The gut microbiome as a modulator of healthy ageing

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Abstract | The gut microbiome is a contributory factor in ageing-related health loss and in several non-communicable diseases in all age groups. Some age-linked and disease-linked compositional and functional changes overlap, while others are distinct. In this Review, we explore targeted studies of the gut microbiome of older individuals and general cohort studies across geographically distinct populations. We also address the promise of the targeted restoration of microorganisms associated with healthier ageing.

Everyone ages but the deleterious effects of ageing on physical and intellectual function are not experienced uniformly. Delayed age-related decline, or 'healthy' ageing, is evident in many people. Determinants of healthy ageing include genetic, environmental and lifestyle factors, the latter presenting an opportunity for intervention. The microbiome transduces environmental signals, conditions host immune, metabolic and neurological function, and modifies the risk of disease, including age-related diseases. However, the microbiome has a reciprocal relationship with age: it changes as the host ages and is altered in age-related disease, but it also modifies age-related impairment of the host.

The importance of these relationships is underscored by changing global demographics. It is projected that, in the next 30 years, the number of people aged ≥65 years will more than double, reaching 1.5 billion globally, the majority of whom will be in less-developed countries[1]. Therefore, costs of health care will escalate enormously unless the determinants of healthy ageing are comprehensively addressed. While stressors, such as the disproportionate effects of COVID-19 on older people, reveal the fragility of healthy ageing, it is the tenuousness or lack of physiological reserve in older people that may enable minor alterations relative to a non-diseased state in the microbiome to have a significant beneficial or negative influence on physical and cognitive function. In this respect, ageing presents an opportunity, tantamount to a human model for calibrating microorganism–host interactions in health and disease.

We have previously addressed the elements of a healthy microbiome while highlighting that a single universal healthy microbiome configuration does not exist[2]. In this Review, we focus on the dynamic relationship between the gut microbiome and ageing, including age-related disease, and explore prospects for microbial manipulation to promote healthy ageing. Throughout this Review, we specifically concentrate on the gut microbiome but the concepts are likely to be applicable to microbial communities at other body sites.

The gut microbiome in health and disease

Animals harbour complex communities of gut microorganisms that show host specificity in their composition and function, and which co-evolved with their multicellular hosts[3], shaped by habitual diet. In the human gut, the resident community of microorganisms reputedly numbers approximately 2,000 bacterial species[4] and might outnumber the human cell count and number of different coding genes[5]. Interest in the human gut microbiome accelerated when culture-independent methods revealed that the microbiome was different in patients with a range of disorders compared with healthy individuals (reviewed in REF.[6] and summarized in TABLE 1). However, few mechanisms that link pathophysiology with specific microbial metabolites lost from the normal microbiome or gained by the disease-associated microbiome have been identified[7–10], a challenge exacerbated by the difficulty in defining a health-associated microbiome[2]. Despite these challenges, microbiome–health interactions can be assigned to a number of broad categories that are useful for considering (as we do later in this Review) how these relationships might go awry in ageing and what the effect on the host would be.

Metabolic effects. Microorganisms have developed a remarkably broad capacity to use inorganic and organic molecules as nutritional substrates; in the mammalian gut, they can catabolize plant-derived polysaccharides and resistant starches that would otherwise be unavailable to the host. Flint et al. have reviewed how changes in gut microbial metabolism of short-chain fatty acids (SCFAs), vitamins, lipids, gases, cholesterol and atherogenic compounds can alter host susceptibility to obesity and metabolic syndrome, irritable bowel syndrome,

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Key points

- The gut microbiome is a transducer of environmental signals, modifies the risk of disease across all age groups and changes with host age.
- Age-related alterations in the gut microbiome are influenced by personal factors, including progressive physiological deterioration, as well as by lifestyle-linked factors such as diet, medication and reduced social contact.
- Age-related and disease-related deterioration in the gut microbiome of older people reflect overlapping interactive but distinct processes.
- Resetting gut microbiome-derived signals of 'unhealthy' ageing through personalized or subpopulation-level microbiome-associated interventions is a new area of research informed by large shotgun metagenomics-based studies and data analytics.
- Gut microbiome-based therapeutics for older people will need combined approaches, including dietary intervention with microbial restoration of lost strains.

and cardiovascular disease[11]. Despite the scale and complexity, analytical advances have catalogued the most abundant microbial metabolites that affect the host (reviewed in REF.[12]) as well as the microbial enzymes involved. Key metabolites include SCFAs, amino acids, bile acids, vitamins, tryptamine, histamine, serotonin, dopamine, para-cresol and phenylacetylglutamine[12]. Thus, in addition to contributing to flux in host metabolic pathways, many microbial metabolites can affect the activity of the liver and the endocrine system[13]. The role of the microbiome in a multifactorial disorder such as metabolic disease is, of course, complex, and it is unclear whether the candidate mechanism and effector molecule (branched-chain amino acid production) is the sole microbial factor in type 2 diabetes mellitus[14,15].

Anti-inflammatory or immunomodulatory effects. Several microbial metabolites, including SCFAs, are anti-inflammatory[16] but other microbially derived macromolecules, such as exopolysaccharides, also downregulate gut inflammation[17,18]. Depletion of taxa that have anti-inflammatory properties, such as *Faecalibacterium prausnitzii*, was highlighted in early reports of gut microbiome alterations in patients with inflammatory bowel disease[19]. The anti-inflammatory effects of this anaerobe have been attributed to secreted peptides[20], salicylic acid[21] and even the bacterial quorum-sensing molecule *N*-acyl homoserine lactone[22].

Neurological effects. Gut bacteria have been proposed to affect behaviour, cognition and mood by three pathways: the enteric nervous system; uptake of microbial metabolites into the circulation; and by modulating immunoinflammatory responses in the central nervous system (reviewed in REF.[23]). This modulatory effect of the gut microbiome on the central nervous system has been exploited for the development of specific probiotics (also known as psychobiotics) that are reported to alleviate stress[24]. Whether manipulation of the microbiome in humans can favourably influence mental health and neurological disorders remains to be proven. However, this research is germane to ageing, because even in the absence of severe underlying pathology, as occurs in Alzheimer disease or Parkinson disease, cognitive impairment is very common.

Probiotics
Microbial species (or lineages) that have a beneficial effect on the host.

Psychobiotics
Subgroup of probiotic bacteria that have a beneficial impact on the gut–brain axis resulting in improved behaviour or cognitive function.

Age-related influences on the microbiome

Ageing is a progressive loss of homeostasis, impaired function and vulnerability to death. Age-related diseases include infectious, neoplastic, metabolic and degenerative disorders with frailty and cognitive decline. At a fundamental level, the molecular and cellular hallmarks of ageing in mammalians have been identified[25] but these are accompanied by changes in the microbiome, which in turn affect the rate of age-related decline (FIG. 1).

Age-related changes in the microbiome are highly variable, influenced by both personal and external environmental factors. For example, the gut microbiome is predictably affected by progressive deterioration in the physiology of the alimentary tract. These changes include increased ageing-associated inflammation, genomic instability, cellular (and mitochondrial) dysfunction, reduced proteostasis and epigenetic dysregulation, which further lead to the onset of chronic diseases, metabolic disorders and impaired gut–brain communication (reviewed in REFS[26,27]). The attendant effects on host behaviour and lifestyle (increased frailty, medication intake, surgery, reduced physical activity and quality of diet) can further exacerbate the effects on the gut microbiome. Lifelong personal lifestyles, particularly diet, also shape the composition and function of the microbiome in older people[28] but represent opportunities for healthy behaviour change. Less well explored is the influence of social interactions on the composition of the microbiota and how society cares for its older people (FIG. 2).

Increasing evidence suggests that transmission of microorganisms among individuals living in a social group has important health benefits[29]. While the spread of pathogens within social groups has received close scrutiny, strain tracking studies have shown that commensals and mutualistic microorganisms are also shared within social networks[30]. The microbiomes of individuals living in the same home tend to have compositional similarities, in comparison with those from people in other households[31,32]. Household pets can contribute to microbial sharing by acting as vectors of transmission[33]. Moreover, individuals with larger social networks seem to have more diverse gut microbiomes[34]. However, the collective microbial metacommunity of a family (the 'social microbiome') changes over time, with opportunities for microorganism acquisition diminishing, sometimes abruptly, for older people. Additional change depends on whether the individuals reside alone or in institutional care[28] (FIG. 2). Aloneness and loss of group living are among the social changes that have become characteristic of modern socioeconomically developed societies and contrast with the past and with traditional, non-industrialized ethnic groups such as the Hutterites and the Irish Travellers[29,30,35].

Gut microbiome changes with ageing

Microbiome studies focused on older people can be classified into two broad categories: reports of differences in gut microbiome composition related to age per se, and reports of alterations in the microbiome of older people that are associated with particular ageing-linked disorders (detailed in TABLES 2,3). The main findings

from the two categories of ageing-related studies, a literature-based identification of distinct groups of taxa that show consistent alterations with ageing as well as between healthy and unhealthy ageing, along with the metabolic capabilities of these taxon groups that can direct the host to either a healthy or an unhealthy ageing trajectory, are described in this section.

The first category of studies provides a picture of how the gut microbiome changes with age in general, notwithstanding the fact that these are not longitudinal studies. Particularly noteworthy are studies of centenarians across various geographies and nationalities[36–44]. In general, the composition of the gut microbiome of centenarians (studied in countries/regions including Italy, Russia, China and India) is distinguished by a lower abundance of symbionts associated with health in younger age groups (such as *Faecalibacterium* spp.) and an elevated abundance of both alternative health-associated taxa (such as *Akkermansia* spp.) as well as disease-associated pathobionts. Gut microbiota genes associated with xenobiotic degradation were noted to be in higher abundance in extreme ageing. Interest in these cohorts stems from the assumption that identifying gut microbiome signatures specific to long-living centenarians might be akin to identifying therapeutic microbiome signatures for longevity or healthy ageing. However, this assumption might not be correct because these are all cross-sectional single snapshots and extreme ageing is not equivalent to healthy ageing. In other words, all apparently healthy centenarians might not be equally healthy and some might have progressed into ageing-linked physiological decline (as summarized in FIG. 1).

Despite reservations about the generalizability of such findings in centenarians, there are striking similarities in the extreme ageing-linked microbiome signatures established across different studies. This is despite wide variation in the demographics of the study populations, ranging from a geographically isolated rural population in India to a wealthy semi-urbanized community in Italy[38,43]. We and others have also shown that these taxonomic changes overlap with a generic age-associated microbiome variation pattern[28,45,46] (FIG. 3). These general ageing-related changes are characterized by a loss of dominant commensal taxa (such as *Prevotella*, *Faecalibacterium*, *Eubacterium rectale*, *Lachnospira*, *Coprococcus* and the health-associated genus *Bifidobacterium*)[28,45,46]. These taxa appeared to be replaced by a second group of commensals (such as the putatively beneficial *Akkermansia*, Christensenellaceae, *Butyricimonas*, *Odoribacter* and *Butyricicoccus*) and pathobionts (such as *Eggerthella*, *Bilophila*, Fusobacteria, *Streptococcus* and Enterobacteriaceae)[45,46]. These microbiome alterations encompass those associated

Table 1 | **Representative studies linking human conditions to the microbiome**

Condition or disease	Microbiome alteration	Potential or known mechanism	Comments	Refs
Obesity	Greater abundance of pathobionts and Firmicutes	Calorie harvesting, inflammation, modulating satiety, regulating adipogenesis	Controversial microbial links to complex, that is, multifactorial, disease	157
Type 2 diabetes	As for obesity, with signals related to *Prevotella copri* and *Akkermansia muciniphila*	Unclear; liver signalling, branched-chain amino acids?	Initial success with faecal microbiota transplantation not maintained in later studies	158
Inflammatory bowel disease	Reduced abundance of Christensenellaceae, *Coriobacteriaceae*, *Faecalibacterium prausnitzii*; higher abundance of Actinomyces, *Veillonella*, *Escherichia coli*	Products of colonic inflammation stimulate anaerobic respiration, driving microbiome further towards a pro-inflammatory type	Meta-analysis concedes lack of a unifying taxon signature for inflammatory bowel disease; once inflammation is triggered, the microbiome may be irrelevant for treating inflammatory bowel disease	159,160
Irritable bowel syndrome	*Ruminococcus gnavus* and Lachnospiraceae are more abundant, *Barnesiella intestinihominis* and *Coprococcus catus* depleted	Pathophysiology may involve a reduction of luminal pH by excessive fermentation and sensitization of the enteric nervous system by inflammation	Not all patients with irritable bowel syndrome have an altered microbiome; disruption of the diet–microbiome–metabolome connectivity is a feature of those who do	161,162
Colorectal cancer	Presence of *Fusobacterium nucleatum* and other oral biofilm-forming pathobionts is a feature of tumour microbiome	Inflammation, DNA breakage, mutagenesis	Microbiome alterations linked to colon cancer relate to known risk factors such as diet and inflammation; microbiome also influences the responsiveness of cancers to checkpoint immunotherapy	10
Cardiovascular disease	Bacterial taxa capable of generating trimethylamine from carnitine, choline and glycine betaine	Trimethylamine is a substrate for liver production of trimethylamine oxide, an atherogenic metabolite	Initial controversy due to inverse relationship between choline intake and cardiovascular disease but prospects for druggable targets	7,9,163
Cognitive function, behaviour and mood	Diverse observations and metabolites reported but a catalogue of gene products with neuroactive potential identified	Effects on neurodevelopment, neuroplasticity, degree of myelination, peptide binding to immune cells and vagus nerve endings, other brain signalling effects	Plausible leads but a paucity of compelling human studies	8,164

These studies represent selected examples of conditions or diseases in which the causality of the microbiome as a contributing or mediating factor was demonstrated. The studies and reviews provided are those that describe the mechanism, and the bacteria specified are those that show constant association across studies.

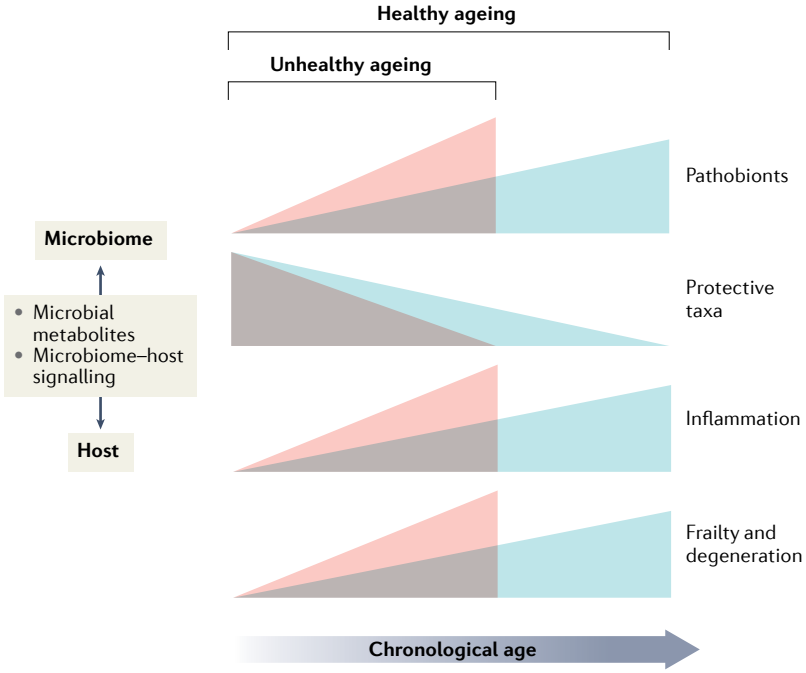

Fig. 1 | **Microorganism–host signalling as a contributor to healthy or unhealthy ageing.** Chronological age is accompanied by changes in host–microorganism homeostasis that determine, in part, the rate of physical and cognitive decline. Lifestyle and environmental effects on the microbiota can delay (healthy ageing) or accelerate (unhealthy ageing) deterioration in the host and foreshorten life expectancy.

with ageing in general as well as those associated with ageing-linked decline in health.

Identifying the microbiome elements associated with healthy and unhealthy ageing can be achieved by longitudinal studies tracking individuals over an extensive period of time and by relating the gut microbiome composition measured at intermediate time points to the final physiological or clinical status. A widely used alternative is to adopt a cross-sectional study design that stratifies older populations based on indices of unhealthy ageing as well as of healthy ageing and that identifies the corresponding taxonomic markers (TABLE 3). One of the primary ageing-linked physiological hallmarks is the onset of frailty. Using various measures of stratification, studies by our group on the ELDERMET cohort of 500 people in Ireland and studies by others in geographically distinct populations, have uncovered consistent frailty–microbiome associations[28,45–51]. Similarly, studies investigating microbiome alterations in other disorders associated with an unhealthy ageing trajectory, including reduced physical activity, cardiometabolic disorders, cognitive decline, migraine, reduced bone mass density, obesity, metabolic syndrome and related comorbidities, chronic kidney disease, and pre-mortality, have all been linked with microbiome changes akin to those broadly associated with frailty[52–64]. These are primarily characterized by loss of commensals that are reduced with ageing in general and a gain of pathobionts (such as *Eggerthella*, *Desulfovibrio*, Enterobacteriaceae family members, disease-associated *Clostridium* species, such as *Clostridium hathewayi*, *Clostridium clostridioforme*, *Clostridium symbiosum*, *Clostridium bolteae* and *Clostridium citroniae*, and *Ruminococcus torques*) (FIG. 3). By contrast, the third group of commensal microbial markers (such as *Akkermansia*, *Odoribacter*, *Butyricimonas*, *Butyrivibrio*, *Oscillospira*, Christensenellaceae and Barnesiellaceae) increase with ageing (in general ageing studies; TABLE 2) but decrease in some age-related disorders (or conditions associated with unhealthy ageing; TABLE 3). These taxa represent taxonomic 'milestones' that increase in abundance in a healthy ageing trajectory but are lost when there is a shift to a state of physiological decline.

Thus, based on a detailed literature survey of taxonomic alterations reported in these two kinds of studies, three kinds of taxonomic groups can be identified (FIG. 3; Supplementary information). Group 1 includes taxa that are lost with ageing and especially during unhealthy ageing. Group 2 consists of pathobionts that increase with ageing, especially in unhealthy ageing. Group 3 are healthy ageing-associated taxonomic checkpoints that become more abundant with age but are lost during unhealthy ageing.

Given the consistency of microbiome alterations across multiple studies of ageing-linked disorders, similar to what has been reported in meta-analyses performed across multiple cohorts[65,66], it is likely that overlapping or shared microbiome alterations are, in part, a consequence of general physiological decline, including inflammation and localized loss of the mucosal barrier. These findings have been summarized extensively by DeJong et al.[67]. In this context, a 2020 meta-analysis of >2,500 metagenomic datasets from individuals between 20 and 89 years of age, including some with major diseases, found shared and disease-specific microbiome alterations in addition to substantial variations according to chronological age[46]. Six groups of microbial taxa were identified based on their disease-linked abundance alterations in different age categories. These groups were referred to as groups G1 (disease-elevated across all age groups), G2 (elevated in multiple diseases only in older individuals aged ≥60 years), G3 (elevated across multiple diseases only in young or middle-aged people aged between 20 and 60 years), L1 (depleted in multiple diseases across all age groups), L2 (depleted in multiple diseases only in older individuals aged ≥60 years) and L3 (depleted in multiple diseases only in young or middle-aged people aged between 20 and 60 years). Notably, a subset of these taxa that were enriched in multiple diseases across all ages (the G1 group) was also associated with increased frailty in individuals in the ELDERMET cohort. These frailty marker taxa (all belonging to the unhealthy-associated pathobiont group; FIG. 3) were also linked with detrimental metabolic functionalities such as the production of hydrophobic bile acids, ethanol, trimethylamine and para-cresol (FIG. 4). Thus, although it is difficult to distinguish between causal and consequential microbiome changes, there are 'usual suspects' with functionalities that might contribute to a vicious cycle of progressive decline in health. Only a small proportion of the taxa-to-metabolic associations in our study could be verified by interrogating faecal and/or serum metabolomic data. However, as discussed later, these findings have been corroborated in several studies.

TABLE 4 presents other examples of microbial metabolites and macromolecules that have demonstrated activities upon host metabolism, inflammation and disease risk. Urolithins are an interesting class of gut microbiome-derived compounds that have been hypothesized to be potentially distinguishing markers between healthy young and aged (especially unhealthily aged) individuals[68]. Urolithins are produced by gut microorganisms from dietary plant components, especially ellagic acid. Previous studies have shown that individuals can be divided into three groups, known as urolithin metabotypes (UM), based on their urolithin patterns: UM-A, UM-B and UM-O[69]. Although the prevalence of the health-associated UM-A metabotype was reported to decrease with age, UM-B, which is associated with colorectal cancer and metabolic syndrome, showed a progressive increase in prevalence with age. Notably, UM-B was characterized by increased levels of iso-urolithin A, whose production has been attributed to an unknown member of the pathobiont Eggerthellaceae family[70].

Cytolethal-distending toxin-producing *Campylobacter* strains, colibactin-producing *Escherichia coli* strains and *Fusobacterium* are other Group 2 pathobiont lineages (observed to be increased with age) that can induce colorectal carcinogenesis either by DNA-damaging toxins or hyper-inflammatory cell surface proteins, as shown primarily in cell and organoid systems and animal models[71–73]. Pathobiont-mediated physiological decline in ageing might be driven not only by the production of detrimental metabolites but also by the consumption of beneficial metabolites. For example, *Desulfovibrio* can dissimilate SCFAs such as butyrate[74], a gut microbiome-derived metabolite that has been shown to have a multitude of properties to prevent ageing-linked physiological decline[75].

In apparently healthy older people, the loss of prominent butyrate producers belonging to Group 1, such as *Faecalibacterium*, *Roseburia*, *Coprococcus* and *Eubacterium* spp. (especially *E. rectale*), typically associated with health in younger individuals, is less severe. The benefits of prolonged retention of a 'youth-like' microbiota have been further shown in a large-scale mouse study where faecal microbiota transplantation (FMT) from young mice reversed ageing-linked deterioration in peripheral and brain immunity and attenuated selected age-associated impairments in cognitive behaviour in aged mice[76]. Furthermore, studies on humans have observed that, in apparently healthy older people, the loss of Group 1 members is compensated for by the gain of alternate butyrate-producing Group 3 taxa such as *Odoribacter*, *Butyricimonas*, *Butyrivibrio* and *Oscillospira* (TABLES 2,3). Butyrate is a potentially pivotal inhibitor of unhealthy ageing by virtue of multiple properties that delay the ageing host from shifting towards physiological decline. These properties include preventing inflammation (by multiple means, including acting as an energy source for colonocytes, improving barrier function and downregulating endocannabinoid-regulated adipogenesis), insulin

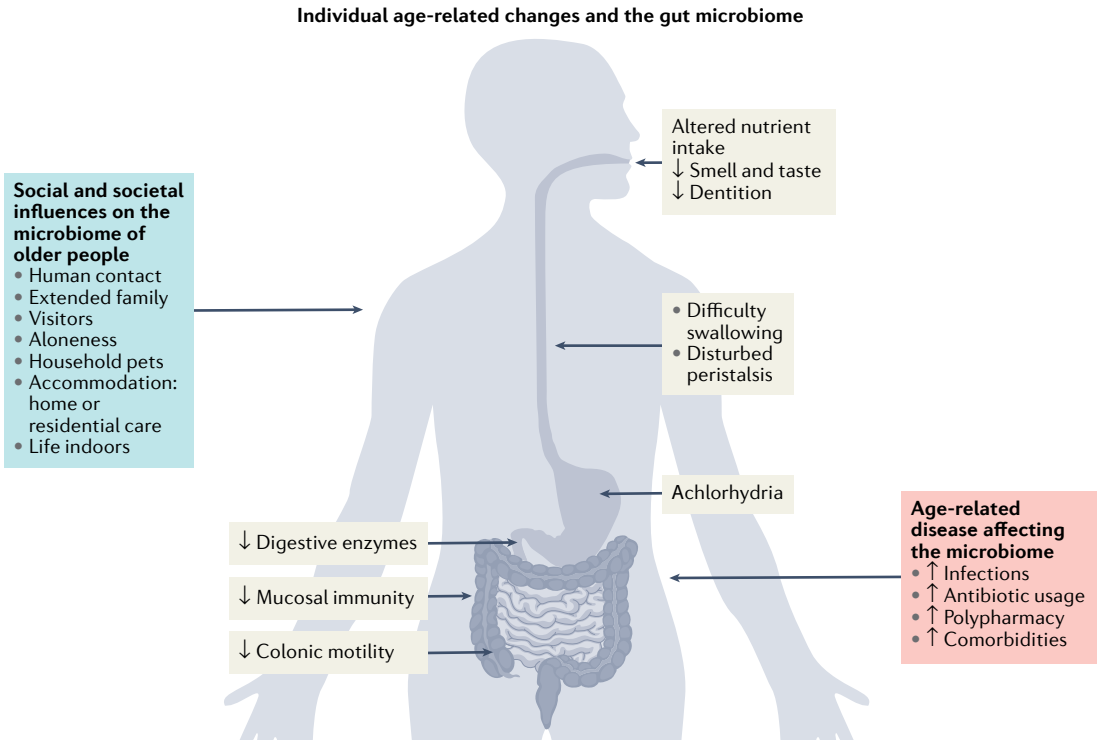

Fig. 2 | **Physiological, social and disease-related influences on the microbiome of older people.** Progressive decline in physiological function along the alimentary tract changes the internal microenvironment in all individuals to a variable degree and indirectly affects nutrient intake by older people. Additional variables that shape the microbiome of older people include lifelong lifestyle choices, contact with the external microenvironment, social networks (the social microbiome), and the reciprocal influences of age-related diseases and their treatment.

Table 2 | **Studies investigating gut microbiome alterations in older people**

Study (cohort size[a])	Country/ region	Molecular technique	Main microbiome alterations in older people
Ruiz-Ruiz et al.[165] (10)	Spain	Proteomics	Tryptophan and indole decreased
Iwauchi et al.[166] (29)	Japan	16S	Pathobionts, *Akkermansia*, Christensenellaceae, *Butyricimonas* increased
Xu et al.[36] (115); Odamaki et al.[37] (77)	Japan[b]	16S	Pathobionts, *Odoribacter*, *Butyricimonas*, Christensenellaceae, *Lactobacillus*, *Oscillospira*, *Oxalobacter*, *Butyrivibrio* increased; core SCFA producers and *Bifidobacterium* decreased
Biagi et al.[38] (54)	Italy[b]	16S	Pathobionts, *Akkermansia*, *Odoribacter*, *Oscillospira*, *Butyricimonas*, Christensenellaceae, Mogibacteriaceae increased; core SCFA producers decreased
Wu et al.[40] (42)	Italy[b]	16S	Pathobionts, *Methanobrevibacter smithii* increased; core SCFA producers decreased
Rampelli et al.[39] (51)	Italy[b]	Shotgun	Core SCFA producers decreased; pathobionts, *Methanobrevibacter smithii*, *Akkermansia muciniphila*, xenobiotic degradation, LPS biosynthesis, phenolic metabolites in serum increased
Wu et al.[40] (46)	Italy[b]	ITS	No significant differences
Collino et al.[167] (233)	Italy[b]	Metabolomics/ HITChip	Para-cresol sulfate and phenylacetic acid increased; butyrate-producing bacteria decreased
Kong et al.[41] (121)	China[b]	16S	Alpha diversity, *Akkermansia* and Christensenellaceae increased
Wang et al.[99] (15)	China[b]	16S	*Akkermansia*, *Parabacteroides*, *Paraprevotella*, pathobionts increased; core SCFA producers decreased
Wang et al.[42] (198)	China	16S	*Butyricimonas*, *Butyricicoccus*, *Odoribacter*, *Alistipes*, *Christensenella*, *Barnesiella*, pathobionts increased; core SCFA producers and *Megamonas* decreased
Bian et al.[100] (284)	China[b]	16S	No significant differences
Zhang et al.[97] (750)	China	Shotgun	Pathobionts, *Lactobacillus salivarius*, *Butyrivibrio crossotus*, *Subdoligranulum variabile*, *Roseburia hominis*, *Coprococcus catus*, *Clostridium sachharolyticum*, *A. muciniphila*, *Victivallis vadensis* increased; *Bifidobacterium* and gender-associated gut microbiome differences decreased
Ke et al.[168] (77)	China	Metabolomics	TMAO increased
Kim et al.[169] (47)	South Korea[b]	16S	Pathobionts, *Akkermansia* and Christensenellaceae increased; core SCFA producers and *Prevotella* decreased
Park et al.[96] (70)	South Korea	16S	*Prevotella copri*, pathobionts, *Roseburia inulinivorans* increased; core SCFA producers, *Megamonas*, *Alistipes*, *Bacteroides uniformis*, *B. vulgatus* decreased
Tuikhar et al.[43] (30)	India[b]	16S	*Akkermansia*, *Butyricimonas*, Ruminococcaceae increased; Prevotellaceae and core SCFA producers decreased
La-Ongkham et al.[95] (47)	Thailand	16S	Pathobionts, *Eubacterium eligens*, *Bacteroides thetaiotamicron*, *B. uniformis*, *B. caccae*, *B. ovatus*, *Parabacteroides distasonis* increased; other core SCFA producers decreased
Rahayu et al.[98] (40)	Indonesia	qPCR	Pathobionts, *Bifidobacterium* decreased; multiple *Lactobacilli* increased
Kashtanova et al.[44] (42)	Russia[b]	16S	*Lactobacillus*, *Roseburia*, *Christensenella* increased; *Dorea*, *Dialister*, *Ruminococcus* decreased
Huang et al.[170]; Cuesta-Zuluaga et al.[101]	Multiple (meta-analysis)	16S	Core gut microbiome members decreased
Galkin et al.[171]	Multiple (meta-analysis)	Shotgun	Pathobionts and *Bifidobacterium bifidum* increased
Wilmanski et al.[103]	United States	16S	Increased gut microbiome uniqueness (or dissimilarity) and decreased *Bacteroides* associated with ageing; faster shift towards uniqueness in healthy ageing cohort

Pathobionts include one or more taxa belonging to the following lineages: *Desulfovibrio*, *Bilophila*, *Eggerthella*, all *Enterobacteriaceae*, *Campylobacter*, *Fusobacterium*, *Streptococcus*, *Anaerotruncus*, *Bacteroides fragilis*, *Campylobacter*, *Actinomyces*, *Corynebacterium*, *Staphylococcus*, *Parvimonas*, *Porphyromonas*, *Flavonifractor*, *Ruminococcus torques*, *R. gnavus*, *Clostridium asparagiforme*, *C. hathewayi*, *C. bolteae*, *C. citroniae*, *C. clostridioforme*, *C. symbiosum*, *C. hylemonae*, *C. scindens* and *C. difficile*. Core SCFA producers include members of the following genera: *Faecalibacterium*, *Roseburia*, *Eubacterium*, *Dorea*, *Coprococcus* and *Blautia*. ITS, internal transcribed spacer; TMAO, trimethylamine-*N*-oxide; HITChip, Human Intestinal Tract Chip; LPS, lipopolysaccharide; qPCR, quantitative polymerase chain reaction; SCFA, short-chain fatty acid. [a]Indicates that the cohort size does not include individuals <50 years of age. [b]Indicates that the study includes centenarians.

resistance (by regulation of B1 cell activity), cancer onset (by acting as a histone deacetylase inhibitor and facilitating programmed cell death) and cognitive decline (by acting as a putative negative regulator of amyloidosis and neuro-inflammation)[75,77–80]. Besides the butyrate producers, multiple studies have associated the Group 3 member *Akkermansia* (especially the species *Akkermansia muciniphila*) with beneficial traits (some causally using preclinical studies), including facilitating the growth of butyrate producers (by producing acetate), which results in reduced loss of colonic bilayer (thereby reducing inflammation), reduced activation of B1a cells (thereby preventing insulin resistance), prevention of cellular senescence (tryptophan mediated as well as through modulation of bile acid profiles), ameliorating progeroid symptoms as well as markedly reducing

insulin resistance and the risk of cardiometabolic disease in humans with overweight or obesity[79,81–85]. Surprisingly, *A. muciniphila* has also been reported as more abundant in faecal samples of patients with multiple sclerosis and Parkinson disease than of healthy individuals as controls[86,87]. However, it is still uncertain whether the

Table 3 | **Targeted studies investigating gut microbiome alterations related to specific aspects of ageing-related health loss**

Aspect of unhealthy ageing	Study (cohort size[a])	Country/ region	Molecular technique	Main alteration associated with condition investigated
Frailty	Claesson et al.[28] and Jeffery et al.[45] (both 178)	Ireland	16S	*Parabacteroides*, *Anaerotruncus*, *Coprobacillus* increased; core SCFA producers and core gut microbiota decreased
Frailty	Ghosh et al.[46] (189)	Ireland	Shotgun	Pathobionts with production capacity of multiple detrimental metabolites increased; SCFA producers decreased
Frailty	Jackson et al.[47] (728)	United Kingdom	16S	Pathobionts increased; SCFA producer *Faecalibacterium prausnitzii* decreased
Frailty	Lim et al.[48] (176)	Korea	Shotgun	Pathobionts increased; *Prevotella copri*, SCFA producer *Coprococcus eutactus* decreased
Frailty	Maffei et al.[49] (~80)	United States	16S	Pathobionts, *Ruminococcus*, *Coprobacillus* increased; alpha diversity decreased
Frailty	Zhang et al.[102] (27)	China	16S	*Ruminococcus torques*, Atopobiaceae increased; *Gemella*, *Eubacterium ruminatium*, *Azospira* decreased
Frailty	Picca et al.[51] (35)	Italy	16S	*Ruminococcus* increased; Christensenellaceae, *Barnesiellaceae* reduced
Reduced physical activity	Langsetmo et al.[52] (373)	United States	16S	*Coprobacillus*, *Eggerthella*, *Anaerotruncus*, *Megasphaera* increased; *Cetobacterium*, *Faecalibacterium*, Lachnospiraceae, *Prevotella* decreased
Reduced physical activity	Fart et al.[53] (98)	Sweden	Shotgun	*Bilophila* positive; *Faecalibacterium prausnitzii* decreased
Cardiometabolic disease	Taniguchi et al.[54] (33)	Japan	16S	*Clostridioides difficile* increased; *Oscillospira* decreased
Cognitive decline	Verdi et al.[172] (NA)	United Kingdom	16S	Pathobionts, *Ruminococcus*, *Lactobacillus*, *Blautia* increased; *Prevotella*, *Odoribacter*, Christensenellaceae, *Barnesiella*, *Butyricimonas*, *Lachnospira*, alpha diversity decreased
Cognitive decline	Anderson et al.[55] (37)	United States	16S	*Akkermansia* and Lentisphaerae decreased
Cognitive decline	Manderino et al.[56] (43)	United States	16S	*Akkermansia* decreased
Parkinson disease	Heinzel et al.[173] (666)	Germany	16S	Pathobionts increased; *Prevotella*, core SCFA producers, *Bifidobacterium*, *Odoribacter*, *Victivalis* decreased
Alzheimer disease	Haran et al.[57] (108)	United States	Shotgun	Pathobionts increased; *Butyrivibrio*, core SCFA producers and *Adlercreutzia equolifaciens* decreased
Migraine	Chen et al.[58] (108)	United Kingdom	Shotgun	Pathobionts increased; core SCFA producers, *Butyrivibrio*, *Bifidobacterium adolescentis*, other SCFA producers decreased
Reduced bone mass density	Das et al.[59] (181)	Ireland	16S	Pathobionts, *Lactobacillus* increased
Reduced bone mass density	Li et al.[60] (102)	China	16S	*Roseburia*, *Bifidobacterium* and *Lactobacillus* decreased
Visceral fat deposition	Le Roy et al.[61] (1,760)	United Kingdom	16S	Pathobionts increased; *Oscillospira*, Christensenellaceae, Ruminococcaceae, Lachnospiraceae decreased
Obesity and metabolic syndrome	Zhong et al.[62] (382)	Ireland	16S	*Collinsella*, *Bifidobacterium*, *Paraprevotella* increased; *Akkermansia*, *Faecalibacterium*, *Prevotella* decreased
Comorbidity	Singh et al.[174] (65)	United States	16S	Pathobionts increased; *Akkermansia* decreased
Chronic kidney disease and frailty	Margiotta et al.[63] (64)	Italy	16S	Pathobionts and *Lactobacillus* increased; *Roseburia*, *Faecalibacterium* and *Prevotella* decreased
Mortality (among centenarians)	Luan et al.[64] (75)	China[b]	Shotgun	*Akkermansia muciniphila*, *Alistipes* sp., *Bacteroides* sp., *Butyrivibrio crossotus*, *Prevotella stercorea* decreased
Progeria	Bárcena et al.[84] (14)	Spain	16S	*Akkermansia* decreased
Comorbidities (among long-living individuals)	Zhang et al.[102]	China	Shotgun	Pathobionts and xenobiotic degradation pathway genes increased

Pathobionts include one or more taxa belonging to the following lineages: *Desulfovibrio*, *Bilophila*, *Eggerthella*, all *Enterobacteriaceae*, *Campylobacter*, *Fusobacterium*, *Streptococcus*, *Anaerotruncus*, *Bacteroides fragilis*, *Campylobacter*, *Actinomyces*, *Corynebacterium*, *Staphylococcus*, *Parvimonas*, *Porphyromonas*, *Flavonifractor*, *Ruminococcus torques*, *R. gnavus*, *Clostridium asparagiforme*, *C. hathewayi*, *C. bolteae*, *C. citroniae*, *C. clostridioforme*, *C. symbiosum*, *C. hylemonae*, *C. scindens* and *C. difficile*; core SCFA producers include members of the following genera: *Faecalibacterium*, *Roseburia*, *Eubacterium*, *Dorea*, *Coprococcus* and *Blautia*. NA, not available; SCFA, short-chain fatty acid. [a]Indicates that the cohort size does not include individuals <50 years of age. [b]Indicates that the study includes centenarians.

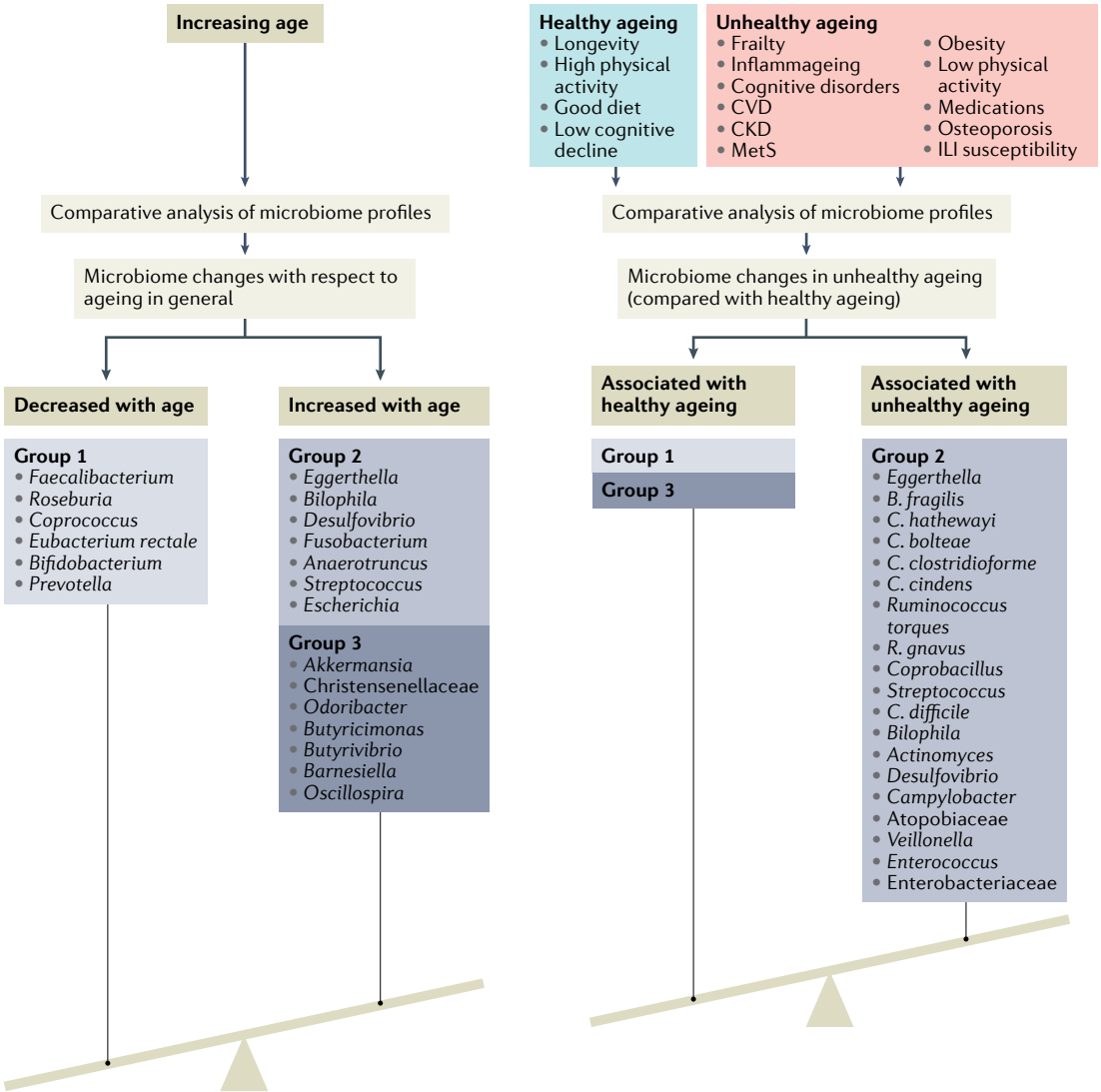

Fig. 3 | **Microbiome alterations in ageing (and unhealthy ageing).** Consistent patterns of microbiome taxa alterations associated with ageing (in general) and across various aspects of healthy versus unhealthy ageing in humans. There are primarily two categories of gut microbiome versus ageing studies in humans: studies that investigate changes in the gut microbiome composition across the age landscape and studies that investigate alterations between apparently healthy and unhealthy older individuals. Based on an extensive literature survey of these two kinds of studies (TABLES 2,3), three major groups of taxa showing consistent patterns of alteration (increasing or decreasing abundance in older people; increasing or decreasing abundance with unhealthy ageing) could be identified (Supplementary information). Group 1 taxa decreased with age and were associated with healthy ageing. Group 2 consisted of the pathobionts that increased with age and were associated with unhealthy ageing. Group 3 increased with age but were observed to be depleted in unhealthy ageing. It is important to note that these three groups are defined only with respect to ageing-linked microbiome alterations (from the context of this Review). CKD, chronic kidney disease; CVD, cardiovascular disease; ILI, influenza-like illness; MetS, metabolic syndrome.

Internal transcribed spacer
A method of microbial community profiling similar to 16S profiling that relies on specific PCR-based amplification, sequencing and characterization of the internal spacer DNA regions between the small subunit rRNA components (16S for bacteria/archaea and 18S for eukaryotes) and the large subunit rRNA components, and is typically used for the characterization of the mycobiome of a given environment due to the superior phylogenetic granularity afforded by the sequence.

positive association of *Akkermansia* in these diseases is a cause or an effect of a variable host-specific immune response[88]. Other Group 3 members, Barnesiellaceae and Christensenellaceae, have been associated with increased physical activity and are depleted in humans with sarcopenia, inflammation and mitochondrial DNA damage[89–92]. Strains belonging to the family Odoribacteriaceae (related to another Group 3 member *Odoribacter*) were shown to produce a novel bile acid called isoallolithocholic acid, which was enriched in the faecal metabolome of centenarians and showed antimicrobial effects on multidrug-resistant pathogens, including *Clostridioides difficile*[93] (FIG. 4). Further evidence for the microbiome basis of longevity comes from a study in *Caenorhabditis elegans*, where screening a mutant bank of *E. coli* identified 12 microbial genes associated with longevity, including those linked to colanic acid secretion, which in turn beneficially regulate mitochondrial gene transcription and the unfolded protein response by facilitating transcription of mitochondrial chaperone genes[94].

Despite generally consistent findings, ageing-linked microbiome alterations also exhibit variation across studies. One example is *Roseburia*. While ageing studies

Inter-study variations
Variations in the patterns of gut microbiome alterations detected across different studies investigating the same (or similar) host trait or phenotype (for example, age, BMI, a disease or disorder, or specific dietary patterns).

Shotgun metagenomics
A method of community genomic profiling that involves the extraction and sequencing of the entire genomic content of all cells belonging to a given microbial community.

in Italian, Irish and Thai individuals have reported a statistically significant reduction of *Roseburia* spp. abundance with age[28,38,40,95], studies of Russian, Chinese and Korean populations have observed an increase[44,96,97]. In addition, contrary to many ageing-associated studies[2,46,95], the pathobiont *Bacteroides fragilis* decreased with age in a general ageing-associated study in a healthy Indonesian cohort[98]. These variations might reflect differences in ethnicity, diet, geography and lifestyle; even within cohorts of the same nationality and region, different signatures have been reported. For example, considerable inter-study variability has been found in Chinese populations[41,42,97,99,100]. Inter-study variations might also relate to the physiological status of the ageing population (different degrees of frailty within older people) or might be methodological such as due to sample collection regime, sample storage, DNA extraction protocol or sequencing platform.

In addition to ethnogeographic variance, age and sex might interact in determining gut microbiome composition. A large-scale shotgun metagenomics study of >2,500 individuals in China identified sex-specific signatures, which diminished with age (a pattern replicated in two other large cohorts from the Netherlands and Israel)[97]. A similar sex-related pattern was found in the American Gut project[101]. Younger individuals were observed to have more pronounced sex-dependent differences in microbiome composition (beta diversity) compared with older people, with women having a higher microbiome alpha diversity than men.

There are challenges in defining cohorts of healthy and unhealthy ageing individuals, especially in different age groups of older people. Individuals who do not age healthily at early stages of ageing (for example, 60 or 70 years of age) are unlikely to survive to become centenarians. However, physiological decline and a shift to an unhealthy ageing trajectory can occur at any age (even among centenarians). For example, in the Italian super-centenarian cohort (age >105 years) previously analysed by Biagi et al. (84 individuals) and Rampelli et al. (69 individuals), approximately 40% of the individuals had dementia and >55% of the individuals were receiving anti-hypertensive and/or cardiovascular therapy[38,39]. Thus, the marked increase of opportunistic allochthonous bacteria (that is, those not normally replicating stably in the gut) along with the increased abundance of bacterial genes involved in xenobiotic degradation pathways is more likely a consequence of the unhealthy ageing phenotype setting among a subset of these super-centenarians (rather than being a sign of longevity). Further highlighting this observation, a pilot study from China investigated the microbiome shifts in healthy ($n = 37$) and unhealthy individuals ($n = 9$) among long-living people (aged 90 years and above)[102]. The unhealthy ageing phenotype in this study was defined by the presence of comorbidities encompassing gastrointestinal diseases, cardiovascular disorders, diabetes, arthritis and respiratory diseases. Notably, the patterns of taxonomic alterations observed were similar to those observed in other studies investigating various healthy versus unhealthy ageing phenotypes: that is, an increase in pathobiont lineages, such as *Streptococcus*, *Actinomyces* and *Eggerthella*, along with an increase in the proportional abundance of genes involved in xenobiotic degradation pathways. Thus, increased chronological age does not indicate healthy ageing. It is imperative that, irrespective of the age group investigated, studies investigating microbial determinants of healthy ageing stratify individuals on the basis of various indices of physiological well-being and frailty prior to selecting appropriate study populations. Furthermore, the observed microbiome alterations will also depend on the index of unhealthy ageing used to stratify individuals. Nevertheless, despite inter-study differences, the patterns of microbial alterations discussed previously are of sufficient consistency to suggest biological relevance and a plausible basis for the design of microbiome-based interventions against unhealthy ageing.

In addition to the consistent taxonomic alterations summarized previously, a 2021 study (investigating two US-based population-level cohorts of 3,653 and 907 individuals) linked the degree of 'uniqueness' (a measure indicating how dissimilar an individual's microbiome is from their nearest neighbour in the given population cohort) of the microbiome with ageing[103]. This finding appeared to be driven by loss of the core genus *Bacteroides* and agrees with the taxonomic alterations associated with ageing discussed previously

Table 4 | **Reported links between metabolites associated with major microbiome gain/loss groups and ageing-associated conditions**

Taxon groups	Metabolites	Linked disorder	Association	Refs
Group 2 (unhealthy ageing-associated pathobionts)	Trimethylamine	Cardiovascular disorders	Putatively causative	175–177
		Cognitive disorders		178,179
		Inflammation, oxidative stress		180
		Osteoporosis		180
		Colorectal cancer		181
		Chronic kidney disease		182
	Para-cresol	Inflammation, oxidative stress		183
		Cognitive disorders		184
		Chronic kidney disease		182
	Deoxycholic acid and lithocholic acid	Cognitive disorders		185
		Colorectal cancer		186,187
	Lipopolysaccharide	Metabolic syndrome		188
		Inflammation, oxidative stress		
	DNA-damaging toxins	Colorectal cancer		71–73
Group 1 and Group 3 (commensals associated with younger age groups and healthy ageing)	Butyrate	Cognitive disorders	Preventive	77,78
		Insulin resistance		75,79
		Obesity		189,190
		Inflammation, impaired barrier function		191
	Acetate	Insulin resistance		192

Groups are as defined in FIG. 3 and Ghosh et al.[46].

Prebiotic
Nutritional supplements designed to increase the abundance of specific target groups of beneficial microorganisms based on restricted catabolic ability in certain taxa.

Synbiotics
Single or consortia of beneficial microorganisms that are administered in combination with prebiotics.

Postbiotics
Deliberately inactivated (heat-killed) microbial cells, cell components or microbiota-derived metabolites that confer health benefits.

Quantitative polymerase chain reaction
(qPCR). A method of real-time microbial quantification in a given sample that relies on measuring the change in PCR amplicon copy number (in this context, amplified by primers designed against a phylogenetic marker gene corresponding to a given species or a microbial group) during the cycles of the PCR; copy numbers of phylogenetic marker gene regions belonging to microbial lineages in higher abundance are expected to be amplified faster, resulting in a faster increase in the strength of fluorescent oligonucleotide probes targeted at these lineages.

Fluorescence in situ hybridization
(FISH). A method of physically locating or enumerating the population of a specific microorganism or cell type by using fluorescent oligonucleotide probes that bind to specific genomic sequences in the target cells with high sequence complementarity; it can be used to measure either specific bacterial species or overall bacterial cell abundance (by using probes towards the universally conserved regions of the 16S rRNA gene).

(depletion of dominant Group 1 commensals compensated for by increased Group 3 commensals and Group 2 pathobionts). However, the link between uniqueness and healthy ageing is likely to be context dependent and additional studies are required to determine the validity of uniqueness as a marker of healthy and unhealthy ageing in different populations. More importantly, the enrichment or depletion of specific taxonomic lineages might be more valuable metrics of healthy ageing than summary statistics such as uniqueness or diversity.

Microbiome-based ageing interventions

Although functional versatility is a defining characteristic of the human gut microbiome, the responsiveness of the gut ecosystem to therapeutic modulation is unclear, particularly in older people, largely because the broader ecological effects of an intervention on core taxa have not been determined. Microbiome-directed or microbiome-derived therapeutic interventions include prebiotic supplements (designed to increase the abundance of specific target groups of beneficial microorganisms), single or combined beneficial taxa alone or with a prebiotic (synbiotics), postbiotics, or FMT and health-linked dietary regimens that can theoretically facilitate a broader shift in the gut microbiome towards health. The aim of such interventions is to break the self-perpetuating cycle of ageing-linked physiological decline facilitated by a disease-susceptible microbiome. From this perspective, we propose that the increased abundance of Group 2 taxa and the depletion of Group 1 and Group 3 (discussed previously) are analogous to 'buttons' that drive unhealthy ageing. Thus, resetting these buttons should be the key goal of any microbiome-associated therapeutic intervention targeted to healthy ageing. While numerous studies of variable size, quality and design have addressed the onset of unhealthy ageing and the microbiome of rodents[91], it is doubtful whether they can be extrapolated to humans. Therefore, we restrict this commentary to studies in humans.

Microbiome-based interventions to promote healthy ageing in older people are a work in progress; selected key studies are presented in TABLE 5. Only five studies have a cohort size of >100 individuals[104–108]; excluding an across-study meta-analysis[109], the mean cohort size of the other older people-focused interventions is only approximately 36 (this is excluding FMT intervention case studies involving either a single patient or less than 5 patients). In addition to the low sample size, many studies used low-resolution microbiome profiling, including quantitative polymerase chain reaction (qPCR) and fluorescence in situ hybridization (FISH)[105,110–114]. The questions posed by intervention studies also vary, with some limited to effects on host physiology rather than associated microbiome alterations[106,109,115–118]. Most studies in this list involving postbiotics and FMT have not performed any microbiome profiling and have relied on changes in host physiology and well-being (TABLE 5). Efficacy metrics have also varied from improvements in inflammatory status, insulin resistance, frailty and cognitive function to responsiveness to vaccines, which has been disappointing with regard to achieving superior vaccine response after microbial/prebiotic supplementation[119,120] but have shown promising results in the case of postbiotic interventions such as administration of heat-killed Lactobacilli[121,122]. Encouraging reports of other postbiotics, such as using butyrate to treat inflammatory diseases (such as ulcerative colitis) in younger cohorts ($n = 216$) and cognitive decline in mice models[123,124], remain to be assessed in older people. In addition, FMT in older people has largely been limited to the treatment of *C. difficile* infections[108,125,126]. While promising, such strategies risk transmission of pathobionts or detrimental microbiota in the context of an older host[127]. FMT for other age-associated disorders in older people has been limited to isolated case reports[23,128].

Notwithstanding these limitations, there are grounds for optimism for microbiome-targeted interventions, particularly in the context of unhealthy ageing. In pilot studies of polyphenol-rich foods, prebiotics and some probiotics that included sub-cohorts of older and younger individuals, efficacy was more pronounced in older people, especially in the unhealthy group with frailty, insulin resistance and obesity, than in healthy and younger individuals, particularly for polyphenol-based and prebiotic/oligosaccharide-based interventions[118,129–134]. A popular but perhaps simplistic explanation for these effects emphasizes increased intestinal permeability in older people[135]. Increased gut permeability is associated with inflammageing, frailty and insulin resistance[67,136]. However, a polyphenol-based pilot intervention found that the age-linked beneficial effects included changes in the microbiome[129]. The study investigated the effects of daily freeze-dried blueberry powder consumption for 6 weeks on the faecal microbiota of 17 women in two age groups (aged 21–39 years and 65–77 years, respectively). Specifically, consumption of blueberries (a source of polyphenols) was associated with increased microbiome alpha diversity and increased abundance of butyrate-producing Group 1 taxa (*Faecalibacterium* and *Coprococcus*) and Group 3 taxa (*Butyricimonas* and *Barnesiella*), which was accompanied by increased antioxidant activity with respect to baseline compared with placebo controls.

Similar improvements in microbiome composition have also been reported for other interventions in older people (TABLE 5). The quality of evidence for 'resetting' microbiome alterations in unhealthy ageing varies with the type of intervention, duration and dosage. For example, for interventions with probiotics and prebiotics, the primary beneficial objective has been to increase the abundances of the putatively beneficial taxa (primarily Lactobacilli and Bifidobacteria) in addition to the butyrate producers (from Group 1 and Group 3; FIG. 3) and *Akkermansia* (Group 3) (FIG. 5). These lineages not only have beneficial attributes, such as promoting insulin sensitivity and maintaining intestinal barrier integrity[137,138], but also cross-feed the butyrate producers by providing acetate[139]. Additionally, lactobacilli facilitate butyrate uptake in colonocytes by induction of monocarboxylate transporter 1 (MCT1) as demonstrated in Caco-2 human colon cell lines (graphically summarized in FIG. 5)[140]. Many prebiotic and probiotic studies

Table 5 | **Intervention studies targeting the microbiome in older people**

Study	Duration and cohort size	Intervention type	Molecular technique[a]	Study aim or system targeted	Effect on microbiome	Physiological effects
Ghosh et al.[104]	1 year, 612	Diet (Mediterranean diet)	16S	Inflammageing, cognitive function, disease incidence and frailty	Diversity, keystone taxa, Group 1 taxons, *Prevotella*, SCFA producers positive; Group 2 pathobionts negative	Improvements in all health measures associated with an intermediate microbiome response
Mitchell et al.[193]	10 weeks, 28	Diet (protein rich)	16S	Generation of volatile toxic compounds	No significant changes	No significant changes
Nagpal et al.[145]	6 weeks, 17	Diet (Mediterranean diet plus keto diet)	16S	Alzheimer disease	Group 3 taxons, faecal butyrate, propionate positive	Butyrate negatively associated with amyloid β-40/42, *Proteobacteria* positive
Ntemiri et al.[129]	6 weeks, 17	Diet (blueberry intake)	16S	Inflammation, insulin resistance, oxidative stress	Group 1 and Group 3 taxons positive	Antioxidant activity increased (only in older people)
Igwe et al.[194]	8 weeks, 31	Diet (QGP intake)	16S	Cognition, inflammation	No significant changes	No significant changes
Del Bo et al.[130]	8 weeks, 66	Diet (PR diet)	16S	Inflammation, insulin resistance, cardiometabolic health	Group 1 and other SCFA producers increased	Zonulin, blood pressure (in women) decreased; effects pronounced in individuals with higher baseline BMI and insulin resistance
Ostan et al.[105]	8 weeks, 125	Diet (nutraceutical supplementation)	qPCR	Inflammation, insulin resistance	NA	Insulin resistance, inflammatory markers decreased
Ruiz-Saavedra et al.[195]	NA, 73	Adherence scores to a healthy diet (scores)	16S	Cognition, blood glucose and pressure	NA	All measures of blood measure decreased (only in older people)
Zengul et al.[196]	NA, 29	Diet (dietary fibre)	16S	Breast cancer	Group 2 pathobionts decreased	Serum oestradiol decreased
Cancello et al.[133]	4 weeks, 20	Diet plus MS	16S	Obesity, insulin resistance, inflammation	Group 3 and Group 1 members increased; Group 2 pathobionts decreased; effect was higher in individuals with obesity	Weight decreased, *Faecalibacterium* and *Akkemansia* negatively associated with obesity measures
Qu et al.[109]	NA, 689	MS (meta-analysis)	NA	Inflammation	NA	High variability; no significant association
Buigues et al.[117]	13 weeks, 50	Prebiotics (inulin, FOS)	NA	Cognitive decline, frailty	NA	Physical frailty measures decreased
Theou et al.[134]	13 weeks, 50	Prebiotics (inulin, FOS)	NA	Frailty	NA	Reduction in frailty; effects pronounced in individuals who are frail
Tran et al.[131]	26 weeks, 37	Prebiotics (consortia)	16S	Inflammation	Specific Group 1 members and *Parabacteroides* increased in individuals who are frail	Inflammatory CXCL11 decreased in individuals who are frail
Alfa et al.[141]	12 weeks, 84	Prebiotics (amylose, amylopectin)	16S	Inflammation, insulin resistance	*Bifidobacterium*, *Prevotella*, Group 2 taxa, butyrate, *Desulfovibrio* increased	HOMA-IR reduced; effect pronounced in older people
An et al.[197]	4 weeks, 48	Prebiotics (SBP, maltodextrin)	16S	Generation of volatile toxic compounds	NA	NA
Chung et al.[198]	10 days, 21	Prebiotics (AXOS, maltodextrin)	16S	SCFA and microbiome status	*Bifidobacterium*, *Prevotella*, *Oscillibacter* increased	Higher SCFA linked with higher *Prevotella*

Table 5 (cont.) | **Intervention studies targeting the microbiome in older people**

Study	Duration and cohort size	Intervention type	Molecular technique[a]	Study aim or system targeted	Effect on microbiome	Physiological effects
Watson et al.[199]	5 weeks, 20	Prebiotics (inulin)	16S	Stool characteristics	No significant changes	No significant changes
Birkeland et al.[200]	6 weeks, 25	Prebiotics (inulin)	16S	SCFA and microbiome status	*Bifidobacterium adolescentis*, Group 1, *Bacteroides ovatus* increased; *Ruminococcus* decreased	SCFA increased
Leblhuber et al.[114]	4 weeks, 20	MS (consortia)	qPCR	Alzheimer disease	*Faecalibacterium* increased	Zonulin decreased; zonulin negatively correlated with CDT, MMSE
Gao et al.[201]	NA, 33	MS (consortia)	16S	Inflammation	*Blautia*, *Faecalibacterium*, Pathobionts increased	IL-1β decreased
Kim et al.[142]	12 weeks, 53	MS (*Bifidobacteria*)	16S	Brain function	*Clostridiales*, *Eubacterium* increased	Serum BDNF decreased
Eloe-Fadrosh et al.[202]	12 weeks, 12	MS (*Lactobacilli*)	16S, RNA sequencing	NA	Differential gene transcription	NA
Spaiser et al.[113]	3 weeks, 32	MS (consortia)	16S, qPCR	Inflammation	*Bifidobacterium*, *Faecalibacterium* increased; *Escherichia* decreased	Anti-inflammatory IL-10 increased
Nyangale et al.[143]	4 weeks, 36	MS (*Bacillus coagulans*)	FISH	Inflammation	*Faecalibacterium prausnitzii* increased	Anti-inflammatory IL-10 increased and pro-inflammatory TNF increased
Sanborn et al.[106]	13 weeks, 145	MS (*Lactobacilli*)	NA	Cognitive decline	NA	Improvements in multiple scores of cognitive function
Costabile et al.[144]	3 weeks, 37	MS (*Lactobacilli*)	16S	Inflammation, cardiometabolic health	*Parabacteroides*, *Oscillospira*, *Desulfovibrio* increased	LDL/total cholesterol, C-reactive protein reduced
Björklund et al.[112]	2 weeks, 47	MS (*Lactobacilli*) plus prebiotic (lactitol)	qPCR	Gut microbiome alterations	Reduced loss of SCFA producers	NA
Nilsson et al.[115]	1 year, 70	MS (*Lactobacilli*)	NA	Bone loss	NA	Reduced loss of bone mineral density
Nyangale et al.[111]	4 weeks, 6	MS (*B. coagulans*) plus prebiotic (GOS/FOS)	FISH	Inflammation, SCFA status	Group 1 SCFA producers increased	Faecal SCFA increased
MacFarlane et al.[110]	4 weeks, 43	MS (*Bifidobacteria*) plus prebiotic (inulin)	FISH	Inflammation	*Actinobacteria* and *Firmicutes* increased; *Proteobacteria* decreased	SCFA increased; TNF decreased
Akatsu et al.[121]	12 weeks, 15	Postbiotic (heat-killed *Lactobacilli*)	NA	Influenza vaccine response	NA	Significant improvement in antibody titre for all three variants (H1N1, H3N2 and B)
Maruyama et al.[122]	12 weeks, 45					
Shinkai et al.[107]	20 weeks, 300		NA	Improved immunity	NA	Significantly reduced incidence of common cold
Kotani et al.[203]	12 weeks, 80		NA	Salivary IgA and mucosal immunity	NA	Improved IgA production and mucosal immunity
Andreux et al.[204]	4 weeks, 60	Postbiotic (urolithin A)	NA	Mitochondrial and cellular health	NA	Improved mitochondrial gene expression and fatty acid oxidation

Table 5 (cont.) | **Intervention studies targeting the microbiome in older people**

Study	Duration and cohort size	Intervention type	Molecular technique[a]	Study aim or system targeted	Effect on microbiome	Physiological effects
Agrawal et al.[108]	NA, 146	FMT	NA	CDI treatment	NA	Significantly improved cure rates
Bamba et al.[125]	NA, 4					
Friedman-Korn et al.[126]	NA, 34					
Xie et al.[128]	NA, 1			Treatment of alopecia areata (hair loss disease) and non-infectious diarrhoea		Amelioration of diarrhoeal systems, new hair growth
Cai et al.[205]	NA, 1		16S	Treatment of depression	*Firmicutes* increased; *Bacteroides* reduced	Improved appetite; increased physical activity, happiness; improved PHQ scores

Group 1 indicates multiple taxa belonging to the younger age group-associated Group 1 as highlighted in FIG. 3. Group 2 indicates multiple taxa belonging to the pathobiont Group 2 as highlighted in FIG. 3. Group 3 indicates multiple taxa belonging to the healthy ageing-associated Group 3 as highlighted in FIG. 3. AXOS, arabinoxylan-oligosaccharide; BDNF, brain-derived neurotrophic factor; CDI, *Clostridioides difficile* infection; CDT, clock drawing test; FISH, fluorescent in situ hybridization; FMT, faecal microbiota transplantation; FOS, fructo-oligosaccharide; GOS, galactooligosaccharide; HOMA-IR, homeostatic model assessment — insulin resistance; LDL, low-density lipoprotein; MMSE, mini-mental state examination; MS, microbial supplementation; NA, not available; PHQ, Patient Health Questionnaire; PR, polyphenol-rich; QGP, Queen Garnet plum; qPCR, quantitative polymerase chain reaction; SCFA, short-chain fatty acid; SBP, sugar beet pectin. [a]Molecular technique refers to the technique used for microbial profiling in each study (NA indicates that there was no microbial profiling performed as part of the given study).

report beneficial effects on host physiology, such as reduction in insulin resistance, zonulin gene expression (a marker of barrier function), inflammation, and levels of brain-derived neurotrophic factor (BDNF), which is upregulated during neuro-inflammation. In addition, improved cognitive function and cardiometabolic health, previously associated with butyrate, have been reported[106,111,113–115,130,132,141–144] (FIG. 5).

Akkermansia (Group 3; or the species *A. muciniphila*, specifically) is another hallmark of healthy ageing. Administration of live or pasteurized *A. muciniphila* for 3 months has been reported to reduce body weight, insulin resistance and levels of cardiometabolic risk factors in a Belgian cohort of 32 individuals ranging between 18 and 70 years of age[85]. While prebiotics and probiotics have been consistently linked with beneficial Group 1 and Group 3 taxa, there are conflicting reports on their influence on the abundance of pathobionts (Group 2 taxa). A significant reduction in Proteobacteria[110,113] should be weighed against a reported increase of pathobionts such as *Desulfovibrio*, *Streptococcus* and *Enterococcus*[114,132,144].

In contrast to supplements, whole diet-based interventions promise a fundamental resetting of gut microbiome composition[104,105,130,145]. However, most studies have focused on the intake of specific dietary components, often in small studies of short duration (TABLE 5). To address this limitation, a multi-national European consortium conducted the first comprehensive older people-targeted NU-AGE Mediterranean Diet (MedDiet) intervention[104]. The MedDiet regimen is characterized by increased intake of vegetables, fruits, legumes, fish, olive oil, and nuts and reduced consumption of red meat, dairy products and saturated fats. The MedDiet has been linked with reduced mortality and reduced onset of multiple chronic diseases and frailty[146]. The study included 323 test individuals and 289 control individuals, all of whom were pre-frail individuals over age 65 years followed for 1 year. Adherence to the MedDiet for a 1-year intervention period was correlated with retention of microbiome composition compared with that of the control individuals who maintained their normal diet; the intervention arm individuals also retained higher levels of health metrics, including levels of pro-inflammatory and anti-inflammatory cytokines, cognitive function and physical frailty, and incidence of non-communicable diseases[104]. The study provided the first clear evidence that a MedDiet can help to 'reset' the microbiome alterations that initiate the vicious cycle of unhealthy ageing-linked physiological decline (FIG. 5). Strict adherence to the diet was associated with retention of the keystone taxa (such as *Faecalibacterium prausnitzii* and *Bacteroides thetaiotamicron*) constituting the core gut microbiome and with retention of microbiome diversity. The Group 1 butyrate producers, including *Faecalibacterium prausnitzii*, *Roseburia hominis*, *E. rectale* and *Prevotella copri*, were positively associated with MedDiet adherence. By contrast, pathobionts (the Group 2 taxa group), such as *R. torques*, *Collinsella aerofaciens*, *Clostridium ramosum* and *Flavonifractor plautii*, were negatively associated. Taxa that were negatively associated with the MedDiet had greater genomic coding capacity for producing potentially harmful metabolites such as deoxycholic acid, lithocholic acid and para-cresol, all of which are linked to unhealthy ageing (as described in FIG. 4).

To quantify the beneficial microbiome alterations associated with MedDiet intake, we computed MedDiet-associated microbiome scores, whereby higher scores were linked with physiological well-being (including negative associations with inflammatory markers and incidence of chronic diseases, colorectal cancer, and frailty and positive associations with cognitive scores). Thus, it seems that adherence to the MedDiet initiated specific changes in microbiome composition, resulting in an altered set of microbial metabolites promoting

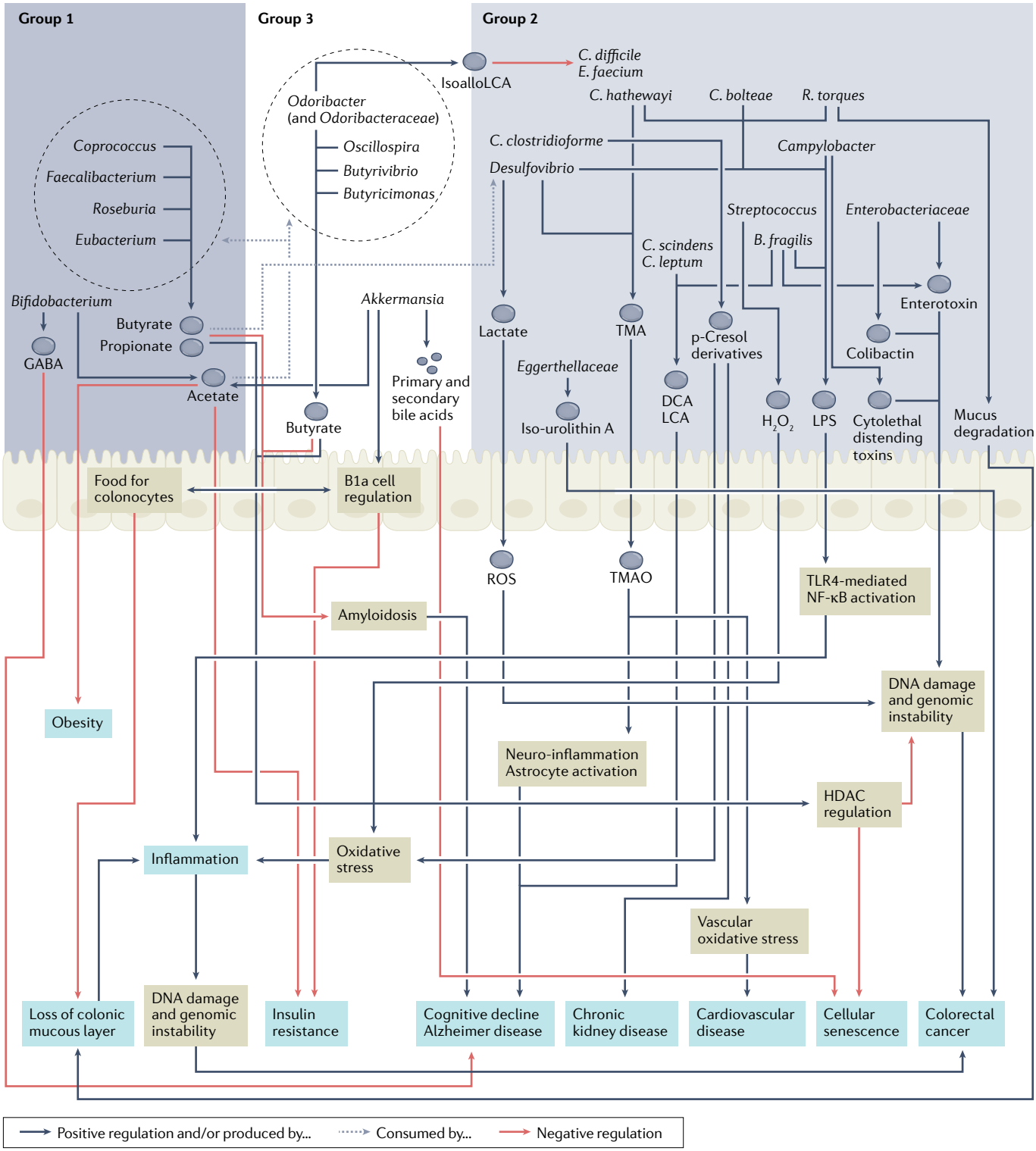

Fig. 4 | **Functional implications of microbiome alterations on host physiology in ageing.** Metabolic capabilities of the three taxa groups are linked to unhealthy ageing-linked decline in host physiology. Graphic summary of the key metabolites or effectors produced by the three taxa groups and the effect each of these microbiome-derived entities has in either negatively or positively regulating various ageing-linked diseases and disorders. DCA, deoxycholic acid; HDAC, histone deacetylase; IsoalloLCA, isoallolithocholic acid; LCA, lithocholic acid; LPS, lipopolysaccharide; p-Cresol, para-cresol; ROS, reactive oxygen species; TMA, trimethylamine; TMAO, TMAO, trimethylamine-*N*-oxide.

improved host health, as previously hypothesized[147]. The findings have been validated in several MedDiet intervention studies (not specifically focused on older people), which showed consistent enrichment of *Faecalibacterium* and *Roseburia* and depletion of pathobionts (such as *R. torques* and *R. gnavus*, *Collinsella*, members of

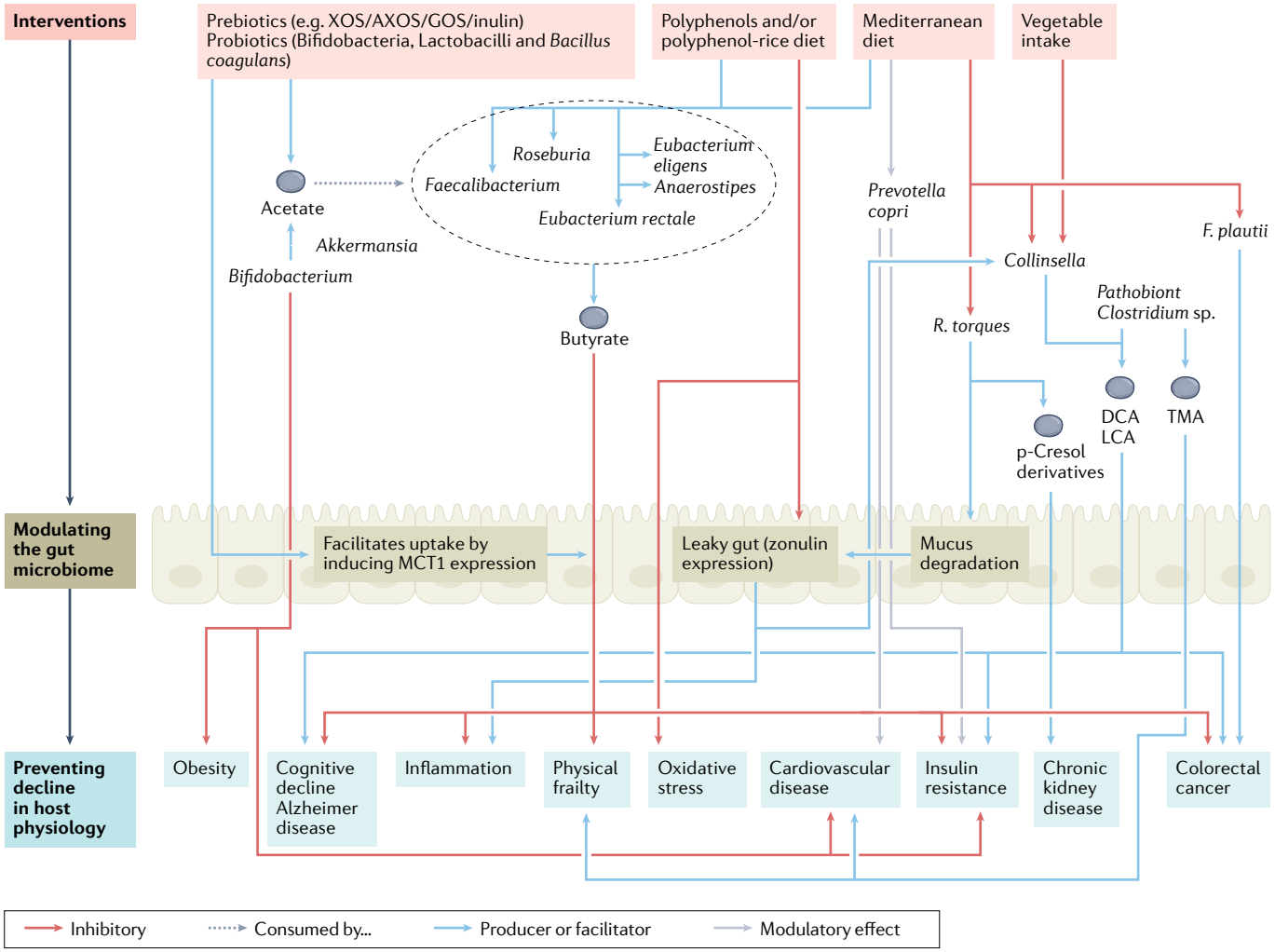

Fig. 5 | **Microbiome-associated interventions to prevent unhealthy ageing.** Pictorial summary showing examples of how specific intervention strategies have been shown to alter the microbiome alterations that prevent the ageing-associated decline in host physiology. AXOS, arabinoxylan-oligosaccharide; DCA, deoxycholic acid; GOS, galactooligosaccharide; LCA, lithocholic acid; MCT1, monocarboxylate transporter 1; p-Cresol, para-cresol; TMA, trimethylamine; XOS, xylooligosaccharides.

pathobiont *Clostridium*, and *Flavonifractor plautii*), along with a decreased capacity for production of deoxycholic acid and lithocholic acid[148–150] (FIG. 5). These studies also highlighted the predictive value of the baseline gut microbiome for responsiveness to the MedDiet, with a higher abundance of *Prevotella copri* at baseline linked with less responsiveness for some health metrics (such as insulin sensitivity, reduced inflammation, and lower triglyceride and total cholesterol levels)[148,149]. By contrast, other reports have observed a positive association between *Prevotella* abundance and lower cardiometabolic disease risk[151,152]. Strain-level variations within this lineage probably account for these apparently contradictory results as observed previously for *Faecalibacterium prausnitzii*[149]. Levels of the pathobiont *Collinsella* were also found to mediate the beneficial effect of dietary vegetable intake on lymphocyte counts[153].

In summary, beneficial responses to microbiome-based dietary interventions have been achieved, but transduction of dietary signals to the physiological homeostasis of the host can be highly personalized, depending on baseline microbiome composition[152]. This observation calls for a better understanding of age-related deterioration in the microbiome and for precision in the selection of interventions matched for an individual's stage in that process of decline. Stages of microbiome deterioration associated with unhealthy ageing probably first include a reduction in the abundance of specific core or keystone species, followed by complete loss of the keystone members and surrounding community structure, enabling the outgrowth of pathobionts[45,154] (FIG. 6a). Simple dietary interventions are likely to fail or have limited efficacy in individuals whose diet-responsive keystone core species are low or already lost. Such scenarios will require combinatorial therapy involving diet adjustment supplemented by microbial restoration of keystone taxa at the central nodes of microbiome networks. Individuals with less microbiome deterioration might still be responsive to dietary interventions alone.

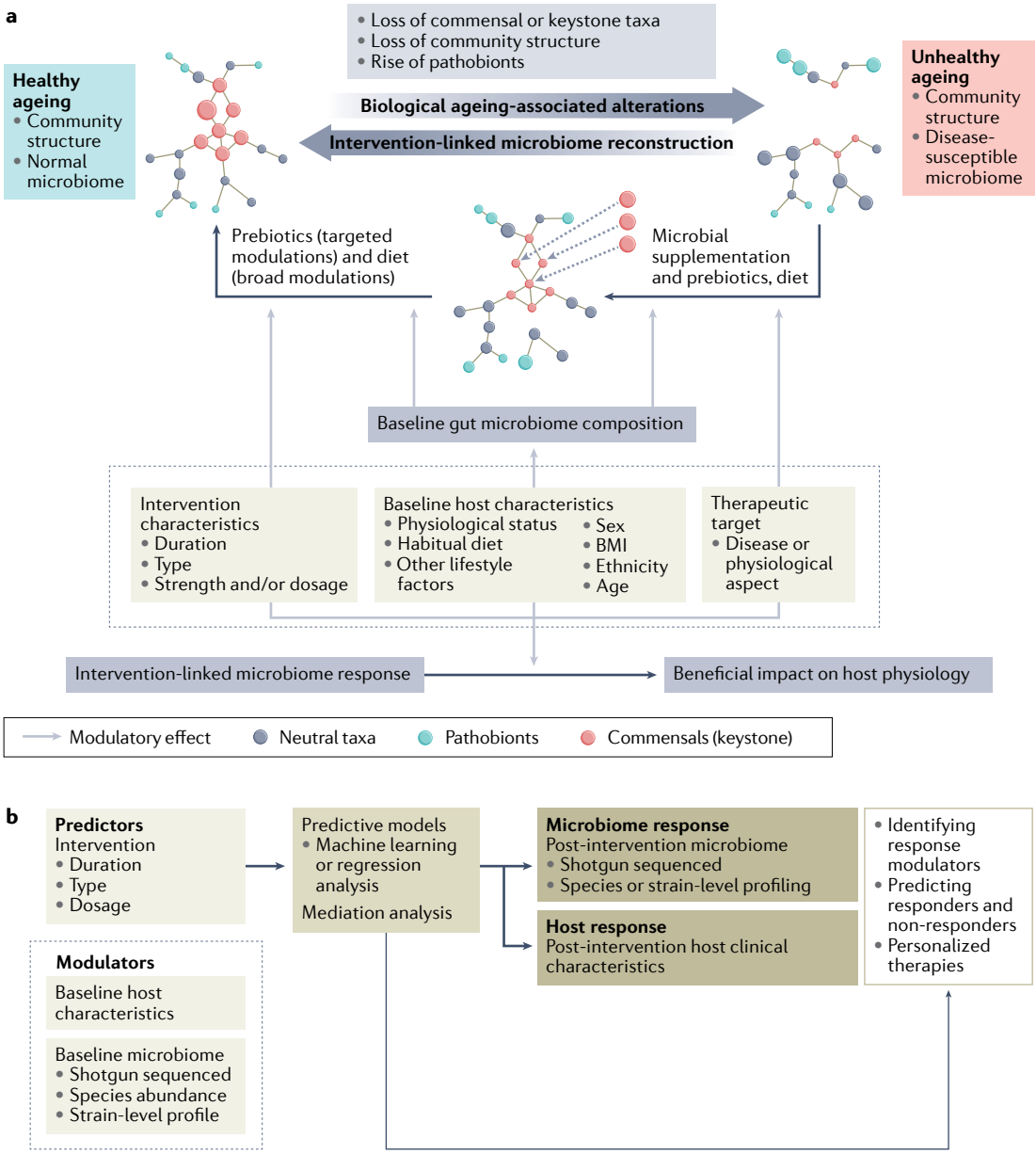

Fig. 6 | **Strategies for the formulation of personalized microbiome reconstruction strategies in older people.** **a** | Conceptual framework for applying personalized microbiome reconstruction strategies tailored to the extent of microbiome decline in a person with unhealthy ageing, and the factors that mediate the response of the host to these intervention strategies. **b** | Graphical summary of the protocol to identify interactions between host phenotype, baseline microbiome and the overall response to an intervention and to enable the design of predictive strategies for host response and personalized intervention therapies.

As previously noted for *Prevotella*, the host response to a given dietary intervention depends not only on the presence or absence of responsive taxa but also on the representation of specific strains of neutral taxa that are seemingly not related to the intervention. However, a variety of factors, including habitual diet, influence the baseline strain-level composition of the gut microbiome, which requires shotgun metagenomics for monitoring (FIG. 6b). None of the microbiome-based interventions in older people to date has used this approach. Designing the near-perfect intervention would require elucidation of the interactions among host phenotype (physiology, demographics and lifestyle), the baseline gut microbiome, the intervention characteristics, and longitudinal responses in the microbiome and host physiology (FIG. 6). Predictive models based on machine learning could be used to identify the factors driving the personalized response to diet as well as to dissect the interactions between the type of intervention, duration and dosage, the modulators (baseline host characteristics including diet and baseline microbiome composition), and the personalized response (at the level of the microbiome and host response). They can also be used to assess the translatability of these interactions across different sub-populations (FIG. 6b).

Similar strategies have already been adopted in population-level studies of diet–microbiome–host

16S profiling
(Also known as 16S rRNA sequencing). A method of microbial community profiling that relies on specific polymerase chain reaction (PCR)-based amplification, sequencing and phylogenetic profiling of either the full length or specific sub-regions of the 16S rRNA marker gene across all bacterial and archaeal cells present in a given sample.

interactions in younger people, which predict cardiometabolic and post-prandial responses dependent on baseline host and microbiome characteristics[152]. In addition, a shift from low-resolution methods, such as 16S profiling, to shotgun metagenomics will be essential for resolving species-specific and strain-specific variations. Thus, incorporating predictive strategies for the design of microbiome-based studies will help realize the promise of combining precision with preventive medicine for healthy ageing.

Conclusions and future projections

Microbiome science is maturing through a phase of wonderment to a stage where we know enough to know how much we do not know. The metric for true advances will be the clinical impact rather than technical or computational wizardry. However, convincing evidence for the efficacy of microbiome-based therapies in humans is sparse. Curiously, ageing might offer the greatest opportunities and prospects for success. The tenuous homeostasis and limited physiological reserve of some older people might mean that relatively small improvements in the microbiome can have a profound functional influence on the individual.

Although lifelong human studies of the microbiome are logistically complex, surrogate models of accelerated human ageing, such as progeria and Down syndrome, might be informative but are still works in progress. In addition, strain-level resolution in microbiome-based diagnostics and therapeutics is highly desirable but not yet uniformly performed.

Translating knowledge of the microbiome for clinical benefit in older people will require answers to other lingering questions. Can the gut ecosystem of older people be 'rewilded' with missing or lost strains? What is the optimal way for restoring the microbiome? What are the dietary requirements for maintaining a restored microbiota? Will the food industry formulate new products informed by microbiome science as well as age-related physiology? Of course, delayed onset or outright prevention of unhealthy ageing and microbiome decline is preferable to therapeutic intervention. However, people will not return to ancestral or other diets simply because microbiome scientists think they are good for one's ecosystem!

Better public health messaging and food policies will be required, supported by an unassailable evidence base. Moreover, the social determinants of health will always trump personalized predictors of response. Eating healthily is unaffordable for many. This fact has been shown in the United Kingdom, one of the most affluent countries/regions on the globe[155,156], where families in the lowest decile for household income would have to spend almost three-quarters of disposable income on food if they were to comply with national dietary guidelines, whereas the comparable figure for the wealthiest decile is only 6%[155,156]. Dietary and other microbiome-based strategies for healthy ageing should be weighed against such social inequity and should seek to develop realistic solutions.

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All authors contributed equally to all aspects of the manuscript.

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