are being considered (as discussed below), such dietary components need also to be included in the equation.

EFFECT OF VARYING DIETARY INTAKE OF FODMAPS ON GUT MICROBIAL COMMUNITIES

Several studies have examined the effect of varying dietary FODMAP intake in healthy subjects or those with IBS. There has been heterogeneity of methods employed for microbial profiling, of targeted phyla, genera and species, and of the nature of the comparator diets. Defining what changes relate specifically to changes due to reducing FODMAPs, due to increasing FODMAPs or to both is not simple, but consistent messages have emerged.

1. **Richness and alpha diversity:** These have not been altered by a diet low in FODMAPs [8,9,23,39,41,42], except for one study in which increased richness and diversity was noted [30]. This was of significance since maintenance of microbial diversity is believed to be a key for a healthy microbiome. In contrast, diversity was reduced in association with a high FODMAP diet in one study [43].
2. **Absolute abundance of faecal bacteria depends upon the FODMAP availability:** Since FODMAPs are a major source of energy for microbes, reduced abundance or density has been a consistent observation [8,9,42,44] in faeces during a low FODMAP diet compared with habitual or comparator diet. Furthermore, the LPS content of faecal water from 6 subjects with IBS-D was elevated compared to that of 6 healthy controls, but reduced to normal after 4 weeks of a low FODMAP intake concomitant with reduced IBS symptoms [34]. *Bacteroides* contributes to the majority of LPS in faeces [45].
3. **The phylum *Actinobacter* and genus *Bifidobacterium*:** Across nearly all studies, *Actinobacter* and the genus *Bifidobacteria* have reduced relative abundance during low FODMAP intake relative to control arms where FODMAP intake is normal or high [23,39,40,42-44,46] and their normalisation can be achieved with supplemental FOS [24].

Likewise, the intake of supplemental FOS, inulin or GOS is bifidogenic in the vast majority of multiple studies [47].

4. **Butyrate-producing *Firmicutes*.** Alteration of the abundance of saccharolytic *Firmicutes* might be anticipated as its fermentative substrate is increased or decreased. In a cross-over feeding study involving 30 patients with IBS, 8 healthy controls and 9 with Crohn's disease, the relative abundance of a broad grouping of saccharolytic bacteria, *Clostridium* cluster XIVa, was consistently higher in the dietary arm in which FODMAP oligosaccharide intake was highest than observed when consuming a low FODMAP or their habitual diet (as detailed above) (8,9). These findings were more consistent with a prebiotic effect of the extra oligosaccharides than a loss of prebiotic effects during the low FODMAP diet. However, when specific members or families of *Firmicutes* are examined, consistent effects have not been observed. The effect of FODMAPs on *F. prausnitzii* is of particular interest due to its high abundance (>5% of faecal bacteria), its ability to produce butyrate and putative anti-inflammatory effects, and its reduction in patients with inflammatory bowel disease. Its relative abundance was suppressed in association with a low FODMAP diet and corrected with supplementation with FOS in one study [39], but neither effect has been observed in other studies [8,9,23,42-44]. Members of the family of *Lachnospiraceae* have exhibited diverse responses to FODMAP supplementation. *Anaerostipes*, which can ferment inulin directly and utilise products of bifidobacterial fermentation of inulin, exhibited consistent increase in abundance in response to inulin [47,48]. However, *Roseburia* was suppressed in the high FODMAP arm or when FOS supplementation was given [41].
5. ***Akkermansia muciniphila*:** This is one of the few members in the gut of the phylum, *Verrucomicrobia*, and is a mucus-associated bacterium that degrades mucus, stimulates mucous secretion and has syntrophic interactions such as production of butyrate from acetate, amongst other effects [7]. Since its faecal abundance is reduced in pathological situations such as inflammatory bowel disease and type 2 diabetes, it is putatively regarded as health-promoting bacterium [7]. Increased relative abundance of *A. muciniphila* was consistently demonstrated in a feeding study involving patients with Crohn's disease and IBS as well as healthy subjects in association with a moderate FODMAP diet compared with that during habitual and low FODMAP diet periods where the oligosaccharide intake was lower [8,9]. In other studies, inulin, FOS and GOS supplementation have all been associated with increased relative abundance of *A. muciniphila* [7]. However, other studies

in which diets varied in FODMAP content have not shown associated changes in its abundance [39-41].

6. **Bilophila:** This genus in the Proteobacteria phylum is bile-tolerant and its most studied species, *Bilophila wadsworthia*, is saccharolytic and sulfate-reducing. It generally comprises <0.1% of the gut microbiota and, not surprisingly, was associated with consumption of an animal-based diet in the classic study of David et al.[47,48] *Bilophila* had increased relative abundance in association with low FODMAP intake in two studies [42,52] and was suppressed with the intake of GOS and inulin supplements [48,52]. These observations are consistent with the apparent inverse relationship between carbohydrate and protein fermentation according to availability of substrate (discussed below); increased protein fermentation would increase the availability of sulphite as an electron acceptor to facilitate the anaerobic respiration by *Bilophila* [53].
7. **Other bacteria:** *Ruminococcus* spp. have been variably affected with apparent increases in relative abundance in association with low FODMAP ingestion in two studies [8,44]. Other alterations reported have not been reproduced.

Conclusions

Difficulties in interpreting microbial structure in the setting of altering FODMAP intake is confounded by heterogeneous methodologies and poorly documented background dietary intake. The effect of collinearity that besets dietary studies is ever-present. For example, polyphenols, potentially powerful factors that change microbial structure [54], are not measured or considered in most dietary intervention studies that concentrate on carbohydrate content. Fervour around the positive and negative health-related effects associated with microbial community structure with alteration of the intake of dietary FODMAPs, either by modification of the diet or by the intake of supplementary prebiotics, seems generally to be poorly supported. Microbial diversity and richness are not affected. The major changes evolve around the relative abundance of *Bifidobacteria*, but the implications of this for health remain more emotive than based upon data with health outcomes. While variations in abundances of saccharolytic bacteria will occur, details of the microbial changes do not appear to be predictable and reliable diet-related expansion of specifically targeted bacteria such as *F. prausnitzii* has not occurred. Suppression of *A. muciniphila* by reducing FODMAP exposure has not been consistently observed, but higher intake of FODMAPs or specific FODMAP prebiotics is likely to relate causally to its specific expansion if the background diet is low in these prebiotics. The stimulation of *Bilophila* may reflect the exhaustion of carbohydrate

substrates in the distal colon that might better reflect the amount of less rapidly fermented fibres (resistant starch and non-starch polysaccharides) than FODMAPs (i.e., a manifestation of collinearity – see discussion below), and might represent a biomarker of inadequate fibre intake in this setting.

EFFECT OF VARYING DIETARY INTAKE OF FODMAPS ON THE INTESTINAL MICROENVIRONMENT

The functional interaction of the microbiota with its non-microbial environment might provide greater insight into beneficial or harmful effects of altering FODMAP intake, especially with the redundancy of functional capability of the microbiota that may not be reflected in microbial community structure. A major limitation of understanding effects on the soluble microenvironment is access to the contents in the free-living ambulatory state, other than those of the rectum and faeces. While the development of telemetric capsule technology may overcome some of the limitations [55], observations in experimental animals remain an important source of information from which concepts in humans are built and subsequently evaluated regarding their applicability.

Several quantifiable molecules or functional activity in the luminal compartment provide information regarding microbial metabolic activities that may have importance to the function and/or health of the intestinal mucosa. The putative importance of and the effects of varying FODMAP intake on these are outlined below.

Biomarkers of carbohydrate fermentation

Key biomarkers of carbohydrate fermentation are the concentration or production of the short-chain fatty acids (SCFA), acetate, propionate and butyrate [56], together with luminal pH that is largely dependent upon the SCFA, although other weak acids, such as lactate and succinate, and mildly basic molecules like ammonia may also contribute to luminal pH.

Relevance: *Luminal pH* influences absorption of charged molecules. For example, lowering luminal pH in response to prebiotic use inhibits absorption of ammonia and similar molecules (a mechanism for alleviating portosystemic encephalopathy in patients with portal hypertension) and may improve calcium absorption, although the physiological importance of colonic calcium absorption may not be as important to humans as it is to rats[57,58]. The microbial communities are affected by luminal pH [59]; growth and fermentation of saccharolytic bacteria is inhibited with lowering of the pH, which might be viewed as a self-

regulating system [60]. Epithelial and mucosal effects of lowering luminal pH are discussed below.

The major *short-chain fatty acids* (SCFA) in the intestinal lumen are acetate, propionate and butyrate, which are present in relative high concentrations in the colonic lumen (10-50 mmol/L). These are major end-products of carbohydrate fermentation by saccharolytic bacteria. The relevance of SCFA to human health was recently extensively reviewed [56]. In terms of the colonic epithelium, there are three scenarios: (a) Deficiency of SCFA due to loss of dietary substrates and reliance upon on endogenous carbohydrate sources leads to mucosal atrophy. This only occurs in patients on extreme restriction of oral carbohydrates (e.g., nil orally) or where the 'faecal stream' is diverted away from a segment of colon. (b) Normal delivery of carbohydrate substrates from dietary intake where variations in colonic delivery of carbohydrates will lead to alterations of SCFA production and delivery to the mucosa within a modest range; (c) Delivery of high amounts of carbohydrates leads to excessive production of SCFA, which are toxic at high concentrations [56]. Such toxicity is well documented in direct studies of cell lines in vitro, and directly in a pig model, in which reversible injury to the epithelium, barrier dysfunction and mucosal inflammation occurred at $\text{pH} \leq 5.0$ [61]. Whether toxic concentrations of SCFA are reached in the proximal colon is unknown due to the inability to currently measure SCFA concentrations in any part of the bowel except faeces, but caecal pH is often less than 5.0 on pH motility capsule telemetry [62].

Effect of FODMAPs: In the proximal colon, reducing FODMAP intake will be likely to reduce SCFA production, concentration and delivery to the mucosa, but animal studies have shown that, despite low fermentable load, caecal SCFA concentrations remain substantial [63,64]. Human data are largely restricted to studies in faeces, where reducing FODMAP intake had minimal effect on SCFA concentrations or daily output in some studies [8,42] and reduced them in others [38,39,42]. The reason for such heterogeneity might reside not in variations in FODMAP content, but in the dietary intake of longer-chain fibres (non-starch polysaccharides and resistant starch) that are more slowly fermented and are the substrates responsible for more distal fermentation [64]. The studies in which the fibre content was carefully matched showed no diet-related differences in SCFA concentrations in the faeces [8,42], whereas the studies in which the faecal levels were reduced did not control NSP and resistant starch content [39,40]. Because of the ready absorption of SCFA from the lumen, the faecal concentrations of SCFA are likely to reflect fermentation occurring in the distal colon. In this way, reduction of their

content would reflect low levels of non-starch polysaccharides and resistant starch reaching those regions. Foods naturally high in FODMAPs are often also sources of longer-chain dietary fibre, the content of which may be concomitantly reduced in the strict restriction phase of the FODMAP diet [66].

Increasing delivery of FODMAPs to the proximal colon will increase production of SCFA with its subsequent increased concentrations and delivery to the mucosa. Luminal pH will also fall, but this is exaggerated if a large amount of fermentation is rapidly occurring; the production of lactate, which is not absorbed [67], overtakes its consumption by other bacteria. High FOS intake in humans is likely to be fermented in the proximal colon, but one study in which concomitant fibre intake was high led to evidence of increased distal fermentation as shown by the increased faecal lactate concentrations [37]. However, SCFA concentrations were not assessed.

Biomarkers of protein fermentation

Proteins, which are usually plentiful in the colonic lumen due to natural maldigestion of up to 20% of that consumed and endogenous proteins, are diverted from being a major amino acid source for structural metabolism to being a major source of energy supply for gut microbiota when carbohydrates are depleted. Unique products of such fermentation include branch-chain fatty acids (BCFA) such as isobutyrate and isovalerate, nitrogenous compounds such as ammonia, cresols and phenols, and sulphur-containing compounds such as hydrogen sulphide [38,68]. The nitrogenous products, particularly ammonia, tend to increase the luminal pH. It is not surprising then that the amount of protein fermentation increases in the distal colon (as carbohydrate substrates are relatively exhausted) and that the pH rises [38]. A study ex vivo using fresh faecal slurries showed that FOS rapidly and potently inhibited hydrogen sulphide production even when stimulated by addition of cysteine to the slurry [68]. Thus, the concept is that the fermentative and other metabolic behaviour of the gut microbes depends upon the balance of available carbohydrate and protein substrates.

Relevance: Protein fermentation is, in general, unfavourable to health due to the associated toxicity of nitrogenous metabolites and of hydrogen sulphide, although the latter may help in regulating cellular processes of the gut such as inflammation, motility, epithelial secretion and nociception [66] when present in low concentrations. However, volatile products such as hydrogen sulphide are foul smelling as the alternative name for protein fermentation, putrefaction, indicates. There is evidence that concomitant hydrogen sulphide and nitric oxide

production induce the biochemical lesion that characterises the colonic epithelium in human ulcerative colitis [71] and that hydrogen sulphide influences visceral sensitivity unfavourably in rodents [72]. This apparent paradox is reflected in the concept that enhancing production of hydrogen sulphide in the colon might be beneficial by potentially reducing dysbiosis and helping mucus layer reconstitution [70]. In contrast, reducing its production might have benefits where hydrogen sulphide is believed to be pathogenic, such as in ulcerative colitis or pouchitis [71,73]. The development of ways to accurately measure its production in vivo might resolve this apparent paradox.

FODMAP effects: As outlined above, evidence of enhanced protein fermentation and sulphide reduction in the faeces have been documented via increased concentrations of BCFA concentrations and promotion of a sulphate-reducing genus, Bilophila, in association with low FODMAP intake. One study also found faecal SCFA concentrations to be concomitantly reduced. However, when intake of NSP and resistant starch were carefully controlled, no alterations in BCFA, phenols or cresol were observed [8,9]. The effect of enhancing FODMAP intake on protein fermentation in the proximal colon is best illustrated by the use of lactulose in patients with portosystemic encephalopathy. The absorption of nitrogenous metabolites is reduced not only by altering their charge in the more acidic environment, but also by the diversion of fermentative activity away from proteins and the utilisation of amino acids for the synthesis of structural proteins by the bacteria (the so-called ‘nitrogen sink’) [74].

Gas production

Major products of microbial fermentation are gases, principally hydrogen, methane and carbon dioxide, in addition various trace gases such as hydrogen sulphide, nitric oxide and sulphur-containing compounds. This has been the subject of a recent extensive review [55].

Relevance: Hydrogen, carbon dioxide and methane are particularly important due their volumetric effects; stimulation of intestinal mechanoreceptors is a major mechanism for the genesis of abdominal symptoms in patients with functional bowel disorders, especially in the presence of visceral hypersensitivity. Methane reduces the volume occupied by hydrogen and carbon dioxide from which it is generated by methanogens and, therefore, can reduce mechanoreceptor stimulation. Intestinal gases may also have non-volumetric, so-called gasotransmitter, effects. Methane slows motility in the colon and is associated with constipation [75]. It might also have anti-oxidant and anti-apoptotic effects as described in ischaemia-reperfusion animal models [76]. The potential antioxidant action of hydrogen in

reducing hydroxyl radicals and peroxynitrite has translated into protection of various tissues from oxidant injury in both animal models and in metabolic illnesses in humans [77,78]. The benefits have generated from delivery of relatively small amounts of hydrogen-infused water orally or from inhaled gas. Whether the large amount of hydrogen produced in the colon during fermentation likewise exerts anti-oxidant effects locally or systemically is not known, but is an intriguing concept. As outlined above, roles for hydrogen sulphide in modulating motility and visceral sensitivity have been documented in rats [70,72].

FODMAP effects: Enhancing fermentation by increasing the delivery of FODMAPs to the colonic microbes leads to the increase in hydrogen and methane production [22]. Conversely, reducing FODMAP intake will reduce gas production, a putative mechanism underlying the efficacy of the low FODMAP diet in patients with IBS. In one study, the ingestion of a diet high in FODMAPs led to preferential production of hydrogen over methane and vice versa in healthy subjects, although the effect was not so evident in patients with IBS [22]. The suppressive effect of FODMAPs on hydrogen sulphide production has been examined in faecal slurries [69], but, to date, an effect altering this aspect of the diet on hydrogen sulphide production in the colon has not been studied directly, due to difficulties in reproducibly measuring hydrogen sulphide, a very reactive gas [55]. However, the expansion of bacteria with sulphate-reducing ability, such as *Bilophila*, in association with a low FODMAP intake (with or without lowering of NSP intake) [42,51] is suggestive that dietary FODMAPs may be associated with hydrogen sulphide production in vivo.

Metabolome in general

There is great interest in the effects of altering FODMAP intake on metabolic patterns in the urine and faeces. The spectra/patterns of *volatile organic compounds (VOCs)* in the faeces and urine (without specifically identifying key molecules) appear to reflect the presence of colorectal cancer, and the presence and degree of intestinal inflammation [79,80]. There is also evidence that the pattern of faecal VOCs might predict response to a low FODMAP diet in patients with IBS [81]. Hence, VOCs are of great interest as diagnostic and prognostic tools. However, their relevance to pathogenesis, such that their modulation might have therapeutic or preventive potential, is unknown. There is little in the way of information on how altering FODMAP intake affects patterns of VOCs. Urinary patterns on mass spectrometry analysis might also give insight into mechanisms of action or prognostic information in subjects on high and low FODMAP diets. Analysis of individual molecules identified three with consistent

differences, the most intriguing being histamine [44]. However, the origin of the histamine has yet to be defined; it could be of mast cell origin or from the microbiota.

EFFECT OF VARYING DIETARY INTAKE OF FODMAPS ON MUCOSAL BIOLOGY

The influence of varying dietary FODMAP intake on mucosal biology is important to define since modulation of FODMAP intake underpins dietary approaches to patients with functional gut symptoms. Such symptoms are common in patients with chronic intestinal disorders like inflammatory bowel disease), coeliac disease and post-infectious IBS that are characterised by impaired barrier function, intestinal injury and mucosal inflammation.

Clues to a potential pathogenic relationship between high intake of dietary FODMAPs and intestinal barrier dysfunction, injury and inflammation have been derived from studies in animals where greater control over dietary intake can be exerted, higher doses of FODMAPs can be tolerated, and the structural and functional integrity of the proximal colon where FODMAPs are generally fermented can be effectively interrogated. Several key observations have been reported.

- Lactulose, FOS and inulin at generous doses (estimated to be about 4% of food weight) fed to rats with a low calcium dietary background were associated with increased epithelial permeability, caecal inflammation, and increased susceptibility to and severity of experimental Salmonella infection [29,82,83]. Evidence of epithelial injury comprised increased mucus production and epithelial turnover. Surfactant activity of the faecal water was also increased. All this occurred despite increasing the relative abundance of Bifidobacteria. Similar findings were reported in mice by another investigating group [30]. Excessive fermentation occurring in the supplemented animals was reflected in elevated caecal lactate levels, indicating consumption of lactate by bacteria was overwhelmed by the marked fermentative activity. High calcium intake negated the effects [83], possibly via the buffering capacity of calcium phosphate or via stimulation of fermentation in a low calcium environment [84]. While the rat studies were criticized because of the low calcium background [85], they have provided unequivocal evidence that excessive fermentation can have deleterious effects in rats and mice.
- In a detailed analysis of the effect of altering dietary FODMAP intake in rats, a high FODMAP intake – usually comprising FOS or lactulose or multiple FODMAPs at doses

between 4-10% of the diet -induced mild mucosal inflammation, impaired epithelial barrier function and increased visceral sensitivity when compared with a low FODMAP intake [34]. Mechanistic studies identified a higher lipopolysaccharide (LPS, endotoxin) content of the faeces as the likely inducer of the abnormalities by virtue of amelioration of the effects with neutralising antibodies to endotoxin. Together with the effects of human faecal water in those rats outlined above, the results indicated that high intake of FODMAPs might induce mild mucosal inflammation, barrier injury and visceral hypersensitivity. In other words, FODMAPs might be causal in IBS not just an opportunity for symptom alleviation.

- In a Chinese study, mice subjected to water-avoidance stress developed intestinal inflammation and visceral hypersensitivity. These were exaggerated by FOS supplementation (8 g/kg body weight, estimated to be about 2% of food intake, although the weight and amount of food consumed was not reported), which increased fermentation in the caecum [31].
- In pigs whose caecum was intubated to enable control of caecal pH using acetic acid in an attempt to mimic differing degrees of fermentation, a caecal pH of 5 or less was associated with reversible epithelial apoptosis and histopathological abnormalities, and with impairment of barrier function [61]. Whether the use of acetic acid alone was appropriate or toxic independently of its effect on pH was not addressed. Similarly, in rats on a low calcium intake, rapid fermentation of inulin was associated with low caecal pH and increased epithelial turnover [84]. Likewise, chicory fructans also induce colonic epithelial apoptosis [33].
- In a French study of mice fed a diet very low in fermentable non-digestible carbohydrates, supplemental FOS (10% of food weight) and lactose induced increased mast cell density, visceral sensitivity and thinning of the mucus layer [32]. The novel finding in this study was that an inhibitor of glycation, pyridamide, inhibited the effects associated with FOS ingestion. The dose of FOS was high enough to show evidence of fermentation throughout the entire colon.
- In models of murine colitis, inulin or FOS, given at 7.5% of the diet (erroneously reported in the paper as 75%), exacerbated colitis, while pectin at a similar amount did not [86]. Evidence that this effect was associated with elevation of luminal butyrate concentrations was supported by amelioration of the deleterious effects when butyrate production was inhibited or when butyrate concentration in the caecum were boosted at least 4-fold by supplementation with tributyrin. Such data support the notion that boosting luminal

butyrate as a therapy for patients with ulcerative colitis may be not a wise choice [87], especially as the luminal levels are already elevated in active disease [88].

- That the effects of excessive fermentation are not restricted to short-chain (FODMAP) carbohydrates, the effect of rapid fermentation in the colon was also demonstrated in rats fed diets containing uncooked potato starch, rich in readily fermentable resistant starch [36]. Marked increases in epithelial turnover without increasing length of crypts suggested epithelial injury was occurring [89]. Tumour development in response to dimethylhydrazine was also accelerated. All of these effects were reduced by concurrent administration of wheat bran, which slowed proximal fermentation and shifted it distally, supporting the notion that regional excessive fermentation has detrimental effects on mucosal biology.

Translation of these observations to alterations in FODMAP intake in humans is compounded by different colonic biology, physiology and microbiology, by the relatively high doses given, the very low fermentable load in the control diet, and the observed fermentation of the FODMAP throughout the colon, which is not observed in human studies under physiological conditions. However, several observations provide at least some support for the relevance of animal data in human subjects.

- FODMAP ingestion greater than that of the habitual diet has some evidence that it may be associated with induction of subtle mucosal inflammation. The effects of altering the intake of FODMAPs in patients with inflammatory bowel disease have been reported by several groups. Randomised controlled trials of low FODMAP intake have demonstrated symptomatic benefit in patients with well-controlled inflammation and ongoing symptoms likely to be functional [9,29] as they have in observational real-world cohorts [90]. No differences were observed in faecal calprotectin in these studies. However, challenging patients with inactive inflammatory bowel disease (normal faecal calprotectin) with individual FODMAPs in a double-blind placebo-controlled study on a background low-FODMAP diet identified that fructans (12 g/d) led to a very modest but consistent elevation of faecal calprotectin from mean of 30 to 73 $\mu\text{g/g}$ [91]. The increment in calprotectin was not one that had clinical significance in the setting of established inflammatory bowel disease, but the findings did suggest subtle changes might be occurring in the mucosa associated with greater fermentation of the fructans. In contrast, dietary supplementation with FOS tended to reduce circulating inflammatory cytokines in patients with mildly

active Crohn's disease in a pilot study [92], but this was not confirmed in a subsequent well-powered study in which background diet was not controlled [12]. In the setting of patients with IBS-D where mucosal inflammation is subtle at worst, circulating concentrations of interleukin-6 and interleukin-8 were consistently higher in association with the habitual diet compared with a low FODMAP diet. The FODMAP intake on habitual or low FODMAP diet was not quantified. However, supplementing them with 16 g/d FOS whilst consuming the same low FODMAP diet did not elevate the cytokines, raising doubt about the link between the FODMAP intake and the cytokine levels [39]. In a subset of patients with IBS fed high and low FODMAP diets (although the FODMAP content was not estimated or quantified), urinary histamine levels were markedly different with high levels being associated with the high FODMAP arm [44]. If the origin of the histamine was the mucosal mast cell, then this would suggest differences in inflammatory status. Thus, there is some, albeit weak, evidence that increasing the dietary FODMAP intake induces subtle increases in mucosal inflammation in the colon. Deeper analysis of epithelial or barrier changes, of effects on enteric nervous function (such as visceral sensitivity), or of histological analysis of cellular components of inflammation have not been reported.

- Faecal water from patients with IBS on their habitual diet not only induced similar inflammatory lesions in the rat colon as did the rats' own high FODMAP diet, but also induced visceral hypersensitivity [34]. Furthermore, the changes were also shown to be causally due to LPS in the faecal water. Faeces from those patients after following a diet low in FODMAPs did not show injurious effects on the rat colons and had considerable reduction of LPS content.
- There is some evidence that FODMAPs may or may not be associated with colonic epithelial injury in humans. In 64 healthy men, intake of 20 g/d of FOS caused increased faecal mucus output, suggestive of epithelial irritation [37]. However, unlike the rat studies, colonic epithelial barrier function, as measured by EDTA permeation into the urine, and cytotoxicity of faecal water were not altered. The background diets were not controlled apart from avoiding high calcium and fructan-containing foods. The calcium content of the faeces was much higher than that observed in the rats and dietary fibre intake was high, which may have pushed the site of fermentation around the bowel, particularly as the faecal lactate levels were high. Hence, the experiment did not mimic conditions in rats that induced changes. In an evaluation of biomarkers of epithelial injury in the intestine, the

effect of reducing FODMAP intake during a randomised controlled blinded rechallenge cross-over trial of two doses of gluten and placebo in patients with self-reported gluten sensitivity, serum levels of the pan-epithelial marker, sydecan-1, fell by about 30% when FODMAP intake was reduced with no change following the rechallenges with gluten/placebo [93]. The small intestine-specific marker, intestinal fatty acid binding protein, was not significantly altered and markers of bacterial translocation likewise not affected. These findings suggested that colonic epithelial injury was reduced when the subjects with IBS reduced FODMAP intake.

- As outlined above, caecal luminal pH may reach 5.0 or less in patients with IBS [63]. In pigs, lowering of caecal pH using acetic acid induces reversible epithelial injury and barrier dysfunction in pig caecum, but whether that adequately mimics the luminal environment in IBS is debatable [61].

In summary, animal studies clearly indicate that doses of FODMAPs well above those consumed by humans when delivered to the caecum may be injurious to the colon, induce inflammation and increase mast cell density, impair barrier function and heighten visceral sensitivity. The underlying mechanisms might include excessive fermentation with luminal pH being too low and SCFA concentrations being in the toxic range. These events are illustrated in Figure 2. The evidence to suggest similar events might be occurring in humans who are healthy, or who have IBS or inflammatory bowel disease, and consume doses of FODMAPs that exceed those in the average diet is weakly supportive with also negative studies.

FODMAP HYPOTHESIS REVISITED

The original hypothesis speculated that dietary FODMAPs might be pathogenically related to the development of Crohn's disease affecting the terminal ileum and proximal colon [1]. The more recent detailing of the neurobiological changes that can be induced by FODMAPs outlined above now lead to an extension of this hypothesis to IBS. That the intake of high quantities of FODMAPs are insults to the proximal colon potentially inducing reversible changes to the structure and function of the mucosa has been confirmed and now the mechanistic basis of such events extend beyond the products of excessive fermentation and now include the promotion of high luminal content of LPS and glycation of other products (Figure 2). These insults will not manifest in serious acute illness, but their cumulative effect might lead to functional alterations in the behaviour of the ENS with ongoing visceral hypersensitivity and chronic, but subtle inflammation in the intestinal wall. Hence, as shown

in Figure 3, FODMAPs delivered repeatedly at doses higher than the average FODMAP intake might be causally related, at least in part, to pathophysiological abnormalities that characterise IBS. Consistent with this notion and its potential reversibility is the observation that most patients are able to derestrict FODMAP intake considerably without losing symptom control in the maintenance (personalised) phase of the FODMAP diet. It is indeed possible that the avoidance of excessive FODMAP intake is avoiding intermittent insults to the mucosa and permitting the recovery from inflammation and damage with its associated downgrading of visceral hypersensitivity. As for all hypotheses, these effects may not be occurring and there has been no direct study of this concept. Such pursuit would be worthwhile.

The major problem to this hypothesis is that the dietary aspects of the interventional studies are not ideally expressed or designed. In humans, the lack of quantification of FODMAP intake associated with interventions, the often poor attention to other dietary components with which FODMAPs potentially interact (such as full description of the amount and type of dietary fibre taken) and the lack of consideration of the background diet in supplementation studies all compromise interpretation of the results. In animal work, it is difficult to make sense of the effects reported due to the excessive amount of FODMAPs included in the diets, which is generally many fold greater than that observed in the human diet, even with supplementation, the somewhat obtuse way that their content in the diets is being expressed, poor characterisation and, at times, inappropriate design of the control diets and the almost absence of dose-dependent studies. Effects on intestinal biology then may represent pharmacological effects rather than realistic physiological variance applicable to humans.

CONCLUSIONS

Dietary FODMAPs are modulators of multiple aspects of gastrointestinal function. The majority of attention is paid to their role in symptom generation in patients with IBS, leading to the widespread and efficacious use of the FODMAP diet. The effects of this dietary approach on gut microbiota has highlighted the presence of FODMAPs with prebiotic effects in food. This has led to fear-mongering that reducing FODMAP intake will lead to a dysbiotic gut microbiota with associated adverse health effects. Apart from reduced relative abundance of Bifidobacteria associated with extreme restriction of FODMAPs, changes to microbial communities have generally been overinterpreted and are not alarming, but should remind health professionals about the importance of reintroduction of FODMAPs in their clinical management of patients with IBS. Trials of supplemental prebiotics have seldom taken into

account background dietary prebiotic intake, which may influence effects of those prebiotics with associated disappointing efficacy on both microbial communities and health outcomes. The potentially adverse effects of the intake of FODMAPs in excess of those usually consumed on gut health, apart from inducing symptoms, have received minimal attention in humans, despite considerable old and new information of the injurious effects that excessive fermentation of carbohydrates can have on mucosal health and of the effects on gut motility have in experimental animals. The potential that such effects might occur in humans too (although evidence is weak at best) may be of more relevance now that there is increasing awareness of food-related prebiotics and the introduction of prebiotics into the food supply. A hypothesis that excessive intake of dietary FODMAPs might be contributing to the apparent increase in functional intestinal disorders is entertained and adds to the original 'FODMAP hypothesis' suggesting their involvement in the pathogenesis of Crohn's disease. To gain data to support or deny such hypotheses, there are limitations on our ability to interrogate what is happening in the caecum of ambulatory, free-living humans who are healthy or have gastrointestinal disorders in terms of key indices such as SCFA concentrations and mucosal histology. Technological advances such as those in the telemetric capsule space will assist in this way [94,95]. Future research needs to pay more attention to the quantitative and qualitative details of both the dietary interventions being used and the background diet. With the hazards associated with translating animal observations to the human condition, it is essential that the potential dangers of excessive carbohydrate fermentation in the intestines are properly investigated in the human.

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PRG: Conceived the concepts, wrote the paper

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PRG has (i) served as a speaker, a consultant and an advisory board member for Allergan, Janssen, MSD, Pfizer, Anantara, Atmo Biosciences, Immunic Therapeutics, Novozymes and Takeda and Bristol-Meyers Squibb, and has received research funding from MSD and Atmo Biosciences; and (ii) PRG owns stocks and shares in Atmo Biosciences. (iii) Monash University financially benefits from the sales of a digital application, on-line education and booklets on the FODMAP diet. He has published two educational/recipe books on diet.

EPH: has (i) served as a speaker, a consultant and an advisory board member for Ferring, Janssen, Abbvie, Takeda and Shire

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FIGURE LEGENDS

Figure 1. Average FODMAP oligosaccharide intake according to dietary style with and without an oligosaccharide supplement (e.g., fructo-oligosaccharide or inulin) of 8 g/d. Intake values are estimated from dietary intake studies where oligosaccharide content has been measured [23-25] and the amount of supplement is typical of prebiotic studies. Supplementation or fortification of foods with oligosaccharides leads to total daily intake of FODMAP oligosaccharides that is considerably greater than that achieved from eating unsupplemented food.

Figure 2. Synthesis of data from animal studies. High intake of rapidly fermented carbohydrates that are delivered to the colon induce excessive fermentation with luminal

effects that injure reversibly the mucosal barrier with subsequent effect of the enteric nervous system to induce visceral hypersensitivity. Limited studies in humans addressing these pathways are inconclusive.

Figure 3. Alternative effects of elevated FODMAP intake:

A. Higher FODMAP intake might have prebiotic effects with associated health benefits. While increasing the relative abundance of Bifidobacteria is well documented, supportive human data for health benefits are limited.

B. Higher FODMAP intake might pathogenically contribute to IBS. Based largely on animal studies with limited human supportive data, higher daily FODMAP oligosaccharide intake may lead to reversible mucosal injury. Repeated intakes above this critical level will cause repeated insults and lead to changes in the enteric nervous system manifesting as elevated visceral sensitivity.

Figure 1. Average FODMAP oligosaccharide intake according to dietary style with and without an oligosaccharide supplement (e.g., fructo-oligosaccharide or inulin) of 8 g/d. Intake values are estimated from dietary intake studies where oligosaccharide content has been measured [23-25] and the amount of supplement is typical of prebiotic studies. Supplementation or fortification of foods with oligosaccharides

leads to total daily intake of FODMAP oligosaccharides that is considerably greater than that achieved from eating unsupplemented food.

Figure 2. Synthesis of data from animal studies. High intake of rapidly fermented carbohydrates that are delivered to the colon induce excessive fermentation with luminal effects that injure reversibly the mucosal barrier with subsequent effect of the enteric nervous system to induce visceral hypersensitivity. Limited studies in humans addressing these pathways are inconclusive.

Figure 3. Opposing hypothetical effects of elevated FODMAP intake:

A. Higher FODMAP intake might have prebiotic effects with associated health benefits. While increasing the relative abundance of Bifidobacteria is well documented, supportive human data for health benefits are limited.

B. Higher FODMAP intake might pathogenically contribute to IBS. Based largely on animal studies with limited human supportive data, higher daily FODMAP oligosaccharide intake may lead to reversible mucosal injury. Repeated intakes above this critical level will cause repeated insults and lead to changes in the enteric nervous system manifesting as elevated visceral sensitivity.

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