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Beneficial modulation of the gut microbiota

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ABSTRACT

The human gut microbiota comprises approximately 100 trillion microbial cells and has a significant effect on many aspects of human physiology including metabolism, nutrient absorption and immune function. Disruption of this population has been implicated in many conditions and diseases, including examples such as obesity, inflammatory bowel disease and colorectal cancer that are highlighted in this review. A logical extension of these observations suggests that the manipulation of the gut microbiota can be employed to prevent or treat these conditions. Thus, here we highlight a variety of options, including the use of changes in diet (including the use of prebiotics), antimicrobial-based intervention, probiotics and faecal microbiota transplantation, and discuss their relative merits with respect to modulating the intestinal community in a beneficial way.

KEYWORDS

Microbiota; gut health; microbial modulation; faecal microbiota transplant; diet; antimicrobials

INTRODUCTION

Humans are now thought of as "superorganisms" on the basis of the genetic potential encoded within our resident microbial populations in addition to our own genome. It has been suggested that our microbiota develops with us and alters its own composition and gene expression in response to changing environmental conditions [1]. The largest and most varied of the human-associated microbial communities exists in the gastrointestinal (GI) tract.

The gut microbial population is made up of approximately 1000 species from relatively few phyla. The most abundant species are members of the phyla Firmicutes and Bacteroidetes, with smaller numbers being representatives of the Proteobacteria, Fusobacteria, Cyanobacteria, Verrucomicrobia and Actinobacteria, amongst others [2]. The gut microbiota is composed mainly of anaerobes, which outnumber facultative anaerobes and aerobic bacteria by approximately 2-3 orders of magnitude [3]. It has been noted that, although there is great inter-individual variation in the composition of the gut microbiota, there are a conserved set of encoded functions shared between individuals referred to as the core gut microbiome [4], suggesting that it is the functionality of the microbiota rather than its composition that is of greatest importance to the host. The functions and pathways encoded in the core microbiome are thought to confer the greatest benefit to the host and are probably essential for the correct functioning of the gut. Some well-studied benefits include protection against potential pathogens, digestion of polysaccharides, production of essential vitamins, stimulation of angiogenesis, regulation of fat storage and modulation of the host's immune system [5]. Recent studies have also shown that the gut microbiota influences the gut-brain axis and shapes stress-related symptoms such as anxiety and pain tolerance [6].

Advances in high throughput sequencing technologies (HTS) and tools enabling comparative analysis of the large amount of data that are generated by these technologies have led to a better understanding of what constitutes a 'healthy" gut microbiota. One of the most interesting observations drawn from the data generated is that the resident microbiota encodes > 100 fold more genes than the human genome [7]. The genes present in the microbiome are responsible for many functions essential

to host survival but which are not encoded within the human genome. Due to the range and importance of the metabolic and biochemical processes carried out by the microbiome it has been referred to as "our hidden organ" [8].

While the "healthy" gut microbiota is seen to be a stable community, there are stages within the life cycle of humans during which there can be dramatic alterations in the structure and function of this population. These "natural" changes begin with initial colonisation immediately following birth and subsequent development of the microbiota over the first two years of life. The earliest colonizers are usually members of the enterococci and enterobacteria followed by strict anaerobes such as *Bifidobacterium*, *Clostridium* and *Bacteroides* spp. once the initial oxygen supply present has been depleted [9]. Despite this general pattern, it is important to appreciate that the method of delivery and subsequent feeding type have a profound effect on the initial populations [10]. Once the infant reaches two years of age the microbiota has already begun to transform into its adult form, which is thought to be relatively stable before it undergoes a final "shift" when entering old age [11]. Indeed, with respect to the latter phenomenon, a study by Claesson and colleagues that compared the gut microbiota of individuals ages 65 or older to 9 younger control subjects has highlighted significant changes in community structure associated with ageing, specifically an increase in the abundance of *Bacteroides* spp. and distinct shifts within the *Clostridium* genus [12]. It has been hypothesised that alterations in the elderly microbiota are due to physiological changes in the elderly gastrointestinal tract such as chronic low-grade inflammation, in addition to dietary habits [13].

It has been well established that the human gut microbiota is integral to human health, and, as will be discussed below, it also plays an important role in gastrointestinal disease. It is therefore reasonable to assume that modulation of the gut microbiota can be used as a therapeutic approach to treating chronic gastrointestinal diseases. Thus, this review is focussed primarily on the methods that can be employed to modulate the gut microbiota while highlighting the benefit of guiding community structure towards a more desirable state.

ROLE OF THE GUT MICROBIOTA IN GASTROINTESTINAL DISEASE

There are a growing number of gastrointestinal conditions that have been linked with alterations in the gut microbiota. To properly implement strategies to modulate the gut microbiota as a therapeutic tool, it is first necessary to understand the role of the gut microbiome in specific GI, and other, diseases. Given the recent rapid expansion in the number of disease states that have been linked with alterations in the gut microbiota, it is not possible to address the issue in depth in the confines of this review. Instead, some well-studied examples are discussed below and we refer you to some other recent reviews that address this topic in depth [3,14].

Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is a relapsing disorder characterized by chronic inflammation of the GI tract, and of the colon in particular. The two major types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Evidence suggests that IBD is a complex disease arising from a combination of genetic and environmental factors. From a genetics perspective, genome-wide association studies (GWAS) and subsequent meta-analyses have identified a total of 163 genetic risk loci for IBD [15-17]. A German twin cohort study confirmed the strong genetic element to IBD by observing that monozygotic twins are significantly more likely to be concordant for the disease than dizygotic twins [18]. However, concordance rates between monozygotic twins are nonetheless low (35% for CD and 16% for UC), highlight that environmental triggers do indeed play an important role in both diseases, and in UC in particular.

It is notable that murine studies have revealed that the presence of commensal enteric bacteria is necessary for the development of spontaneous colitis and immune system activation [19] and, indeed, transferring colitogenic gut microbiota into healthy mice can induce spontaneous colitis [20]. Similarly, it has consistently been observed that patients suffering from IBD harbour an altered gut microbiota [21,22], specifically reduced bacterial diversity and changes within the Firmicutes phylum [23]. The changes in microbiota composition appear to be somewhat different between UC and CD. For example, decreased abundance of the butyrate-producing bacteria *Roseburia hominis* and

Faecalibacterium prausnitzii have been observed in UC patients relative to controls [24], while the opposite has been observed in CD patients who possessed increased *F. prausnitzii* levels in addition to a reduced overall diversity [25]. Although these microbial changes could be a result of increased inflammation, evidence suggests that it is more likely that shifts in the microbiota are involved in the disease's pathogenesis, either due to an intolerance to a specific group of commensals or due to an imbalance between protective and harmful members of the population [21,23,26].

Irritable Bowel Syndrome

Irritable Bowel Syndrome (IBS) is a chronic GI disorder that presents with symptoms including abdominal pain, bloating and altered bowel function. IBS is divided into several subtypes based on stool characteristics; diarrhoea, constipated or mixed. It's cause, as of yet, is not fully known and although the aetiology is thought to be a combination of a number of factors, it is hypothesised that perturbations in the normal microbial microbiota play a role in the syndrome's characteristic low-grade inflammation [27]. Indeed, Rajiić-Stojanović et al. used qPCR and phylogenetic microarrays to show that the gut microbiota of IBS patients differed significantly from healthy controls, with IBS sufferers having a 2-fold higher Firmicutes to Bacteroidetes ratio and correlation analysis implicating several groups of Firmicutes and Proteobacteria in IBS pathogenesis [28]. Contrastingly, Jalanka-Tuovinen and colleagues observed that the faeces of diarrhoea-predominant IBS sufferers harboured 12-fold higher levels of several Bacteroidetes members. This group also noted that healthy controls have 35-hold higher numbers of uncultured clostridia [29]. Interestingly, these alterations in the microbiota correlated with changed in expression of host genes involved in amino acid synthesis, cell junction integrity and inflammatory response, suggesting impaired epithelial barrier function in IBS patients. Small intestinal bacterial overgrowth (SIBO), which is characterized by excessive bacteria in the small intestine, has also been put forward as a possible factor in IBS aetiology [30]. Bacterial overgrowth can result in overproduction of gas in the small intestine by degradation of carbohydrates, contributing to the symptoms of IBS [31]. The most commonly isolated bacteria from SIBO patients are *Escherichia coli*, *Streptococcus*, *Lactobacillus*, *Bacteroides* and *Enterococcus* species [32]. However it is not fully understood if any of these microorganisms play a specific role in IBS

progression. It should also be recognised that differences between studies may be due to the causative microorganisms or imbalances differing between IBS subtypes. Regardless, a bacterial role in IBS onset would seem to be clear, as further evidenced by the disease's response to antibiotic therapy [33] and differential expression levels of Toll-like receptors in colonic biopsies of patients with IBS [34].

Obesity

Obesity is a complex disease resulting from a prolonged imbalance of energy input and energy expenditure. Modern dietary and exercise habits are major contributing factors but it is now understood that the composition and function of the gut microbiome plays an important role through a variety of mechanisms [35]. A number of comprehensive reviews focussing on the association between the microbiota and obesity have been published [36,37]. Differences in the gut microbiota between obese and lean individuals have been the subject of great scrutiny. A range of different murine models have been used to this end, including genetically obese [38,39], diet-induced obese [40] and humanized [41] mice. Although a number of studies have reported an increased ratio of Firmicutes to Bacteroidetes in obese mice compared to their lean counterparts, these findings continue to be the subject of much debate in relation to human studies, which have revealed a number of microbial populations that have been associated with obesity [37]. Notably, transplanting the faecal microbiota of obese humans into germ-free mice brought about significant increases in the fat-mass of, and obesity-related metabolic phenotypes in, these mice relative to those which occurred when the corresponding faecal microbiota from lean monozygotic twins was transplanted [42]. Furthermore, a second trial showed that cohousing mice harbouring these two microbial communities prevented development of the obese phenotype, a trend correlating with invasion of specific Bacteroidetes members from lean to obese microbiota [42]. Another recent paper of note has linked the mucin-degrading bacterium *Akkermansia muciniphila* with obesity and type 2 diabetes [43]. The study showed *A. muciniphila* abundance was decreased in obese and type 2 diabetic mice and that prebiotic feeding normalised *A. muciniphila* levels, which in turn correlated with an improved metabolic

profile. Orally administered *A. muciniphila* also reversed high-fat diet induced metabolic disorders in these mice [43]. The results of these, and other studies, make it apparent that the microbiota plays a role in obesity but the specific changes associated with the phenotype are complex and remain unclear.

Type 2 Diabetes

Type 2 diabetes (T2D) is a metabolic disorder with both genetic and environmental influences. It is a major health concern throughout the western world, arising particularly as a result of increasing obesity-related insulin resistance [44,45]. It is evident from a number of studies that the gut microbiome is altered in patients suffering from T2D [46-48], although, as with many obesity-related associations, it is not clear whether these changes are a cause or simply a consequence of the disorder. Nonetheless, it was an interesting development when, in 2010 it was reported that the proportions of Firmicutes, and in particular species of clostridia, were significantly reduced in T2D sufferers compared to healthy individuals [46]. A subsequent, and much larger, metagenome-wide association study of 345 Chinese individuals showed that the gut microbiota of patients with T2D was characterized by a moderate degree of microbial dysbiosis, lower levels of butyrate-producing bacteria and an enrichment of microbial functions relating to sulphate reduction and resistance to oxidative stress [48]. Almost all of the microbial genes enriched in T2D patients were from opportunistic pathogens, including genes from several *Clostridium* spp. as well as *Bacteroides caccae* [48]. These results provided a number of markers that were assessed to determine if they could successfully identify patients with T2D on the basis of an analysis of faecal samples. Notably, this method successfully identified the T2D disease state with 81% accuracy [48], i.e. a greater success rate than using a combination of clinical risk factors and genetic information [49].

Colorectal Cancer

Colorectal cancer (CRC) is the third most common cause of cancer mortality in the world [50]. It is becoming apparent that, even though a single causative microorganism has not been explicitly identified, the gut microbiota plays a role in CRC [51,52]. Wang and colleagues noted that

there was a clear segregation between the microbiota of CRC patients and healthy volunteers, particularly, as was the case for T2D, a decrease in the abundance of butyrate producers and an increase in the incidence of opportunistic pathogens in CRC patients [53]. Members of the *Fusobacterium* genus have also been recently identified as potential causative agents after it was observed that they were enriched in colorectal carcinomas [54], a pattern also noted in other studies [53,55-57]. The authors hypothesised that *Fusobacterium* spp. may contribute to tumourigenesis by an inflammatory-mediated mechanism, a hypothesis supported by a follow-up study which showed that members of fusobacteria could generate a proinflammatory microenvironment through the recruitment of tumour-infiltrating immune cells [58]. *E. coli* has also been linked with CRC in a number of studies. Arthur *et al.* observed that *E. coli* levels were ~100-fold higher in the microbiota of the colitis-susceptible *IL10* $^{-/-}$ mouse strain compared to the wild type [51]. They went on to show that *E. coli* NC101 mono-association significantly promoted development of invasive mucinous adenocarcinomas in azoxymethane treated, *IL10* $^{-/-}$ mice and that deletion of the polyketide synthase (*pks*) genotoxic island from this *E. coli* strain decreased tumour multiplicity and invasion [51]. While further investigations are required, these results suggest that colitis promotes tumourigenesis in mice by altering the composition of the gut microbiota and selecting for members with genotoxic capabilities.

Ultimately, identification of microorganisms, microbial populations or microbial functionalities involved in GI disease is fundamental to developing novel therapies. It is evident that the gut microbiota plays a large role in intestinal health and disease, and therefore manipulation or modulation of this community, is a clinical option that merits serious consideration.

MODULATION OF THE GUT MICROBIOTA

Modulation by Diet

Environmental factors, including dietary intake, can shape the composition of the intestinal microbial community. Indeed, a number of recent studies have highlighted the links between diet and distinct microbial profiles and, in turn, overall gut health [40,59-63]. Having an understanding of how diet influences microbial communities will be of critical importance with respect to employing food to beneficially alter the gut microbiota.

The amount, type and balance of the three main dietary components, i.e. protein, carbohydrates and fat, have a profound impact on the gut microbiota. Short-chain fatty acids (SCFAs), primarily butyrate, propionate and acetate, are the major end products from the microbial degradation of carbohydrates and protein in the gut. SCFAs have a diverse range of physiological effects on the host, with perhaps the most important being their oxidation by mucosal cells to provide energy. An excellent review of the benefits of SCFAs on the host has been published by Macfarlane & Macfarlane [64]. The majority of microbial protein degradation occurs in the distal colon where the pH is neutral and conditions are favourable for the growth of proteolytic bacteria such as *Bacteroides* spp., *Propionibacterium* spp. and *Clostridium perfringens* [65,66]. The main pathway of protein degradation by this population is deamination of amino acids to the aforementioned SCFAs and ammonia [67], high concentrations of the latter have been shown to act as tumour promoters in rats [68]. The range of end products generated by protein digestion is broader than that of carbohydrates (see below) and also includes branched-chain amino acids, phenols, indoles and amines [69]. The majority of studies examining the effect of dietary protein on the gut microbiota have focussed primarily on the detection of altered fermentation products in the cecum [70] and faeces [71]. However, the effects of whey protein isolate on the microbiota have been the topic of some scrutiny in recent years as it has been indicated that dairy products can alleviate several disorders relating to metabolic syndrome [72]. One such study noted significantly increased counts of bifidobacteria and lactobacilli in the faeces of rats whose diets included cheese whey protein isolate or casein supplemented with either threonine or cysteine [73]. Whey protein isolate (WPI) has also been observed to alter the composition of the gut microbiota of mice in a dose-dependent manner [74]. All mice whose high fat diet was supplemented with WPI had significantly increased proportions of

Lactobacillaceae and significantly decreased proportions of *Clostridiaceae* compared to high-fat fed controls, and increasing the amount of total energy derived from WPI caused a more profound shift in the microbiota [74]. Certain components of the normal human dietary intake of carbohydrates cannot by digested directly by the host and act as the major diet-derived energy source for microorganisms in the gut [75]. This fraction, comprised largely of resistant starches and non-starch polysaccharides, is degraded by microbial fermentation to a mixture of gasses and the aforementioned SCFAs. Many such carbohydrates are also referred to as prebiotics. The term prebiotic was introduced by Gibson and Roberfroid in 1995 [76] and are defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host well-being and health" [77]. Prebiotics have most frequently been employed with a view to stimulating the growth of either lactobacilli or bifidobacteria, with many studies focussing on inulin [78-80], oligofructose [81,82] or fructooligosaccharides [83,84]. There is a substantial body of evidence linking prebiotic consumption to human health benefits through modulation of the gut microbiota, with research in this area having been the subject of a number of recent reviews [85-87]. In one particularly notable recent study, it was observed that supplementing the murine diet with SCFAs or fructooligosaccharides caused a shift in microbiota composition which strongly correlated with beneficial changes in body weight, adiposity and glucose control. These physiological changes were brought about via butyrate- and propionate-mediated activation of intestinal gluconeogenesis [88].

The majority of dietary fat is absorbed in the human small intestine but it has been shown that a substantial amount survives digestion and can be recovered in faeces [89]. The undigested portion passes through the colon where it can have a profound effect on the intestinal microbiota. Murphy *et al.* observed that high-fat feeding caused a greater compositional change in the gut microbiota than genetically induced obesity [90], in accordance with a previous study which showed that, when fed a high-fat diet, RELMβ knockout mice showed a significantly altered gut community while staying lean. RELMβ knockout mice were employed as they are known to stay relatively lean when fed a high-fat diet. The authors could therefore conclude that the change in diet, as opposed to the obese

state, was responsible for the observed changes in the microbiota [91]. Many studies have established that mice fed a high-fat diet have significantly dissimilar microbial populations in the gut compared to mice fed on normal chow [38,40,92]. However, a recently published study showed that life-long calorie restriction significantly altered the gut microbiota in mice fed on both high-fat and low-fat diets [93]. This implies that not only the fat content of the diet, but also the number of calories consumed, has the potential to influence the bacterial communities present in the GI tract. The study also linked changes in the gut microbiota to claims that calorie restriction promotes healthy-ageing and increases lifespan in various animal models as the healthiest and longest living mice were those that were fed a low fat diet with calorie restriction [93]. In addition to the studies referenced above, there are many excellent reviews of the effect of dietary fat on the intestinal microbiota [37,94,95].

This specific combination of dietary components can vary according to geographic location, food availability, cultural practices and age and can have a profound impact on the conditions within the gut and the requirements of the microbiota (Table 1 highlights some studies which have investigated this impact). In one instance, the faecal microbiota of European children and children from an African village in Burkina Faso, whose diets differed considerably, was investigated. The diet of the African children was predominately vegetarian; high in starch, fibre and plant polysaccharides and low in fat and animal protein. This diet correlated with a significant increase in the Bacteroidetes:Firmicutes ratio in addition to an abundance of *Prevotella* and *Xylanibacter* when compared to the microbiota of the children consuming a carbohydrate-rich European diet [96]. The *Xylanibacter* genus, which was absent in European children, is known to contain genes for xylan and cellulose hydrolysis and so it was hypothesised that the gut microbiota coevolved with the polysaccharide-rich diet of the Burkina Faso children, allowing them to increase the energy extracted from dietary fibre while also conferring protection from inflammation and non-infectious colonic disease [96]. The comparatively high abundance of *Prevotella* in the faecal microbiota of the African children and the fact that it coincides with a carbohydrate-rich diet is consistent with the observations of Wu *et al.* who found that the overall composition of the microbiota was strongly associated with long-term diet [62]. Specifically, a diet rich in protein and animal fat was associated with higher

proportions of *Bacteroides* while *Prevotella* were more abundant when the diet was enriched with plant-derived carbohydrates [62]. A recent study by De Filippo *et al.* took these investigations a step further by focussing specifically on the effect of diets composed entirely of animal or plants products on the gut microbiota [61]. It revealed that an animal-based diet increased the numbers of bile-tolerant microorganisms present and decreased the numbers of plant polysaccharide degrading Firmicutes. Interestingly, the respective diets brought about a transcriptional response among the gut microbiota that was consistent with previously reported differences in gene abundances between herbivorous and carnivorous animals [61]. In other studies, members of the *Clostridium* clusters IV and XIVa have been found to be enriched in the faeces of omnivores compared to vegetarians and lacto-vegetarians, who generally consume higher proportions of carbohydrates as part of their diet [97-99]. These clusters of bacteria are noted for their ability to convert dietary fibre to SCFAs.

The overall dietary patterns in the De Filippo study above are similar to a study in mice where conventionalised mice were switched from a low-fat diet rich in complex plant polysaccharides (CHO) to an obesity-inducing high-fat/simple carbohydrate "Western" diet [40]. Mice fed on the "Western" diet had a significantly lower level of bacterial diversity, a characteristic seen to be an indicator of an unhealthy microbiota [59]. These mice possessed a significantly higher relative proportion of Firmicutes and lower relative proportions of Bacteroidetes compared to littermates which remained on the CHO diet. This population shift is similar to what is seen in the *ob/ob* mouse model of obesity [38] but differs in that the Firmicutes shift in the genetically-induced obesity model is division-wide whereas the dietary intervention above caused a bloom in a single uncultured clade within the Mollicutes class. A subsequent microbiota transplantation from these diet-induced obese mice into germ-free recipients promoted greater adiposity than transplants from lean donor [38]. A further study by the same group showed that this response of the microbiome to dietary intervention is rapid and can occur within 24 hours [41], a phenomenon also observed by Wu *et al*., [62].

A gut microbiota with decreased diversity has been linked with increased frailty and poorer general health in elderly subjects [60]. In this study, clustering of subjects by diet, residence location and by microbial groupings was apparent. Ultimately, it was evident that subjects that were living in

the community had a healthier and more varied diet than subjects in long-term residential care, which gave rise to a more diverse gut microbiota with significant changes being noted at phylum and family levels. Differences were also apparent at the genus level with long-stay subjects possessing higher levels of *Parabacteroides, Eubacterium, Anaerotructus, Lactonifactor* and *Coprobacillus,* while *Coprococcus* and *Roseburia* (both members of the *Lachnospiraceae* family) were more abundant in community-dwelling subjects [60]. The data also linked microbiota composition to the duration spent in long-stay care. The longer the subject stayed in residential care (and consumed a less varied diet), the more dissimilar their microbiota became to the microbiota of healthy community-dwelling subjects [60]. Another study investigating the temporal relationship between food intake, gut microbiota and metabolic and inflammatory phenotypes reported that individuals with reduced microbial gene richness present more pronounced dys-metabolism and low-grade inflammation than their richer counterparts [100]. This microbiota-associated phenotype was suggested to be a result of long-term dietary habits as it was noted that these subjects seemed to consume less fruits, vegetables and fish than their high gene richness equivalents, i.e. a pattern consistent with that reported by Claesson *et al* [60]. More specifically, the initial sampling of the cohort (49 obese or overweight subjects) showed that subjects with lower gene richness in the gut microbiota presented with increased obesity-associated phenotypes such as higher insulin resistance and increased levels of fasting serum triglyceride, LDL cholesterol and inflammation. Dietary intervention (6 week energy-restricted high-protein diet) increased gene richness significantly in individuals that originally had a low gene count. This increased gene richness remained after the subjects were switched to a 6 week weight-maintenance diet suggesting that dietary intervention as the potential to, at least partially, correct a loss of richness in the microbiota [100].

Given the complexity of the relationship between diet and the gut microbiota, there would seem to be merit in developing and utilising models that allow one to elucidate the specific relationship between specific dietary components and microorganisms. An elegant strategy to facilitate this was provided by Faith *et al.* when they introduced a model community of ten human gut bacteria into gnotobiotic mice and developed a relatively simple statistical model which predicted

over 60% of the species variations that occurred in response to changes in diet [101]. The amount of casein in the diet was observed to be significantly associated with the abundances of all 10 microbial species and highly correlated with the total biomass of the community. Interestingly, *E. coli* and *Clostridium symbosium* were the only two species that had a second dietary variable significantly associated with their abundance, sucrose and starch respectively. The statistical model was subsequently able to determine 61% of the variation of the community members when the host was fed a new, previously unseen diet [101]. These results represent a significant step towards tailoring diet to address chronic microbiota-associated illnesses and a potential evolution of research within the field.

It is clear that microbial composition varies between groups living on different long-term diets. Recent investigations that suggest that short-term dietary changes can also alter the composition, and result in changes to the metabolic activity of the microbiome as a whole, are noteworthy but further investigations are required to determine how best to take advantage of these observations.

Modulation by Antimicrobials

The manipulation of the gut microbiota by antimicrobials is emerging as an attractive therapeutic strategy (Table 2). The success of this approach is likely to ultimately depend on the target specificity of the antimicrobials in question, especially as the undesirable consequences of the overuse of broad-spectrum antimicrobials have become ever more apparent in recent years. For quite some time broad-spectrum antibiotics have been commonly used by clinicians as they can be used in the treatment of a wide range of infections or when the causative bacterium has not been formally identified. However, due to the frequent use of these antibiotics, the spread of antibiotic resistance is now posing a serious problem in health care settings. In addition, antibiotic therapies not only affect the target microorganism but can also perturb the host gut microbial communities. The extent of this damage has recently become more evident through the application of high throughput DNA-based sequencing technologies to assess the composition of gut microbial populations (for review see Cotter

et al. 2012) [102]. Here we provide just a few examples of the negative consequences of the use of broad-spectrum antibiotics on the gut microbiota and, in turn, health.

The widespread use of broad-spectrum antibiotics, such as amoxicillin, to treat childhood infections has been linked to a dramatic decrease in *Helicobacter pylori* carriage [103]. However, studies indicate that those who did not acquire *H. pylori* in childhood were more likely to subsequently develop asthma, hay fever and skin allergies [104], while other investigations suggest that *H. pylori* infection has a protective effect with respect to the development of allergic asthma in mouse models [105]. The use of some broad-spectrum antibiotics, including clindamycin, ampicillin, amoxicillin, cephalosporins and flouroquinolones, can also result in *Clostridium difficile* overgrowth by impacting the resident gut microbiota, followed by antibiotic-associated diarrhoea, pseudomembranous colitis and, potentially, life-threatening complications such as toxic megacolon [106,107]. Low doses of antibiotics have also been used as growth promoters in agriculture since the 1950's despite an unclear understanding of the mechanisms at work. A recent investigation into this effect revealed subtherapeutic antibiotic treatment (STAT) of various antibiotics increased adiposity and hormones related to metabolism in young mice compared to untreated controls [108]. Analysis of the composition and function of the gut microbiota of these animals made it apparent that STAT exposure selected for microbial species that were able to extract more calories from dietary complex carbohydrates that were otherwise indigestible in the control group [108].

When considering these results, it is important to be aware that different broad-spectrum antibiotics differ with respect to their impact on the gut microbiota. Changes to the gut microbiota can also be either long- or short-term. In one instance this was highlighted through murine studies which established that mice treated with a cocktail of amoxicillin, metronidazole and bismuth [3.0 mg, 0.69 mg and 0.185 mg, respectively] daily for 10 days had largely recovered their baseline microbial community structure 2 weeks post-treatment but that treatment with cefoperazone [0.5 mg/ml of drinking water] had long-term effects on community structure and reduced overall diversity [109]. The effect of an antibiotic on the gut microbiota is influenced by several factors including its

antimicrobial effect (bactericidal or bacteriostatic), its mode of action, the structure of the microbiota and the distribution of antibiotic resistance genes among this population [110].

In light of this greater appreciation of the impact of broad spectrum antimicrobials on the gut microbiota, it is apparent that there is value in utilising antimicrobials with a narrow spectrum of inhibition. In addition to existing repositories of narrow spectrum antimicrobials that were not previously commercialised, it is worth noting that the gut microbiota is considered a rich, but yet relatively, underutilised source of antimicrobial-producing, and in particular bacteriocin-producing, bacteria. Bacteriocins are ribosomally synthesised peptides to which the producer has a specific immunity gene and can have either a narrow or broad spectrum of activity [111]. Many bacteriocins have a number of desirable traits, including low toxicity, high potency and, in the case of gut associated strains, the possibility of *in situ* antimicrobial. This combination of traits makes them attractive alternatives to traditional antibiotic therapies. Despite being, as stated above, a relatively underutilised source of antimicrobials, a number of bacteriocins have previously been isolated from mammalian gut microbes [112-115]. Indeed, for example, screening of faecal samples from 266 elderly Irish subjects identified 13 bacteriocin producing strains [115] while a further study lead to the isolation of 23 distinct bacteriocin-producing strains from a range of mammalian gastrointestinal sources [112]. Given that, for a bacteriocin to be produced and be active in the gut, the producer needs to be able to survive in and colonize the human gut and the associated antimicrobial needs to be active in the gut environment, it has been argued that the gut is an ideal source of bacteriocin producers with the potential to alter the gut microbiota [116]. There have already been a number of studies which have highlighted the merits of employing gut-associated bacteriocins, several of which we refer to here. In a distal colon model, the narrow spectrum bacteriocin thuricin CD has been observed to inhibit the growth of *C. difficile* without having any significant additional impact on the other components of the gut microbiota [113]. This contrasted with the significant shift in the relative proportions of the dominant bacterial populations that were observed when the broad-spectrum antimicrobials lacticin 3147, metronidazole and vancomycin, respectively, were employed. Notably, thuricin CD also exhibited a potency comparable to that of the control antimicrobials [113], thereby

establishing that thuricin CD has potential as an alternative to the conventional antimicrobial strategies employed to treat *C. difficile* infection, especially as it is less likely to impact negatively on the commensal gut microbiota and, thus, is more likely to prevent recurrent *C. difficile* infections. While, in the above example, thuricin CD, rather than the associated *Bacillus thuringiensis* producer [106], was employed, there are other examples that have highlighted the merits of using the bacteriocin-producing strain itself. In one such instance, ingestion of the bacteriocin producing probiotic strain *Lactobacillus salivarius* UCC118 provided significant protection against infection by *Listeria monocytogenes* in mice [117]. Production of the Abp118 bacteriocin by UCC118, which has previously been shown to be capable of altering the intestinal microbiota of pigs and mice [118], proved to be the key protective factor as a non-bacteriocin producing mutant failed to confer the same protection. This protective effect was also lost when infection was with a bacteriocin-immune *L. monocytogenes* mutant, thereby confirming that the mode of action was direct antagonism by Abp118 rather than *via* some other indirect effect [117]. In another instance a combination of 5 probiotic strains were employed to control *Salmonella* Typhimurium-induced diarrhoea in pigs [119]. It was subsequently established that the only bacteriocin-producing strain, *L. salivarius* DPC6005, was the dominant member of the cocktail in both the ileum digesta and in the mucosa. It could not be established, however, if bacteriocin production was directly responsible for anti-*Salmonella* activity [120].

In addition to the control of pathogens, antimicrobials have also been investigated with a view to altering metabolic health in diet-induced obese mice [121]. Supplementation of a high-fat diet with vancomycin caused a significant decrease in Firmicutes and Bacteroidetes populations with a corresponding increase in Proteobacteria. This compositional shift was accompanied by a marked decrease in weight gain, fasting blood glucose, plasma TNFα and triglyceride levels compared to the diet-induced obese controls. Although supplementation of the high-fat diet with the bacteriocin-producing probiotic *L. salivarius* UCC118 did not produce any significant changes in the metabolic profiles of the mice, it did result in an increase in relative proportions of Bacteroidetes and Proteobacteria with a corresponding decrease in Actinobacteria. The authors concluded that

antimicrobial strategies have the potential to alter both the composition of the gut microbiota and the metabolic health of the host. However, it was noted that care must be taken when choosing the antimicrobial to be used so as to bring about extended beneficial impacts on metabolic health.

As with diet, the vast majority of work concerning modulation of the microbiota by antimicrobials has taken place in mouse models. Nevertheless, the results are encouraging and suggest that carefully selected antimicrobials represent a viable option with respect to intelligently altering the bacterial populations within the human gut.

Modulation by Probiotics

The World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [122]. Probiotics are becoming increasingly popular and are generally marketed as functional foods or dietary supplements. As it has been recognised that changes in the gut microbiota play a role in GI disease then it is not surprising that probiotics are an attractive option with respect to modulation of the gut microbiome. For a probiotic to successfully exert its benefit on the host's gut microbiota it should be able to remain viable during storage and also be capable of surviving, and potentially colonizing, the host's intestinal environment [123]. The majority of probiotics currently used are members of lactic acid bacteria (LAB) and, more specifically, strains from the genera *Lactobacillus* and *Bifidobacterium* are most commonly used in commercial probiotics. Mixtures of these strains are becoming increasingly popular as researchers gain a deeper understanding of increasing efficacy via possible additive or synergistic effects [124]. Rijkers *et al.* categorised the benefit of probiotics into three levels based on location and method; 1) interference with the growth or survival of pathogenic microorganisms in the gut lumen, 2) improvement of mucosal barrier function or mucosal immune system and 3) influence beyond the gut through the systemic immune system and other organs [125]. A study undertaken by Park et al. found that DIO mice treated with the probiotic strains *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 experienced reduced body weight gain and fat accumulation in addition to lowered plasma insulin, leptin, total-cholesterol and liver toxicity biomarkers compared to a group on

485 the same diet supplemented with a placebo [126]. Supplementation with these probiotic strains also
486 resulted in down-regulation of pro-inflammatory genes in adipose tissue, up-regulation of fatty acid
487 oxidation-related genes in the liver and significant alterations in the diversity and function of the gut
488 microbiota. Similar results were observed by Yadav *et al.*, who found that administration of the
489 probiotic VSL#3 prevented and treated obesity and diabetes in a number of different murine models
490 through modulation of the gut microbiota. In particular, an increase in the number of butyrate-
491 producing bacteria was linked with enhanced secretion of the hunger-reducing hormone GLP-1 as
492 well as upregulation of genesinvolved in GLP-1 synthesis and excretion [127]. McNulty *et al.*
493 observed that, in gnotobiotic mice harbouring a 15-member model human gut microbial community,
494 introduction of 5 probiotic strains isolated from a fermented milk product did not significantly alter
495 the composition of the intestinal microbiota but instead increased the expression of microbial genes
496 involved in carbohydrate and nucleotide metabolism while decreasing expression of genes involved in
497 the metabolism of lipids and amino acids [128]. These metatranscriptomic changes were also apparent
498 in the microbiota of human monozygotic twins when fed the same fermented milk product, primarily
499 upregulation of genes involved in carbohydrate metabolism. In addition to their investigation with a
500 view to contributing to the prevention/treatment of obesity and T2D, it should be noted that probiotics
501 are thought to have the potential to treat a wide range of other conditions such as IBS, allergies, *C.*
502 *difficile* infection, IBD and others by modulation of the gut microbiota as highlighted in a number of
503 recent manuscripts [129-135]. As we learn more about other gut microbes and their role in human
504 health it may emerge that the future of probiotics lies in different, non-traditional probiotics, for
505 example *Akkermansia muciniphila* as mentioned previously [43]. A recent review by Neef and Sanz
506 discusses some of the strains already being investigated and the new techniques employed to assess
507 their impact on human health [136].

508 **Modulation by Faecal Microbiota Transplantation**

509 Following on from the probiotics principle, but on a community rather than strain level, faecal
510 microbial transplantation (FMT) is the process of transplanting faecal bacterial communities from a
511 healthy individual to a recipient whose microbiota has been disrupted or altered. Although still

somewhat in its infancy, FMT is becoming more commonly used as an approach to replenish the gut microbiota in order to alleviate the symptoms of disease. To date, FMT has most commonly been used to treat recurrent *C. difficile* infection (CDI) by replacing populations of commensal bacteria which have been wiped out by antibiotic therapy. Khoruts and colleagues used terminal-restriction fragment length polymorphism and 16S rRNA approaches to compare the bacterial component of a CDI patient's microbiota before and after FMT intervention [137] and found that, before intervention, the microbiota was deficient in both Bacteroidetes and Firmicutes but 14 days post-transplantation the microbiota was changed to closely resemble the donor's microbiota and was dominated by *Bacteroides* spp. [137]. These results are similar to findings by Tvede and Rask-Madsen who reported *Bacteroides* spp. were absent in CDI patients but were replenished after FMT intervention [138]. The composition of the donor's microbiota is the key factor in determining the efficacy of this treatment, as shown by Grehan et al. who collected faecal samples from patients undergoing FMT at 4 time points; pre-treatment and at intervals of 4, 8 and 24 weeks post-treatment to determine the effect of FMT on its microbial content [138]. Using a molecular approach they found that the microbiota was altered by FMT intervention and that at 4, 8 and 24 weeks the community of the recipient was composed predominately of bacteria derived from the healthy donor's samples. Crucially, in addition to bringing about desirable microbiota-related changes, FMT has in a high frequency of cases been successful in controlling CDI. In one such study it was revealed that only 1 of 16 patients treated with FMT experienced a recurrence of colitis during the 90 day follow-up period [139]. Indeed, when many such studies were combined in a systematic literature review by Gough *et al.*, i.e. to examine the effect of FMT on 317 CDI patients across 27 case studies, it was revealed that disease was resolved in 92% of cases [140]. An interesting development in the application of FMT is the use of synthetic microbial communities in place of undefined mixtures from donors (for review see de Vos et al [141]). The synthetic mixtures have the advantage of being controlled, tested extensively for the presence of viruses or pathogens and have the potential to be reproducibly manufactured. Petrof *et al.* showed that a defined mixture of 33 isolates, when administered during a colonoscopy, cured the CDI of 2 patients who had previously failed to respond to antibiotic treatment [142]. 16S rRNA analysis showed that the strains found in the stool substitute were rare in the patient's gut microbiota before

intervention, however following treatment these strains accounted for over 25% of sequences recovered from the gut microbiota. Although FMT has been most extensively studied with a view to CDI treatment, it has, however, also been investigated as a potential treatment option for a range of microbiota-associated diseases including IBD, IBS, obesity, idiopathic thrombocytopaenic purpura and even multiple sclerosis. A recently published review by Borody *et al.* summarises the current state of research and possible future directions of the technique [143].

CONCLUDING REMARKS

It is well established that the gut microbiota influences host metabolism, nutrient absorption and immune function, and that disruption of this balanced community can have very serious health implications. As we gain a deeper understanding of the specific relationships between the gut microbiota and disease, we expose potential therapeutic targets. Intelligent modulation of the intestinal community is a topic that had gained considerable interest and has the possibility to be extremely beneficial for human health.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest in preparing this article.

558 Table 1. Some examples of studies assessing the influence of diet on the microbiota and health of the
559 host.

Diet	Effect on microbiota	Effect on host
Rich in plant-derived polysaccharides [62,96].	Increased Bacteroidetes, decreased Firmicutes [96]. Associated with *Prevotella*-rich enterotype [62].	Faster gut transit time compared to high protein and animal fat diet [62].
Omnivorous compared to vegetarian and lacto-vegetarian [97-99].	Increased *Clostridium* clusters IV and XIVa [97-99].	Not reported
High-fat, simple carbohydrate "Western" diet [38,40].	Increased Firmicutes, decreased Bacteroidetes [38,40].	Diet-induced obesity. Subsequent transplantation of obese microbiota to germ free mice increased adiposity [40].
Reduced carbohydrate intake [63].	Reduced *Bifidobacterium*, *Roseburia* spp. and *Eubacterium rectale* [63].	Not reported
Animal product-based [61]. High protein and animal fat [62].	Increased β-diversity and bile-tolerant bacteria, including *Bacteroides*, decreased Firmicutes [61]. Associated with *Bacteroides*-rich enterotype [62].	Decreased weight independent of calories consumed [61].
Less fruit, vegetables and fish [100].	Reduced microbial gene richness [100].	Increased insulin resistance, fasting serum triglyceride levels, LDL cholesterol and inflammation [100].
Reduced variety due to long-stay care [60].	Increased Bacteroidetes and reduced overall diversity [60].	Increased frailty and poorer general health [60].
Changed from a vegetarian diet to an animal-based diet [61].	Decreased *Prevotella*, increased *Bacteroides* [61].	Not reported

560

561

562 Table 2. Some examples of studies assessing the influence of antimicrobials on the gut microbiota
563 and, where relevant, the host.

Antimicrobial	**Effect on Microbiota**	**Physiological effect on host**
Thuricin CD	Eliminated *C. difficile* without impacting overall microbiota composition [113].	Not examined – distal colon model
Abp118	Protection against *Listeria monocytogenes* infection [117]. Increased Bacteroidetes and Proteobacteria, decreased Actinobacteria [120].	Temporarily reduced weight gain in pigs [117].
Vancomycin	Decreased Firmicutes and Bacteroidetes, increased Proteobacteria [121].	Decrease in weight gain, fasting blood glucose, plasma TNFα and triglyceride levels in DIO mice [121].
Sub-therapeutic antibiotic therapy*	Increased Firmicutes, especially *Lachnospiraceae*, relative to Bacteroidetes [108].	Increased adiposity and bone mineral density in mice [108].
5 strain probiotic mixture**	Reduced shedding of *Samonella enterica* serovar Typhimurium in pigs[119].	Reduced incidence, severity and duration of diarrhoea in pigs. Also, increased weight gain [119].
Lactobacillus gasseri SBT2055, producer of gassericin T bacteriocin	Not reported	Decreased abdominal adiposity, body weight, BMI, waist circumference and hip circumference in human adults [131]. Lower triglyceride levels and reduced expression of lipogenic and pro-inflammatory genes in DIO mice [135].

564 * Penicillin, vancomycin, penicillin plus vancomycin, and chlortetracycline

565 ** *Lactobacillus murinus* DPC6002, *Lactobacillus murinus* DPC6003, *Lactobacillus*

566 *pentosus* DPC6004, *Lactobacillus salivarius* DPC6005, and *Pediococcus pentosaceus* DPC6006

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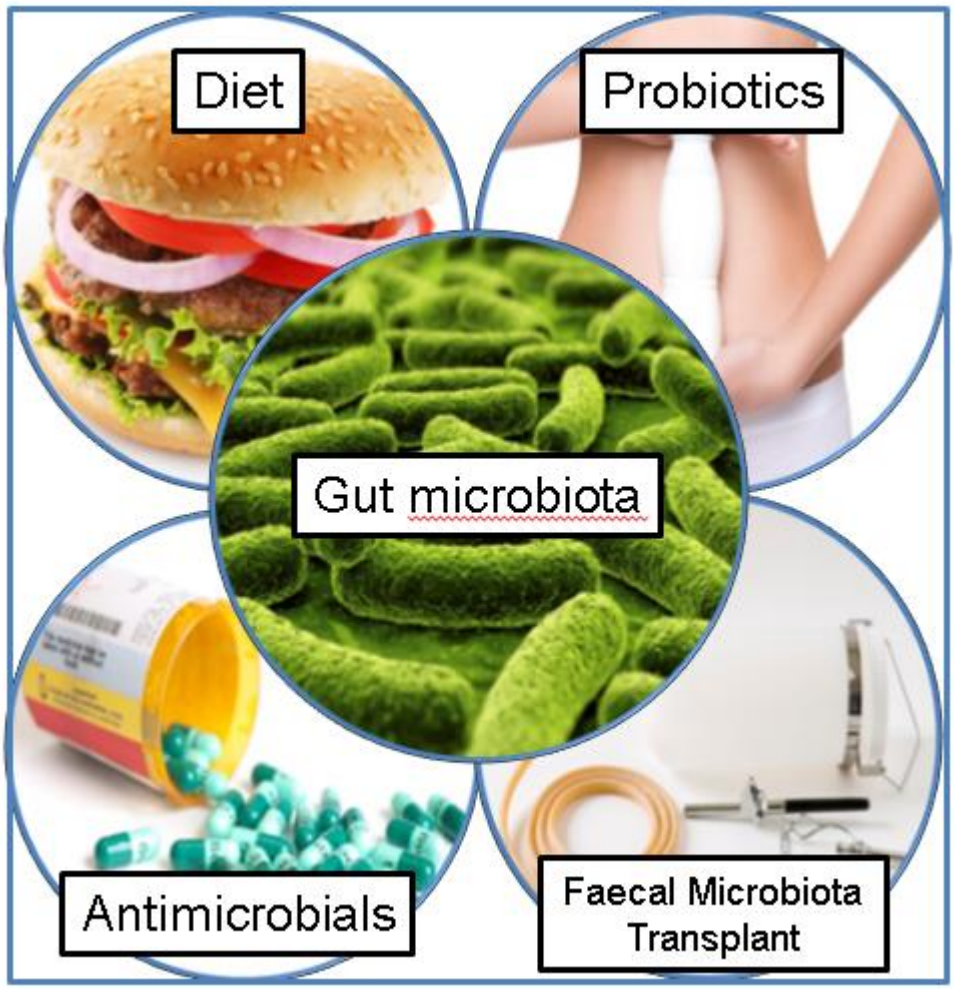

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569 Fig. 1. Potential strategies for manipulation of the gut microbiota.

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