



## Conference on ‘Diet, gut microbiology and human health’ Symposium 2: Changes in the microbiome in disease and lifecourse

### Gut microbiota in older subjects: variation, health consequences and dietary intervention prospects

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Alterations in intestinal microbiota composition and function have been linked to conditions including functional gastrointestinal disorders, obesity and diabetes. The gut microbiome encodes metabolic capability in excess of that encoded by the human genome, and bacterially produced enzymes are important for releasing nutrients from complex dietary ingredients. Previous culture-based studies had indicated that the gut microbiota of older people was different from that of younger adults, but the detailed findings were contradictory. Small-scale studies had also shown that the microbiota composition could be altered by dietary intervention or supplementation. We showed that the core microbiota and aggregate composition in 161 seniors was distinct from that of younger persons. To further investigate the reasons for this variation, we analysed the microbiota composition of 178 elderly subjects for whom the dietary intake data were available. The data revealed distinct microbiota composition groups, which overlapped with distinct dietary patterns that were governed by where people lived: at home, in rehabilitation or in long-term residential care. These diet-microbiota separations correlated with cluster analysis of NMR-derived faecal metabolites and shotgun metagenomic data. Major separations in the microbiota correlated with selected clinical measurements. It should thus be possible to programme the microbiota to enrich bacterial species and activities that promote healthier ageing. A number of other studies have investigated the effect of certain dietary components and their ability to modulate the microbiota composition to promote health. This review will discuss dietary interventions conducted thus far, especially those in elderly populations and highlight their impact on the intestinal microbiota.

#### Diet: Microbiota: Health: Ageing

##### Gut microbiota and its relevance for health

Until recently the human organism has been treated as an exclusively eukaryotic metazoan, whereby the studies of nutrition, health and non-infectious disease were challenging but well defined in terms of cells and external factors. A technological revolution spurred by innovations in DNA sequencing technology after the Human Genome Project ironically provided insights into the

complex community of bacteria that lives on and in the human body. It has been estimated that these bacteria outnumber human cells by a factor of 10 and human genes by a factor of 100<sup>(1)</sup>, and it is now widely accepted that these bacterial communities co-evolved with human subjects and other metazoans in a mutualistic if not truly symbiotic way. The evidence for this stems from the linkage of perturbed microbial communities with a range of symptoms, pathophysiologies and diseases. A microbial

**Abbreviations:** GPR, G-protein-coupled receptor; IBD, inflammatory bowel disease; LPS, lipopolysaccharide.

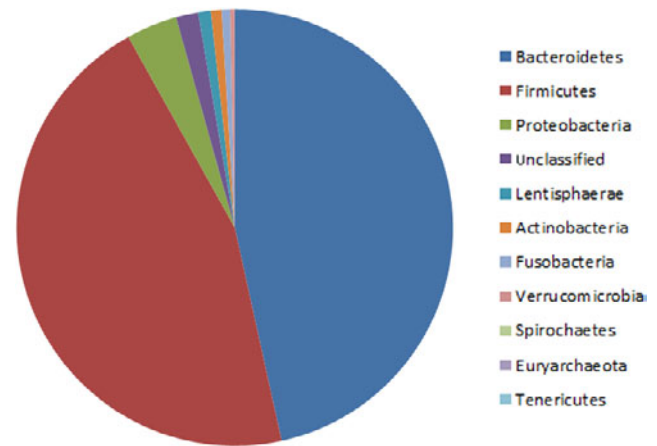
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mechanism that might be involved in multiple such conditions is the documented ability of bacteria to convert dietary ingredients into bioavailable and/or bioactive metabolites, whose lack, due to altered microbiota, leads to symptomatology. This paradigm can have more subtle long-term effects, including CVD, colorectal cancer and malnutrition. Expressed most simply, health studies restricted to diet and host alone have missed a major player in a holistic understanding of how diet affects health and disease. This review briefly summarises what we know about the composition and function of the gut microbiota, how it changes over the lifespan, how it relates to health and disease, and how realistic it might be to accomplish large-scale remodelling of the microbiota by dietary supplementation, in order to improve health.

### Gut microbiota and its development throughout life

The collection of bacteria in a particular environment or site is termed the microbiota; the related and often confused term 'microbiome' refers to the collection of genes or coding capacity that a particular microbiota harbours. As noted earlier, our knowledge of the human microbiome and gut microbiome has expanded dramatically in the past decade because of culture-independent methodologies, whose main features and appropriateness for different research questions were recently reviewed<sup>(2)</sup>. Although it is well established that the mucosal and faecal microbiota differ in composition<sup>(3–5)</sup>, most of our knowledge of gut microbiota is actually derived from studying faecal samples, for pragmatic reasons: colonoscopic biopsy sampling of healthy individuals is not ethical, whereas faecal samples are readily available. Estimates of the complexity of the faecal microbiota vary somewhat depending on the analytical method used, and the bioinformatic processing of the data, but the community is dominated by ten major divisions or phyla<sup>(6)</sup>, among which phylum *Bacteroidetes* and phylum *Firmicutes* together often constitute more than 90% of the total population<sup>(7,8)</sup>. The other major phyla are *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia* and *Lentisphaera*<sup>(6–9)</sup> (see Fig. 1). Two archaeal species, *Methanobrevibacter smithii*, predominantly, and *Methanosphaera stadtmanae*, are present in proportions not usually exceeding 1% in total<sup>(10)</sup>. Development of lower error amplicon sequencing strategies for ribosomal RNA genes has recently estimated that there are about 195 bacterial strains in the faecal gut microbiota, representing about 101 species<sup>(11)</sup>. It has been proposed that the gut microbiota of an individual can be assigned to one of the three so-called enterotypes, *Bacteroides*, *Prevotella* or *Ruminococcus*, depending on the abundance of these microbial genera<sup>(12)</sup>. There is some evidence that rather than a single dominating microbial type, microbiota datasets from large cohorts form gradients rather than discrete clusters<sup>(13,14)</sup>.

Initial studies of the faecal microbiota focused primarily on healthy adults and suggested that the dominant organisms were stably maintained in individuals<sup>(15)</sup>, an observation that has recently been confirmed<sup>(11)</sup>.



**Fig. 1.** (colour online) Phylum-level composition of the human adult faecal microbiota, derived from aggregated data of sixteen healthy controls. Phylogenetic assignments were made using the Ribosomal Database Project classifier as previously described<sup>(9)</sup>.

This stability only occurred if the subjects were not receiving antibiotic treatment, and independent studies have amply demonstrated the significant alteration to microbiota composition caused by antibiotic therapy<sup>(16–18)</sup>. Although the faecal microbiota composition is stable in healthy adults, the two extremes of life, infancy and old age, are characterised by a gut microbiota in flux (reviewed also in<sup>(19)</sup>). Infants are born essentially devoid of microbes, but are colonised by a series of displacements and successions for the first 2 years of life, until an adult-like microbiota has formed<sup>(20–23)</sup>. A distinguishing feature in the infant gut microbiota is the predominance of phylum *Actinobacteria*, specifically *Bifidobacterium* spp.<sup>(24)</sup>. However, the neonatal gut microbiota is very influenced by the mode of delivery, whereby infants delivered by a caesarean section initially have a gut microbiota more similar to the maternal skin and physical surroundings than a vaginal microbiota<sup>(25)</sup>. There is considerable interest in the connection between the composition of the infant gut microbiota and late-life risk for asthma, allergy and autoimmune-type diseases (reviewed in<sup>(26)</sup>), particularly because of the soaring rate of births by caesarean section in many countries<sup>(27)</sup>. Also of particular interest is feeding modality, because human milk oligosaccharides are optimally degraded and metabolised by particular bifidobacteria present in greater levels in healthy vaginally delivered infants<sup>(28)</sup>, supporting the link between breast-feeding and particular bifidobacterial species and strains.

In old age, a process occurs that superficially resembles a mirror image of neonatal gut colonisation. Culture-dependent studies had suggested that the faecal microbiota of older subjects was different from that of younger adults (summarised in<sup>(29)</sup>) but unifying trends in different studies, in different countries, were not identified<sup>(30)</sup>. Although the proportion of *Bacteroidetes* appeared elevated in some studies<sup>(31)</sup>, it was reduced, and *Bacteroides* species diversity was reduced, in others<sup>(32)</sup>. Advancing age in human subjects is often accompanied by frailty, but also by persistent activation of the innate immune



system, the so-called inflammaging, that may contribute to frailty<sup>(33)</sup>. It was therefore interesting that altered microbiota was reported in frail older subjects<sup>(34)</sup>, although the number of subjects was relatively small. Proportions of lactobacilli were significantly depleted (twenty-sixfold), while the abundance was also reduced for the *Bacteroides/Prevotella* group (threefold) and the *Faecalibacterium prausnitzii* group (fourfold)<sup>(34)</sup>. Testing the hypothesis that altered gut microbiota in the elderly might be associated with health loss including frailty and inflammation was one of the aims of the ELDERMET project in Cork, Ireland (see later). Other groups using other culture-independent methods have also identified differences between the gut microbiota of younger *v.* older subjects. Rajilic-Stojanovic *et al.* reported increases in proportions of *Actinobacteria* (*Actinomycetes* and *Atopobium*), streptococci and lactobacilli in ten older subjects compared with ten younger, and reductions in proteobacteria and *Bacteroides*<sup>(6)</sup>. The same phylogenetic microarray was used by Biagi *et al.* to study microbiota changes in older subjects and centenarians<sup>(35)</sup>. In the cluster containing the highest number of centenarians, the microbiota was characterised by higher proportions of Proteobacteria and Bacilli, and decreased levels of Clostridium cluster XIVa bacteria. Decreased *Bacteroides* abundance was not associated with ageing, and centenarians tended to harbour Archaea more frequently than the elderly or young adults<sup>(35)</sup>.

#### Gut microbiota alterations in disease

One of the drivers for research into gut microbiota is the fact that alterations in the normal composition or function have been linked to a wide range of diseases (reviewed in<sup>(36,37)</sup>). It is beyond the scope of this review to describe in detail the evidence for these linkages. The diseases with the strongest evidence for microbiota linkages include obesity (see later), inflammatory bowel disease (IBD), type-2 diabetes, disease-related malnutrition, and there is increasing evidence for a colorectal cancer linkage. IBD can be described as an abnormal immune response to normal gut bacteria, in which pre-disposing genetic factors combine with environmental factors<sup>(38,39)</sup>. The microbiota is different in IBD patients compared with controls<sup>(40,41)</sup>, and loss of *F. prausnitzii*, which ameliorates symptoms in animal models of colitis<sup>(42)</sup>, is a feature of the altered microbiota of some IBD patients. Microbiota alterations have been reported in type-2 diabetes<sup>(43,44)</sup>, and although mechanistic links have yet to be determined, it is relevant that faecal microbiota transplantation has proven effective for treating glucose insensitivity in patients with metabolic syndrome<sup>(45)</sup>. Gordon and co-workers reported an intriguing synergy between a particular microbiota (associated with the severe malnutritional wasting disease Kwashiorkor) and environmental factors (nutrition and disease burden) to produce full symptoms of the disease<sup>(46)</sup>. Colon cancer is the number two ranking cancer diagnosis globally<sup>(47)</sup>, and there is an emerging literature linking the gut microbiota as a risk factor for colorectal cancer, particularly *Fusobacterium nucleatum*<sup>(48–52)</sup>. For this and

most of the other disease linkages, the precise microbial species and mechanisms are unknown. Once they are identified, the potential for nutrition to modulate lifetime risk for these diseases will be an exciting research endeavour.

#### The interaction of gut microbes with nutrition

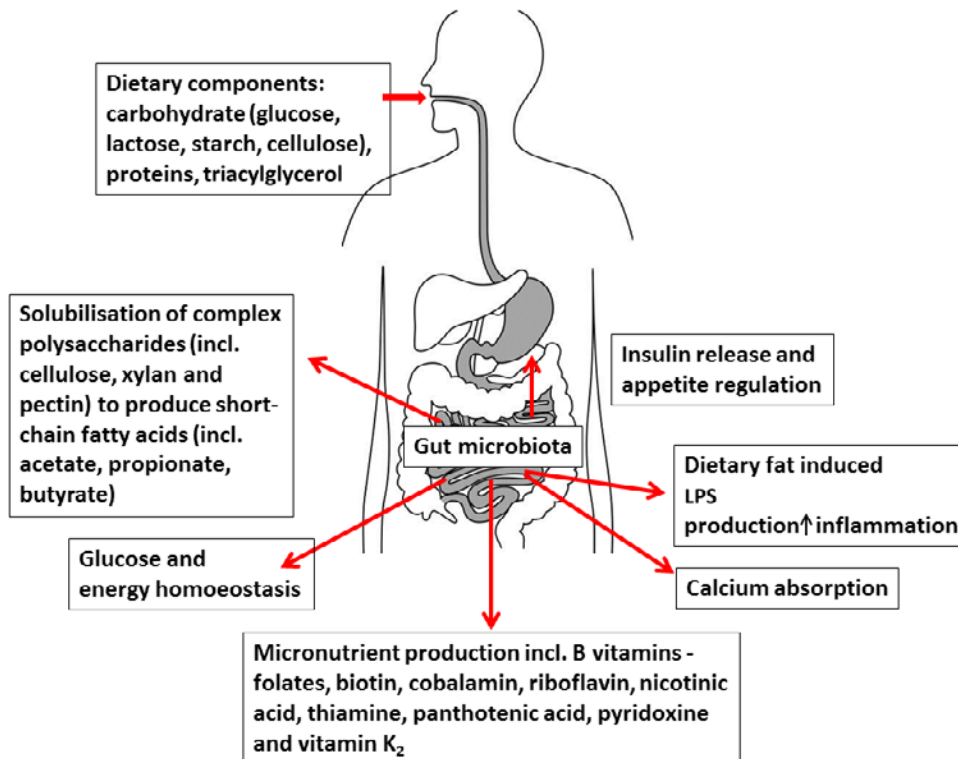
The gut microbiota influences a number of nutrition-related functions, from digestion and energy provision, fermentation, lipid storage, to the production of specific micronutrients (Fig. 2)<sup>(53)</sup>. The preponderance of research focus has concentrated on dietary carbohydrate and lipid metabolism and their direct effects on the gut microbiota, but the gut microbiota also contributes to total host micronutrient production and status including a number of essential B vitamins (including folate, riboflavin, vitamin B<sub>12</sub>, niacin and pyridoxine<sup>(54)</sup>) and vitamin K<sup>(55,56)</sup>. In addition, other activities of the gut microbiota may have indirect diet-dependent interactions. For example, recent evidence suggests that differences in host genotype that affect the mucus carbohydrate composition of the distal gut interact with diet to alter the composition and function of resident microbes in a diet-dependent manner<sup>(57)</sup>.

The gut microbiota and its metabolic activities respond to and alter according to habitual diet; this has been shown across a number of population subgroups, including the elderly. Age-related dietary changes can occur for several reasons, including loss of oral mechanical function, taste and appetite. These changes may result in an increased consumption of low-nutrient dense/high-fat/sugar foods and a reduction in dietary intakes of plant-based foods. The ELDERMET study recently reported different intestinal microbial populations among those aged over 65 years who consume a diet enriched in animal products and food with high glycaemic index. These individuals contained a greater abundance of *Bacteroides* and *Alistipes* species in their microbiota while those consuming a healthier diet (high in plant-based foods) harboured an abundance of bacteria from the genus *Prevotella*<sup>(58)</sup>.

These findings are in agreement with other findings, albeit in different population groups<sup>(59,60)</sup>. In the ELDERMET cohort, change of living environment from community to long-term residential care was associated with a dramatic change in dietary quality and diversity (indicated by a low Healthy Food Diversity index) which correlated with a subsequent reduction in gut microbiota diversity. In addition, those with poorer dietary and microbiota diversity had a concomitant decrease in functional independence (i.e. they were more frail), they had increased levels of serum cytokine markers of inflammation, lowered concentrations of SCFA in their faeces, as well as negative correlations with other clinical parameters<sup>(58)</sup>.

#### Gut microbiota and lipid storage/metabolism

The gut microbiota is thought to influence lipid storage by affecting nutrient acquisition and energy regulation



**Fig. 2.** (colour online) Interaction between diet, gut microbiota and nutritional status (taken with permission from<sup>(63)</sup>). LPS, lipopolysaccharide.

of the host, thereby playing a role in the development of obesity, insulin resistance and diabetes (for a detailed review, see<sup>(61)</sup>). As a product of fermentation, some gut microbes produce SCFA, which are important energy sources for the host. SCFA have been shown to regulate glucose homeostasis by regulating the secretion of a number of gastrointestinal hormones including glucose-dependent insulintropic polypeptide, glucagon-like peptide 1 (an incretin hormone, responsible for increased insulin secretion)<sup>(62)</sup> and glucagon-like peptide 2, and peptide YY<sup>(63)</sup>. However, the molecular pathways underpinning these beneficial effects of SCFA are largely unknown. In addition, free fatty-acid receptors, located on the colonic enteroendocrine L-cells known as free fatty-acid receptor 2 and free fatty-acid receptor 3 (also known as G-protein-coupled receptor (GPR)43 and GPR41), are responsible for the release of glucagon-like peptide 1 among other anorectic hormones<sup>(64)</sup>. In a recent study, Kimura *et al.*<sup>(65)</sup> showed that the SCFA receptor GPR43 links the metabolic activity of the gut microbiota with host body energy homeostasis. GPR43-deficient mice were obese on a normal diet, whereas mice overexpressing GPR43 (and specifically in adipose tissue) remained lean even when fed a high-fat diet. A normal phenotype prevailed in those raised under germ-free conditions or after antibiotic treatment; however, SCFA-mediated activation of GPR43-suppressed insulin signalling in adipocytes, which inhibited lipid accumulation in adipose tissue and promoted the metabolism of unincorporated lipids and glucose in other

tissues. These findings establish GPR43 as a sensor for excessive dietary energy, thereby controlling body energy utilisation while maintaining metabolic homeostasis.

There are three main theories as to how the microbiota might be a determining factor for obesity: energy harvest, signalling and inflammation–adipogenesis. The earliest propounded of these theories was that the obesogenic microbiota was characterised by an altered *Firmicutes*: *Bacteroidetes* ratio. In fact, the contribution of various bacterial groups to the development of obesity in human subjects remains controversial with conflicting evidence regarding the representation of *Bacteroidetes* in obese and non-obese individuals. A study conducted with obese and non-obese adults on low-energy diets revealed that the relative proportion of *Bacteroidetes* was lower in obese individuals compared with lean subjects<sup>(66)</sup>. However, a subsequent study by Duncan *et al.*<sup>(67)</sup> detected no difference in the proportion of *Bacteroidetes* measured in faecal samples from obese and lean individuals on weight-loss diets but did note a reduction in butyrate producing *Firmicutes* in obese subjects. In contrast, Schwartz *et al.*<sup>(68)</sup> found that the ratio of *Firmicutes* to *Bacteroidetes* changed in favour of the *Bacteroidetes* in overweight and obese subjects while recently Simoes *et al.*<sup>(69)</sup> showed that intakes of energy, MUFA, *n*-3 PUFA, *n*-6 PUFA and soluble fibre had significant associations with the stool bacterial numbers. In particular, increased energy intake was associated with reduced faecal proportions of *Bacteroides* spp. in monozygous twins. Finally, a recent interrogation of data from the Human Microbiome Project

and MetaHIT (an eight-country cooperation between Europe and China investigating the role of the metagenome in nutritional status and chronic disease risk) from healthy and obese individuals confirmed differences in phylum-level taxonomic composition variability between lean and obese subjects but no association was found between BMI and taxonomic composition<sup>(70)</sup>. The authors concluded that the effect of the microbiome on obesity may not, in fact be mediated through its taxonomic composition but rather its function, because a discrepancy can exist between closely and distant-related taxa, at a functional level. It is also important to note that the gut microbiota is potentially variable in composition and function and modifiable, depending on diet at the time of analysis; therefore, transient changes in diet affect the gut microbiota; however, longer term effects (of dietary modulation on the gut microbiota) are largely unknown. A 10-d dietary intervention caused alteration in the superficial configuration of the microbiota but not in enterotypes<sup>(60)</sup>, and short-term switch to solely plant or animal diets caused similar general trends in *Bacteroidetes* and *Firmicute* abundance<sup>(71)</sup>.

While the role of the intestinal microbiota in the aetiology of obesity has been elegantly demonstrated in animal models, human studies as yet have failed to concomitantly support the notion of significant phylum-level differences between obese and non-obese individuals and conclude that microbiota variation alone is not likely to cause obesity but overeating and disproportionate consumption of certain food items has the potential to alter the microbiota to one associated with increased energy harvest and reduced diversity. Although distinct taxonomic profiles have been established for lean and obese human in some populations, the relative contributions of the microbiota and diet to lean and obese phenotypes are most likely multi-faceted and regulated by a number of processes<sup>(72,73)</sup>.

It is important to note that the comparison of microbial communities between seemingly disparate phenotype groups merely provides a descriptive platform and cannot, at this point, infer causality. Notwithstanding the primary influencing factors in relation to obesity (diet, lifestyle and genetics), human metagenomic biomarkers have shown much better discriminatory potential between lean and obese individuals compared to human genome markers, which only show modest variability<sup>(74)</sup>. In fact, the specific metagenomic species have been identified which are associated with lower weight gain<sup>(75)</sup>. Furthermore, individuals with low bacterial gene counts are associated with reduced bacterial diversity, and increased inflammation and disease incidence (including type-2 diabetes and IBD)<sup>(76)</sup>. Therefore, low bacterial gene richness may determine the efficacy of dietary intervention in altering clinical phenotypes.

### Gut microbiota and obesity/energy harvest

Evidence from animal models suggests that the microbiota is an important environmental factor effecting energy harvest and storage. The aetiology of

microbiota-induced obesity was established by Backhed *et al.*<sup>(77)</sup> who found that inoculation of germ-free mice with conventional-raised mouse microbiota resulted in insulin resistance and increased body fat despite reduced food intake; this appeared to occur by promoting monosaccharide absorption and inducing *de novo* hepatic lipogenesis. Turnbaugh *et al.*<sup>(78)</sup> confirmed this obesity-induced effect of transplantation of obese microbiota in germ-free animals and concluded that the microbiota is a significant contributing factor in the pathophysiology of obesity. However, a later study investigating the influence of different diets (low-fat, high-fat and Western diets) on body composition in germ-free and conventional mice concluded that the absence of gut microbiota does not provide a general protection from diet-induced obesity and that diet composition affects gut microbial composition to a larger extent than previously thought<sup>(79)</sup>.

Furthermore, evidence that diet plays a pivotal role in the maintenance of a lean-type microbiota has previously been established. Ridaur *et al.*<sup>(80)</sup> showed that the microbiota of obese mice could be transformed to that of lean counterparts by cohousing, resulting in phenotypic rescue. Transformation was primarily associated with invasion by members of the *Bacteroides* species; a decreased abundance of which has been associated with increased energy harvest in human subjects<sup>(73)</sup> and obesity<sup>(72,81,82)</sup>. However, only a diet low in saturated fat and high in fruit and vegetables was responsible for the maintenance of the lean phenotype, whereas those consuming a diet high in saturated fat and low in fruit and vegetables did not maintain the lean phenotype<sup>(80)</sup>. Fleissner *et al.*<sup>(79)</sup> also demonstrated alteration in the faecal microbiota composition of conventional mice fed high-fat and Western diets, with the proportion of *Firmicutes* increasing at the expense of the *Bacteroidetes* with both diets. This infers a diet-dependent effect of successful microbiota transformation and establishes the transmissible and modifiable interactions between diet and intestinal microbiota and their influence on the host biology.

### Gut microbiota, inflammation and adipogenesis

Low-grade, systemic inflammation, initiated by high-fat feeding has been shown to alter the gut microbiota. The first animal study to investigate the effect of high-fat feeding on systemic endotoxemia was conducted by Cani *et al.*<sup>(83)</sup> and identified plasma lipopolysaccharide (LPS) as a major causative factor of systemic inflammation. In addition, this diet-induced increase in LPS appeared to increase the proportion of LPS-containing bacterial species in the gut. These findings suggest that the bacterial LPS production could trigger metabolic diseases characterised by insulin resistance and chronic low-grade inflammation, including obesity and diabetes.

Endotoxemia, namely activation of host-derived inflammatory mediators that induce systemic low-grade inflammation, occurs in obesity, the source and mechanism of which remains controversial. The gut microbiota has been proposed as a source of endotoxin or LPS



which by virtue of being a major component of the Gram-negative bacterial cell wall could potentially influence intestinal permeability with changes in gut bacterial composition<sup>(84)</sup>. In addition, obese or diabetic subjects, or individuals consuming a high-fat diet, have double the circulating levels of serum LPS due to decreased alkaline phosphatase activity (an enzyme involved in intestinal LPS cleavage), increased chylomicron formation and decreased gut barrier integrity<sup>(85)</sup>. Such changes are thought to activate the endocannabinoid system, which consists of bioactive lipids that bind to cannabinoid receptors and promote cell signalling (and are widely expressed in tissues that control the energy balance, i.e. pancreas, muscle, gut, adipose tissue, liver and hypothalamus)<sup>(86)</sup>. This system is thought to regulate gut barrier function and energy homeostasis<sup>(87)</sup> and is particularly sensitive to dietary fat levels; dysregulation is thought to promote adipogenesis and obesity in pathological situations<sup>(88,89)</sup> and may be directly associated with specific changes in the composition of the gut microbiota<sup>(87,90)</sup>.

### Prebiotic interventions in older people

Functional foods such as probiotics and prebiotics are beneficial to the host by promoting health and preventing disease<sup>(91)</sup>. Probiotics are live microorganisms while prebiotics are non-digestible food ingredients which selectively stimulate the growth and/or activity of one or a limited number of bacteria in the colon<sup>(92)</sup>. Prebiotics, given their non-viable nature, offer some technological advantages and may be simpler to exploit than 'living' probiotics, allowing them to be readily incorporated into drinks, confectionary and a range of food products where they are also used to replace sugar, and improve texture and palatability<sup>(93)</sup>. Their inclusion can also improve the nutritional status of a product, e.g. the inclusion of inulin in the manufacturing of ice creams and spreads results in a reduced fat content in the finished product<sup>(92)</sup>. These factors coupled with their health benefits make prebiotics ideal components to incorporate into diets of target populations, from infants to the elderly.

There has been a significant increase in the annual publication rate of studies describing prebiotics and their potential to improve health over the last 20 years, investigating the effects on inflammation, immune system, infections, blood lipids and mineral absorption<sup>(94,95)</sup>. Many prebiotics have been investigated because of their ability to cause a bifidogenic effect in human subjects<sup>(96)</sup>. Fermentation of prebiotics by some microorganisms that are considered beneficial, such as bifidobacteria and lactobacilli, indirectly leads to the production of SCFA by other gut bacteria; these SCFA have been shown to have both immunomodulatory and anti-inflammatory effects<sup>(97)</sup>.

Most prebiotic studies in human subjects have focused on oligosaccharides and inulin, and so the effects reported in elderly subjects are generally concerned with these compounds. Inulin supplementation in a

study of constipated elderly subjects led to an increase in faecal *Bifidobacterium* proportions, and also resulted in a laxative effect when compared to lactose<sup>(98)</sup>. A study in France<sup>(99)</sup> observed increased bifidobacteria and cholesterol excretion (in the stool) in an elderly population receiving fructo-oligosaccharide; baseline levels returned 4 weeks after the wash-out period. Another study with fructo-oligosaccharide ingestion in elderly nursing home patients confirmed its bifidogenic effect and also reported a decrease in IL-6 mRNA in peripheral blood monocytes, indicating its potential to influence the immune system<sup>(100)</sup>. Effects in elderly subjects receiving a galacto-oligosaccharide mixture showed an increase in bifidobacteria levels, a decrease in pro-inflammatory cytokine levels (IL-1b, IL-6 and TNF $\alpha$ ) and an increase in IL-10, an anti-inflammatory cytokine<sup>(101)</sup>. Some studies have indicated that prebiotics such as fructo-oligosaccharide may also influence the antibody response in healthy elderly subjects vaccinated against influenza, but results are conflicting at present<sup>(94)</sup>.

The potential positive effects of these prebiotics have also been demonstrated in particular study populations. For example, galacto-oligosaccharide has been shown to relieve symptoms of people suffering from irritable bowel syndrome,<sup>(102)</sup> while also positively influencing the microbiota and immune function in overweight adults<sup>(103)</sup>.

Dietary supplementation with wheat dextrin in critically ill jejunal-fed patients (including some elderly subjects) was shown to modulate the microbiota and increase SCFA even in the presence of antibiotics and other medications<sup>(104)</sup>. Although further studies need to be carried out, this offers huge potential in reducing the risk of diarrhoea and counteracting the effects of antibiotics.

There are a number of other potential prebiotic compounds that have recently been studied in non-elderly subjects which may have potential in the older age group. Novel fibres such as polydextrose and soluble maize fibre can modulate the gut microbiota with increases in the populations of *Faecalibacteria* for both fibres, and with an increase in *Lactobacilli* in human subjects who consumed soluble maize fibre<sup>(105)</sup>. Animal studies have identified candidates for further investigation, e.g. arabinoxylan that was shown to increase proportions of *Bifidobacteria*, *Bacteroides-Prevotella* and *Roseburia* in obese mice on a high-fat diet, thus conferring both anti-obesity and health benefits<sup>(106)</sup>. Resistant starch has been shown to modulate the microbiota in human subjects (including some elderly subjects)<sup>(107)</sup> and in pigs, with an increase in faecal SCFA levels also being observed in the latter<sup>(108)</sup>. Potential anti-cancer effects have been reported in a number of animal studies including rats with colorectal cancer which received a resistant starch type 3 (Novelose 330)<sup>(109)</sup>. While trials using animal models are an acceptable first step towards identifying dietary candidates for further investigation, it must be acknowledged that this may not necessarily translate in a (young or old) human situation<sup>(93)</sup>. The above ingredients all require further investigation to confirm their beneficial effects and suitability in an elderly population.

Furthermore, the extensive inter-individual variation in gut microbiota composition which exists between similar population sub-groups<sup>(40,70)</sup>, including the elderly<sup>(9,58)</sup> suggests that the response to prebiotic supplementation may be equally varied. The initial gut microbiota composition is evidently important as certain dominant bacterial groups are known to be more nutritionally versatile<sup>(110)</sup> and individuals who harbour such bacteria have previously been reported as 'non-responders'<sup>(96,111)</sup>. Therefore, studies to establish whether a reduced diversity microbiota can be rescued by dietary intervention and thus impact health are warranted. In addition, because individual prebiotics promote various specific individual bacterial species, the effect of combined prebiotic intervention on 'health-promoting' *plyla* are required. Furthermore, the timeframe required to modulate key health-related bacterial species, at an individual level, is unknown and unlikely to be uniform.

### Knowledge gaps/challenges for dietary modulation of the microbiota in older people

Although several studies<sup>(30,58)</sup> have examined the gut microbiota in elderly populations, defining it is still a challenge given the difficulty in comparing individual studies, country effect, inter-individuality and the variation in age of which the elderly study population is set<sup>(112)</sup>. The influence of ageing on bacterial populations, e.g. *Bacteroides* is difficult to determine given the effect of other factors such as antibiotics treatment and hospitalisation which need to be considered<sup>(113)</sup>. In fact, disruption of the gut microbiota with oral antibiotics often precedes the emergence of several enteric pathogens. Recent evidence suggests that gut mucosal carbohydrates (e.g. fructose and sialic acid) can be released and catabolised by the resident microbiota in the presence of antibiotic-associated pathogens (including *Salmonella* and *Clostridium difficile*) and can influence their competitiveness and expansion within the gut<sup>(114)</sup>. These findings may have implications for designing new therapies for the prevention of antibiotic-associated pathogenic infection.

Understanding the relationship between ageing and gut microbiota will require longitudinal, multicentre studies with larger populations using standardised procedures and methods of analysis to allow direct comparison, eliminate discrepancies or identify true variation. This is also the case for identifying future nutritional products which can promote healthy ageing. While a number of studies examining the effect of dietary interventions have been carried out in human subjects (with a limited number in elderly subjects), interpreting the outcome is difficult for a number of reasons including: use of a small study population, lack of a placebo, duration of the intervention and study design<sup>(115)</sup>. The dose-effect and establishing a minimum effective dose is difficult when both variation between studies and dose rates exist<sup>(116)</sup>. Inter-individual variability of a response can also occur, e.g. increased *Bifidobacterium* abundance and decreased *Bacteroides* abundance were not observed

following a galacto-oligosaccharide treatment which included elderly subjects<sup>(117)</sup>. The choice of a particular form of prebiotic could be crucial in certain instances if it is to be effective, e.g. the chain length of arabinoxylan determines the intestinal site of fermentation, and thus the influence on bacterial populations and resulting SCFA levels is modulated<sup>(118)</sup>. Resistant starch types 2 and 4 have different effects on microbiota composition because of their accessibility to the bacteria due to their chemical structures<sup>(119)</sup>. In addition, nutrient stores available to the gut microbiota that may influence the effects of dietary interventions designed to modulate the gut microbial profile warrant further investigation.

As prebiotics are classed as food substances rather than medications by the Food and Drugs Authority (USA), the requirements for manufacturers to conduct large-scale, placebo-controlled trials is small compared to clinical trials of a medicinal product where hundreds of subjects are recruited at a huge cost. However, it is important to note that if a functional claim is to be made for supposedly beneficial prebiotic foods/ingredients, they must demonstrate a positive health effect and not just alter the microbiota composition to be considered therapeutic<sup>(120)</sup>. To date the majority of research on functional foods has mainly focused on the probiotics *Bifidobacteria* and *Lactobacilli*<sup>(121)</sup> and prebiotics oligosaccharides and inulin<sup>(122)</sup>. However, considering our increasing knowledge of the gut microbiota (and the range of species present) other potential contenders such as *F. prausnitzii* (e.g. as a treatment for Crohn's disease<sup>(42)</sup>) are emerging. The development of these functional food ingredients is a personalised healthcare strategy where the effectiveness of the product is consumer dependent, e.g. a prebiotic that promotes a particular bacterium is useless unless the organism is present in the host. It is also important for elderly and unwell populations in particular that frailty, diseases and medications are considered to prevent any adverse effects, highlighting the need for well designed, intervention trials.

### Conclusions

While research indicates reduced intestinal microbiota diversity particularly with advancing years and with reduced dietary diversity, it is important that future research should focus not only on establishing the core gut microbiota in elderly subjects, but also on the impact of long-term dietary intervention on microbiota modification. While we await clarification from intervention studies as to whether a reduced diversity microbiota can be rescued and modified such that it can promote and support better health outcomes in elderly populations, further studies on the elderly gut metagenomic capacity and function are required to identify important bacterial species to target for health promotion. Our expanding knowledge of gut microbiota, its influence on health and ageing is of global importance given that 22% (2 billion) of the population will be aged 60 years and over by 2050 according to the WHO. The development of nutritional products (e.g. prebiotics) which can



improve overall health through influencing the gut microbiota and its effects such as disease prevention and improved immune function will lead to a reduction in hospitalisation, drug intake and financial savings in the healthcare sector. This is an exciting and challenging time for all involved; however, as research continues to examine the interaction between dietary ingredients, the microbiota and influence on health, the key elements leading to the successful development of such nutritional products lie in comprehensive research which is soundly designed and executed thereby removing any ambiguity in the interpretation of results.

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### Conflicts of Interest

None.

### Authorship

All authors contributed to the planning, scoping and writing of this review.

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