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Symposium 3: Diet and gut metabolism: linking microbiota to beneficial products of fermentation

Links between diet, gut microbiota composition and gut metabolism

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The gut microbiota and its metabolic products interact with the host in many different ways, influencing gut homoeostasis and health outcomes. The species composition of the gut microbiota has been shown to respond to dietary change, determined by competition for substrates and by tolerance of gut conditions. Meanwhile, the metabolic outputs of the microbiota, such as SCFA, are influenced both by the supply of dietary components and via diet-mediated changes in microbiota composition. There has been significant progress in identifying the phylogenetic distribution of pathways responsible for formation of particular metabolites among human colonic bacteria, based on combining cultural microbiology and sequence-based approaches. Formation of butyrate and propionate from hexose sugars, for example, can be ascribed to different bacterial groups, although propionate can be formed via alternative pathways from deoxy-sugars and from lactate by a few species. Lactate, which is produced by many gut bacteria in pure culture, can also be utilised by certain Firmicutes to form butyrate, and its consumption may be important for maintaining a stable community. Predicting the impact of diet upon such a complex and interactive system as the human gut microbiota not only requires more information on the component groups involved but, increasingly, the integration of such information through modelling approaches.

Gut bacteria: Detary fibre: Propionate: Butyrate: Lactate

The resident microbiota that colonises the intestinal tract influences our health and nutrition via multiple routes. Most obvious is that it can act as a reservoir for infectious pathogens, but we also know that the more numerous non-pathogenic gut micro-organisms exert a sustained influence on the host's immune system and metabolism through interactions of microbial cell components and gene products (e.g. extracellular polysaccharides and flagella). Just as significant, however, is the combined metabolic activity of the resident gut microbiota, especially that arising from the very dense microbial community that occupies the large intestine. Here the largely fermentative metabolism of undigested food and of host-derived products creates a microbial metabolome (Fig. 1) that in turn impacts on the host metabolome that is detected in faeces, urine and blood[1]. It is increasingly recognised that microbial metabolites have a major influence on host physiology. The major products of carbohydrate fermentation, the SCFA acetate, propionate and butyrate, provide energy sources for host tissues, but also exert anti-inflammatory and anti-apoptotic effects that may be important for the prevention of colorectal cancer and colitis. SCFA may influence the regulation of lipogenesis, whereas their interactions with host receptors have been linked to regulation of hormones that affect satiety[2]. Furthermore, SCFA also play a major role in determining the gut environment, influencing pH, gut transit, nutrient uptake and microbial balance within the large intestine. Apart from SCFA there is a vast array of other potentially bioactive compounds that are generated through microbial activity in the gut, for example from

Abbreviations: HGC, high gene count; LGC, low gene count.
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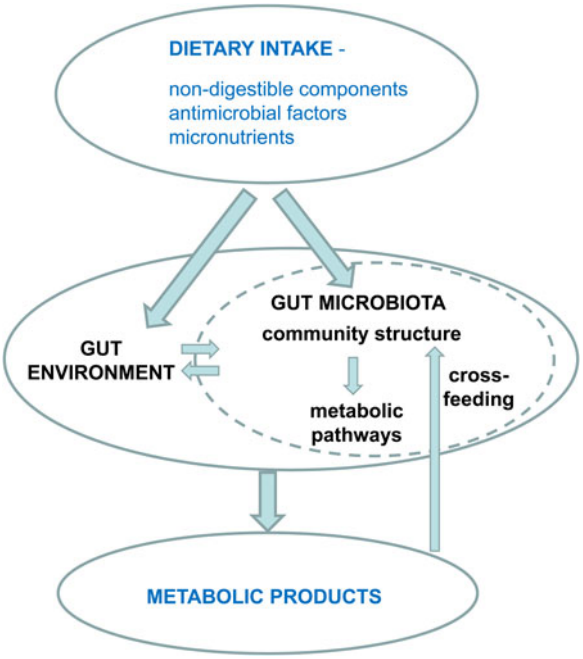

Fig. 1. Impact of diet on microbial metabolites.

protein, dietary phytochemicals and xenobiotics (including drugs).

The formation of microbial metabolites is strongly influenced by dietary intake, particularly of non-digestible dietary carbohydrates, protein and fat. This is dictated primarily by the chemical structures of the substrates themselves and the microbial pathways by which they are processed[3]. It is becoming apparent however that many pathways are limited in their distribution among the various phylogenetic groups of bacteria that dominate the gut microbiota. This has the important implication that changes in the species composition of the gut microbiota, which could potentially come about as a result of individual variation or differences in diet or medication, may change the metabolic profile of the community. The composition of the gut microbiota changes and becomes increasingly complex following birth to adulthood. Within an individual adult, the intestinal microbiota is reasonably stable in composition, but there is potential for change in response to diet change, during periods of gastrointestinal disease and during antibiotic treatment. We will start here by considering recent evidence for the impact of diet upon microbiota composition.

Dietary modulation of gut microbiota composition

Microbial utilisation of dietary polysaccharides

Based on 16S rRNA-targeted molecular analyses, the majority of bacteria detected in faecal samples from healthy human volunteers belong to two phyla, Bacteroidetes and Firmicutes[4–6]. The Gram-negative Bacteroidetes phylum includes the genera *Bacteroides, Prevotella, Parabacteroides* and *Alistipes.* Detailed investigations on human colonic *Bacteroides* isolates show that these organisms possess the capability to utilise a very wide range of substrates of both host and dietary origin[7–9]. Genes encoding polysaccharide utilisation functions in *Bacteroides* spp. are organised into clusters (polysaccharide utilisation loci) that include genes encoding hydrolases and also outer membrane proteins responsible for the initial binding of the soluble polysaccharide and its transfer into the periplasmic space (the region between the outer and cytoplasmic membranes in Gram-negative bacteria)[7]. Much of the hydrolysis of the polysaccharide fragments occurs in this periplasmic space in *Bacteroides* spp. Functional analysis of the original starch-utilisation polysaccharide utilisation loci by Salyers *et al.*[8] showed that this type of organisation results in the sequestration of soluble substrates from the gut lumen, which is assumed to offer a competitive advantage within the very dense microbial community of the large intestine[7,10]. The Firmicutes have received considerably less study although they account for about 70 % of the species diversity within the human colonic microbiota[6]. However, we know that the Firmicutes include several highly abundant species such as *Faecalibacterium prausnitzii, Eubacterium rectale* and *Eubacterium hallii* that have been identified as the dominant producers of butyrate in the colon[11]. They also include species that convert lactate to butyrate or propionate and may help to stabilise the colonic microbiota by preventing lactate accumulation and excess acidity[12]. With regard to substrate utilisation, evidence is emerging that the Firmicutes include some highly specialised degraders of non-digestible polysaccharides that may be regarded as 'keystone' species within the microbiota[13]. Firmicutes typically carry fewer genes concerned with polysaccharide degradation than *Bacteroides* spp.[9], and this is likely to reflect both their smaller genome sizes and their greater nutritional specialisation. Human colonic bacteria that became associated with insoluble starch particles or wheat bran in *in vitro* fermentors included mostly Firmicutes rather than *Bacteroides* spp.[14] and Firmicutes (specifically *Ruminococcaceae*) were also enriched among particle-associated bacteria from human stool samples[15]. The species *Ruminococcus bromii* has recently been shown to play a keystone role in the degradation of resistant starch, as individuals who lack *R. bromii* in their microbiota are unable to degrade resistant starch which is then detectable in their faeces[13]. The enzyme systems involved in polysaccharide degradation by human colonic Firmicutes are less well studied than those of *Bacteroides* spp., but comprise extracellular degradative enzymes and enzyme complexes [10,16,17]. These polysaccharide-degrading Gram-positive anaerobes often produce large multi-domain enzymes that are attached to the cell wall either directly[16] or via extracellular multi-enzyme complexes[18].

Other bacterial phyla within the healthy colonic microbiota include Actinobacteria (that include *Bifidobacterium* spp.), Proteobacteria (including *Escherichia coli*) and Verrucomicrobia (including *Akkermansia muciniphila*). These are typically present

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in smaller numbers than the dominant Bacteroidetes and Firmicutes, but have considerable potential to influence health outcomes. Although only about 30 % of human intestinal species are currently represented by cultured isolates, the most abundant species appear to be well represented[19] and most of the remaining organisms are probably capable of being cultured under appropriate conditions[20,21].

Impact of diet on microbiota composition

Two major mechanisms seem likely to result in shifts in the species composition of the intestinal microbiota. First, different microbial species vary in their capabilities, determined by their genomes, for utilising substrates of dietary and host origin. This is expected to determine the outcome of competition for substrates available in the large intestine, with certain species favoured over others when particular substrates are in greater abundance. This is the principle behind manipulation of the microbiota using prebiotics. Secondly, different species may vary in their tolerance of a wide range of factors in the gut environment, such as high or low pH, high bile salt concentrations or low micronutrient (e.g. Fe) concentrations that tend to limit microbial growth. Recent work with human volunteers and animal models indicates that both of these mechanisms are likely to play an important role in defining species composition.

Human volunteer studies that involve precise control over dietary intake have been used to investigate the impact of different dietary non-digestible carbohydrates on the species composition of the gut microbiota over short time intervals. Changing the major non-digestible carbohydrate from wheat bran to resistant starch (and vice versa) in diets delivering the same intake of protein, fat and total carbohydrate, led to rapid and reversible shifts in the representation of certain bacterial groups within a few days in overweight volunteers[19]. Changes affecting multiple species were detected by 16S rRNA gene sequencing, qPCR and HitChip microarray analysis[19,22]. On the other hand, global analyses of sequence and HitChip data indicated that inter-individual variation played a more major role than dietary change in determining the overall species composition of the microbiota[19,22]. The explanation for this apparent contradiction is twofold. Firstly, many species (especially the less abundant ones) occur only in one or a few individuals. Secondly, it appears that within the microbiota only certain species are responsive to the particular dietary switches, in this case including those bacteria that are specialists at utilising resistant starch or wheat bran. In the present study, many of the diet-responsive species were Firmicutes, notably *Ruminoccocus bromii* in the case of resistant starch diets[19] and various *Lachnospiraceae* for the wheat bran diet[22]. Significant shifts in faecal microbiota composition resulting in decreased butyrate-producing Firmicutes have also been observed with weight loss diets that include decreased amounts of fibre and total carbohydrate[19,23]. In contrast, many dominant species showed limited responses to such dietary switches. These may be considered generalists that have a greater capacity to switch between dietary energy sources, or between host- and diet-derived substrates, as has been elegantly demonstrated using animal models, for example, for *Bacteroides* spp.[24].

Rapid changes in microbiota were also reported recently in another human intervention study involving more extreme diets[25]. A switch between a plant-based diet (high in fibre, low in fat and protein) and an animal-based diet (70 % of energies from fat and 30 % from protein) resulted in an increased proportion of *Bacteroides* spp. and a decreased proportion of many Firmicutes on the animal-based diet. In this case, the change in fat content was proposed to be a major factor[25], mediated via the effect on bile acids, which are known from animal studies to exert an important selective influence upon gut microbiota composition[26].

There is also indirect evidence that variations in habitual dietary intake are responsible for differences in gut microbiota profiles. A survey of ninety-eight US adults[27] showed that individuals whose faecal microbiota is high in *Prevotella* tend to consume more fibre, whereas those high in *Bacteroides* tend to consume more protein and fat, indicating that there is a strong influence of long-term dietary intake upon the relative abundance of these two genera of Bacteroidetes within the gut microbiota. This is in agreement with a previous study in which a group of Italian children showed higher *Bacteroides* and a group of rural African children higher *Prevotella*, with the difference being ascribed mainly to the very different habitual diets of each group[28]. Meanwhile, metagenomic sequence analysis of DNA extracted from faecal samples has revealed a bimodal distribution of low gene count (LGC) and high gene count (HGC) individuals within the general population[29]. The microbiota of LGC individuals tends to be *Bacteroides*-dominated and phylogenetically less diverse than that of HGC individuals, and has lower representation of Firmicutes, including butyrate-producers. Remarkably, the LGC group of individuals was reported to show a higher incidence of obesity and metabolic syndrome than the HGC group[29,30]. When obese LGC volunteers were put onto a controlled weight loss diet, their microbiota diversity increased towards that of the HGC individuals, while the symptoms of metabolic syndrome improved in both HGC and LGC groups[30]. Again, it seems likely that the LGC state is a consequence of dietary intake. The composition of the gut microbiota present in the colon of each individual along with different dietary intakes is likely to impact on microbial metabolic output including the formation of SCFA.

Metabolic activities of the gut microbiota

Formation of propionate and butyrate

The main fermentation products of gut microbial metabolism are the SCFA acetate, propionate and butyrate plus gases. SCFA are absorbed by the host and utilised as an energy source, with butyrate acting as the main

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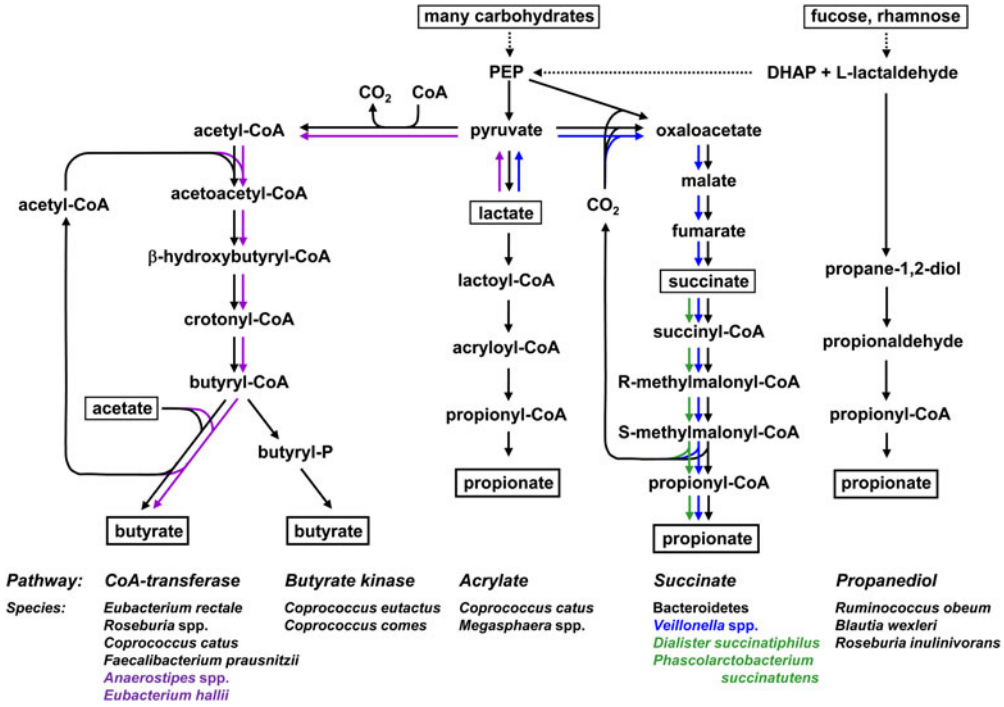

Fig. 2. Metabolic routes for butyrate and propionate formation by representative bacterial genera and species from the human colon. Species shown in purple can utilise lactate to form butyrate; species shown in blue can utilise lactate, and those shown in green succinate to produce proprionate. DHAP, dihydroxyacetonephosphate; PEP, phosphoenolpyruvate.

fuel for the colonic wall[2]. Butyrate has received much attention for its anti-inflammatory and anti-carcinogenic effects[31] and more recently SCFA have been studied for their effects on systemic host metabolism, particularly as satiety-inducing agents[32,33]. In order to increase the concentration of propionate and butyrate in the lower gut by dietary means, it is important to understand which bacteria form these SCFA. Much progress has been made in the identification of butyrate-producing bacteria, revealing that they are present in several different classes within the Firmicutes[11,34]. *E. rectale*/*Roseburia* spp. is an abundant group of butyrate producers (estimated at 2–15 % of the total bacteria[34]) within the *Lachnospiraceae* (clostridial cluster XIVa). These bacteria showed a marked drop in abundance in human volunteers on a high-protein, low-carbohydrate diet and their abundance correlated significantly with faecal butyrate levels[23]. *F. prausnitzii*, another abundant butyrate-producing species within the *Rumincoccaceae* (clostridial cluster IV)[35], has been linked to anti-inflammatory effects[36,37]. Interestingly, *F. prausnitzii* has been shown to use an extracellular electron shuttle to transfer electrons to oxygen, which enables it to grow in the presence of low levels of oxygen[38]. Many other genera within several clostridial clusters produce butyrate, some of which are known to contribute specialist functions including species belonging to *Lachnospiraceae* that can convert lactate into butyrate[39].

Butyrate is formed from two molecules of acetyl-CoA and two different types of reaction contribute to the final step, the liberation of butyrate from butyryl-CoA (Fig. 2). Few bacteria in the gut use the butyrate kinase pathway, whereas the butyryl-CoA:acetate CoA-transferase pathway is utilised by the majority of known butyrate-producing gut strains[11,40]. Butyrate producers are phylogenetically interspersed with non-butyrate producers, making it difficult to quantify them with molecular techniques targeting the 16S rRNA gene. Degenerate primers specifically amplifying the butyryl-CoA:acetate CoA-transferase gene have proven valuable in specifically targeting the butyrate-producing community. This approach currently allows a more in-depth analysis compared with metagenomic mining[11], however, with improved depth and coverage of metagenomic datasets, metagenomic mining will become increasingly attractive to investigate specific functions within the microbiota. It has to be kept in mind however that genes closely related to those under study are often present that may perform a different function. Therefore results based on molecular analysis of genes have to be evaluated critically and backed up by biochemical analysis for less closely related genes. On the other hand, different genes may have evolved to perform the same function. Thus, it appears that a CoA-transferase gene more closely related to propionate CoA-transferase carries out the last step of butyrate formation in butyrate-producing bacteria within the *Erysipelotrichaceae* (clostridial cluster XVI)[41].

Three different biochemical pathways for propionate formation exist within the gut microbiota[42] (Fig. 2). The succinate pathway is present in Bacteroidetes and

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some Firmicutes bacteria within the Negativicutes (*Veillonellaceae*). Bacteroidetes are the second most abundant phylum in healthy human subjects and faecal propionate levels (as a percentage of all fermentation acids) have been found to be significantly correlated with their relative abundance[22]. The level of propionate formation is dependent on the environmental conditions, as succinate may become a major fermentation product instead of propionate. Thus, carbon limitation results in higher propionate production in *Bacteroides ovatus*[43] and vitamin B_{12} is required for propionate formation in *Prevotella ruminicola*[44]. Some Negativicutes bacteria, however, can utilise succinate as a substrate for propionate formation, either in conjunction with other substrates or as the sole energy source[42].

The acrylate pathway (Fig. 2) is utilised for the conversion of lactate to propionate. Based on genomic and metagenomic analyses, this pathway appears to be limited to very few different bacterial genera within the Firmicutes, including *Megasphaera* spp. (*Veillonellaceae*), *Coprococcus catus* and uncultured clones related to *Clostridium lactatifermentans* (both *Lachnospiraceae*)[42].

The propanediol pathway (Fig. 2) operates specifically from deoxy-sugar substrates (fucose, rhamnose) and has been identified in the Proteobacterium *Salmonella enterica* serovar Typhimurium[45] and *Lachnospiraceae* bacteria related to *Blautia* spp. and *Ruminococcus obeum* as well as *Roseburia inulinivorans*[46,42]. The full conversion of the C_5 ring deoxy-sugars to propionate involves a toxic intermediate and thus occurs within polyhedral bodies, the synthesis of which requires approximately ten independent genes encoded on a complex operon. Transcriptional analysis of *R. inulinivorans* revealed that these genes were strongly up-regulated during growth on fucose compared with glucose[46]. *Bacteroides thetaiotaomicron*, which is able to grow on fucose although lacking the genes required for 1,2-propanediol utilisation, has been shown to signal to the host resulting in more fucose production[47]. Phylogenetic distribution of the acrylate and propanediol pathways suggests that they may have been acquired by horizontal gene transfer by some bacteria, providing a competitive advantage by enabling recipients to utilise additional substrates. For instance, many of the glycoconjugates lining the gut epithelium are fucosylated, which may provide an alternative energy source in times of dietary starvation. The succinate pathway for propionate formation and the two butyrate production pathways have not been found to be present within the same species and consequently the ability to produce butyrate and propionate is rarely found within the same organism[42]. Several bacteria carrying either the acrylate or the propanediol pathway, however, are also able to produce butyrate from non-deoxy-sugars, and consequently change their fermentation product profile in response to the growth substrate available. For example, *R. inulinivorans* produces butyrate when grown on glucose, whereas propionate, propanol and butyrate are produced when it is grown on fucose[46].

Lactate formation and utilisation

Although normally detected at low levels in adult faecal samples, lactate is a common end product of bacterial fermentation and is produced, among others, by lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium*. These two genera are considered as key members of the gut microbiota due to their perceived health-promoting effects[48]. Lactobacilli are usually detected in lower abundance than bifidobacteria in stool samples but may be more abundant in the small intestine[49]. Lactate is a major bacterial fermentation product in breast-fed babies that are mainly colonised by *Bifidobacterium*[50–52], whereas the intestinal microbiota of formula-fed infants is more complex, with a lower abundance of bifidobacteria[53]. Breast-fed babies have been reported to show significantly higher levels of lactic acid (mean 8·4 mM) compared with formula-fed babies (mean 1·7 mM) at age 1 month, with the latter indicating a much more mixed acid fermentation. These differences affected the relative faecal pH, with the former pH 5·8 and the latter pH 7·1. The increased faecal lactate and lower pH values were also found in slightly older (age 2–5 months) babies[54].

Lactobacillus species, which are widely used as probiotics, use either the homo-fermentative pathway, yielding 2 mol lactate per mol glucose, or the hetero-fermentative pathway that yields lactate, ethanol and carbon dioxide. *Bifidobacterium* species ferment sugars using the bifid shunt route that yields acetate and lactate in the molar ratios of 3:2. However, it is less well recognised that many Bacteroidetes and Firmicutes that are dominant inhabitants in the large intestine can also form lactate in combination with other fermentation acids (Table 1).

Belenguer *et al.*[12] reported that lactate formation occurred across a pH range between 5·2 and 6·4 in *in vitro* incubations with mixed faecal microbiota, with the highest production occurring under slightly acidic conditions (pH 5·9). At low pH (<5·2) lactate formation was slightly increased but lactate utilisation was strongly inhibited, resulting in lactate accumulation. Normal colonic pH values range between pH 5·5 and 7·5[55] although lower pH values have been reported in severe bowel disease[56] and low pH values in combination with high colonic lactate concentrations (up to about 100 mM) have been associated with ulcerative colitis[57,58]. Faecal lactate concentrations clearly depend on the balance between production by bacteria and by host tissues, microbial utilisation and host absorption. In contrast to the reported beneficial effects of butyrate on gut health, lactate accumulation may be detrimental by causing acidosis and D-lactate can result in neurological problems. In the healthy colon, much of the lactate formed must be utilised by other bacteria via metabolite cross-feeding as faecal concentrations are generally low. Bacteria isolated from the human colon that have been reported to utilise lactate include the acetate producer *Desulfovibrio piger*, which concomitantly forms hydrogen sulphide[59]. Separately, some Firmicutes can form propionate from lactate[60], including *C. catus*[42] as

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Table 1. Major fermentation products of representative dominant species of human colonic bacteria

Genus/species	Phylum (family)	Products (from hexoses)	SCFA utilised
Eubacterium rectale	Firm (*Lach*)	Bu, Fo, La	Ac
*Roseburia inulinivorans**	Firm (*Lach*)	Bu, Fo, La	Ac
Eubacterium hallii	Firm (*Lach*)	Bu, Fo, But	Ac, La
Anaerostipes hadrus	Firm (*Lach*)	Bu	Ac, La
Coprococcus catus†	Firm (*Lach*)	Bu	Ac, La
Ruminococcus obeum	Firm (*Lach*)	Ac, La	
Blautia wexlerae	Firm (*Lach*)	Ac, Su	
Faecalibacterium prausnitzii	Firm (*Rum*)	Bu, Fo, La	Ac
Ruminococcus bromii	Firm (*Rum*)	Ac, Eth	
Bacteroides thetaiotaomicron	Bact (*Bac*)	Ac, Su, Pr	
Bacteroides vulgatus	Bact (*Bac*)	Ac, Su, Pr	
Bifidobacterium adolescentis	Actin (*Bif*)	Ac, La, Fo	
Collinsella aerofaciens	Actin (*Cor*)	Ac, La	

Firm, Firmicutes; *Lach, Lachnospiraceae*; *Rum*, *Ruminococcaceae*; Bact, Bacteroidetes; *Bac, Bacteroidaceae*; Actin, Actinobacteria; *Bif, Bifidobacteriaceae; Cor, Coriobacteriaceae*; Bu, butyrate; Fo, formate; La, lactate; But, butanol; Ac, acetate; Eth, ethanol; Su, succinate.
* *Roseburia inulinivorans* forms propionate and propanol from deoxy-sugars.
† *Coprococcus catus* forms propionate from lactate.

discussed earlier, while lactate is also utilised together with acetate by certain *Lachnospiraceae* including *Eubacterium hallii* and *Anaerostipes* species to form butyrate[12,39,61]. Thus a number of bacterial groups are likely to compete for lactate; in terms of bacterial energy gain, acetate formation from lactate is the least favourable[62].

U^{13}C-labelled DL-lactate provided as the sole added energy source to *in vitro* incubations with mixed faecal microbiota, gave rise to more propionate, formed via the acrylate pathway, than butyrate[12]. In the presence of polysaccharide substrates, however, the reverse was true, with more butyrate than propionate formed from lactate, and this was accompanied by a marked increase in the population of *E. hallii* within the community[12]. In a recent fermentor study, lactate cross-feeding was estimated to contribute to approximately 20 % of the butyrate pool[63]. It is clear that lactate-utilising bacteria, including those producing butyrate, play a key role in modulating the colonic metabolome.

Dietary fibre and phytochemicals

Consumption of fibre-enriched diets is considered to offer several health benefits[64–66]. In addition to increasing faecal bulking and transit rates along the large intestine, fibre delivers a wide range of phytochemicals and bacterially transformed metabolites within the human colon[67] with many of these compounds possessing anti-inflammatory activity[68]. Free phytochemicals are likely to be taken up in the small intestine whereas those that complex with plant polymers will be released and metabolised by the gut microbiota in the large intestine. In contrast, increasing the protein content of diets, particularly with red meat, is associated with increased formation of toxic bacterial metabolites resulting in an increased risk of cancer development[69]. The impact of different protein, carbohydrate and fibre (as non-starch polysaccharide) intakes is shown by a study in which seventeen obese men were provided with an initial maintenance diet (85 g protein, 360 g carbohydrate, 21·9 g non-starch polysaccharide, 116 g fat per d) followed by a high-protein, moderate-carbohydrate (139 g protein, 181 g carbohydrate, 12·8 g non-starch polysaccharide, 82 g fat per d) diet and a high-protein, low-carbohydrate (137 g protein, 22 g carbohydrate, 8·8 g non-starch polysaccharide, 143 g fat per d) diet, in a cross-over design, for periods of 4 weeks each. At the 'normal' intake levels represented by the maintenance diet, the major phenolic metabolites were ferulic acid and its metabolic conversion products that arise from microbial activity[67]. When volunteers were consuming the low-carbohydrate, high-protein diet, however, their faecal samples showed very low concentrations of the fibre-derived ferulic acid and its metabolites and the phenolic acid showing the highest concentration was phenylacetic acid, derived from amino acid fermentation[66,67]. Furthermore, there were increased concentrations of N-nitrosamines and branched chain fatty acids and decreased levels of beneficial products such as butyrate in the stool samples from the volunteers during the period when they were consuming the high-protein, low-carbohydrate diet[67]. Taken together these data suggest a less favourable gut environment, particularly with respect to colon cancer risk, on the high-protein, low-carbohydrate diet and these changes were only partially reversed by the moderate carbohydrate intake.

Predicting the impact of dietary manipulation on microbiota profiles and metabolites

We have seen that microbiota composition is influenced by diet, and that both the substrate and microbiota composition affects bacterial metabolite formation. It is known that prebiotic supplementation can affect both the composition and activity of the gut microbiota. A prebiotic is defined as a 'selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health'[70]. Ideally, prebiotics would be targeted at promoting beneficial bacterial species whose populations are decreased in situations associated with increased disease risk (e.g. metabolic syndrome and ageing). Clearly such specific targeting requires detailed prior knowledge on the substrate utilisation capabilities, and competitive abilities, of the bacterium to be targeted. Substrate utilisation profiles can be established for cultured strains, providing important information on the types of substrate that can be used by a bacterium under ideal growth conditions. Analysis of the supernatant can also provide some indication of the major metabolites produced by a specific bacterium. However, bacteria in the human gut exist within a complex community where there is competition for growth

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substrates, and cross-feeding of metabolites; thus care has to be taken in extrapolating results from pure culture to the *in vivo* situation. This is illustrated by work with fructan-based prebiotics (fructo-oligosaccharides and inulin)[71]. Many studies have focused on establishing an increase in bifidobacterial populations, and sometimes reduced numbers of potentially pathogenic Clostridial species (such as *C. perfringens*), but only a few have investigated the effect on the gut microbiota as a community. Pure culture work has shown however that a number of butyrate-producing Firmicutes are able to use fructo-oligosaccharides directly for growth[72], and genes for prebiotic degradation were identified in a range of abundant commensal bacteria by functional metagenomic screening[73]. Of course, bacteria that are able to utilise prebiotics as substrates for growth in pure culture may not necessarily compete well for the substrate within the mixed community. Nevertheless, a human study investigating the effect of consuming 10 g mixed chain length inulin per day detected increased numbers of both bifidobacteria and *F. prausnitzii*, a butyrate-producing bacterium in faecal samples[74].

Interestingly, fructan-based prebiotics stimulate both bifidobacteria numbers and butyrate production[75] even though bifidobacteria produce lactate and not butyrate. In part, this is likely to reflect the direct stimulation of butyrate-producing species; in addition, other members of the microbial community are able to convert lactate into butyrate, as was discussed earlier. The conversion of lactate into butyrate via bacterial cross-feeding was demonstrated in an experiment in which *E. hallii* and *B. adolescentis* were cultured together and independently on starch[76]. In pure culture *B. adolescentis* grows well, producing acetate, lactate and formate, while *E. hallii* cannot grow on starch. In co-culture, however, acetate declines and lactate completely disappears, being converted into butyrate by the action of *E. hallii*. Meanwhile a second type of cross-feeding was revealed in experiments with *Roseburia* spp., which lack the ability to utilise lactate. These species were only able to utilise fructo-oligosaccharides for growth when co-cultured with a *Bifidobacterium* species because of the release of breakdown products such as fructose, again resulting in butyrate formation[76,77]. Bifidobacteria themselves differ in their competitive ability to utilise fructans of varying chain length for growth, indicating that prebiotic supplementation has strain-specific effects[78,79], and these effects will also be host-specific, depending on the commensal bifidobacteria population. The strain-specificity of substrate degradation is also apparent from co-culture experiments. *R. inulinivorans* was able to outcompete a strain of *B. longum*[80], whereas a different strain of *B. longum* out-competed *R. inulinivorans* during growth on long-chain inulin[79]. Similarly it has been shown that different strains or species of bifidobacteria also differ in their ability to use another class of prebiotics, arabinoxylan oligosaccharides, for growth[81].

More generally, cross-feeding of breakdown products is a widespread feature in the utilisation of complex carbohydrate polysaccharides by gut microbial communities[82,83]. Because Gram-negative *Bacteroides* species are able to sequester soluble polysaccharides and perform substrate degradation largely in the periplasm[7], it is unlikely that many short-chain substrates will escape to be used by other bacteria. In Gram-positive Firmicutes that lack a periplasmic space, however, hydrolysis is more likely to happen extracellularly, with the potential for competition from other bacteria for breakdown products. This has been illustrated in co-culture experiments with the starch degrader *R. bromii*, which is apparently unable to take up glucose and some α(1–6) breakdown products of starch degradation[13].

Conclusion: the need for integrative approaches and modelling

It should be apparent that the complex interactions that occur between gut bacteria make simple predictions of dietary effects extremely difficult. However, the field of theoretical modelling can offer some help and some hope in tackling the behaviour of microbial communities and their metabolites. For example, experimental data from a simple batch co-culture experiment examining lactate cross-feeding have been successfully modelled[84]. A far more ambitious project is to model the whole community by defining a manageable number of functional groups within the gut microbiota. This has met with some success in modelling the impact of a one unit pH change[85] upon the microbial community in an anaerobic continuous flow fermentor system[86]. The models referred to so far aim at quantitative predictions of metabolite concentrations, fluxes and bacterial populations within the system and therefore depend on information from representative cultured bacterial isolates on growth rates, cell sizes, substrate preferences and pH tolerances. Another active, but quite distinct, area of modelling starts from bacterial genomes (genome-scale metabolic models) in order to predict the metabolic outputs of interactions between selected bacterial species[87–89]. Such genome-based modelling is essentially qualitative in nature, but there should be scope for the two approaches to inform each-other in the future[90]. In conclusion, modelling of the complex gut microbial ecosystem appears essential not only for gaining a better understanding of the system and its response to dietary change, but ultimately for designing strategies to alleviate disease and maintain gut health.

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Conflicts of Interest

None.

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Authorship

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