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Review

Gut Microbial Function and Immune Aging: An Evidence-Graded Framework Linking Microbial Metabolites to Healthspan and Age-Related Disease

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ABSTRACT: This narrative review examines how the metabolic output of the intestinal microbiota, rather than its taxonomic composition alone, relates to immune aging and to clinically meaningful geroscience outcomes. We organise the evidence around four questions: which microbial features are reproducibly associated with older age, which host pathways plausibly mediate their effects, how strong the evidence is for each mechanistic step, and what this implies for intervention. Three points structure the synthesis. First, the microbiota of older adults is not a single "dysbiotic" state: much of the reported difference between younger and older cohorts is attributable to frailty, multimorbidity, polypharmacy, residential setting, diet, physical activity, bowel transit and geography rather than to chronological age, and healthy-longevity phenotypes frequently diverge from the frail-elderly phenotype. Second, the dominant mediators—short-chain fatty acids (SCFAs), bile acids, tryptophan and polyphenol derivatives, polyamines and trimethylamine N-oxide (TMAO)—are context-dependent rather than uniformly protective or harmful, their host effects varying with local versus systemic concentration, receptor distribution, immune state, hepatic and renal clearance, and measurement platform. Third, most human data are cross-sectional; causal inference rests largely on rodent transfer experiments whose translational relevance to human healthspan is unresolved. We therefore label the evidence type supporting each major claim, compare cardiometabolic, neurodegenerative, hepatic, musculoskeletal and oncological domains, and appraise microbiota-targeted interventions with attention to strain, dose and formulation specificity, replication failure, endpoint heterogeneity, and safety in frail or immunocompromised older adults. We conclude by proposing an evidence-graded framework mapping defined microbial functions onto discrete features of immune aging and onto validated geroscience endpoints, and by identifying the designs required to move from association to intervention. The causal and clinical evidence base remains limited, and changes in microbiome composition should not be equated with gains in healthspan.

Keywords: gut microbiota; healthy aging, healthspan, short-chain fatty acids, immunosenescence, inflammaging, evidence grading

1. Introduction

Aging is accompanied by reproducible remodelling of the intestinal microbial ecosystem, and this observation has generated a large literature proposing the microbiota as both a biomarker and a modifiable determinant of healthspan [1,2]. The attraction is obvious: unlike most hallmarks of aging, the microbiota is accessible, measurable in a stool sample, and in principle manipulable by diet, live biotherapeutics or faecal

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transfer. The corresponding risk is equally clear. Because microbial composition covaries with almost every exposure that also changes with age—diet, drugs, mobility, comorbidity, care setting—cross-sectional differences between young and old cohorts are unusually vulnerable to confounding, and the step from an observed association to a claim that microbial change contributes to aging biology is frequently taken without the intervening evidence [3].

Several recent reviews have summarised age-associated microbial taxa, microbial metabolites, inflammaging and age-related disease [1,2,4]. Our aim is not to repeat that inventory. Instead we attempt three things that are, in our reading, underdeveloped in the existing literature. We separate chronological aging from biological aging, frailty and disease-associated dysbiosis, and treat the non-age determinants of the older-adult microbiome as part of the interpretive framework rather than as a list of caveats. We attach an explicit evidence descriptor to each major mechanistic claim, distinguishing cross-sectional human association, longitudinal human cohort, human intervention trial, animal transfer or gnotobiotic experiment, and in-vitro or cell-based work. And we align specific microbial functions—fibre fermentation to butyrate, bile-salt hydrolase activity, tryptophan indole production, TMA generation, mucin turnover—with discrete, separately measurable features of immune aging, rather than with "inflammation" as an undifferentiated whole.

Scope and literature identification. This is a narrative review, not a systematic review, and no protocol was registered. We searched PubMed, Web of Science Core Collection and Scopus from January 2000 to March 2026 using combinations of the terms "gut microbiota" OR "gut microbiome" OR "intestinal microbiota" AND "aging" OR "ageing" OR "healthy aging" OR "healthspan" OR "longevity" OR "frailty" OR "centenarian" AND, for mechanism- and disease-specific sections, "short-chain fatty acid", "butyrate", "bile acid", "tryptophan", "indole", "trimethylamine N-oxide", "polyamine", "intestinal permeability", "inflammaging", "immunosenescence", "probiotic", "prebiotic", "postbiotic", "faecal microbiota transplantation", together with disease terms. English-language original research, systematic reviews and meta-analyses were eligible; conference abstracts, preprints and non-English reports were generally excluded. Reference lists of retrieved reviews were screened for additional sources. Where several reports supported the same claim, we preferentially cited human studies in older adults, longitudinal cohorts and randomised trials, and cited animal or in-vitro work only when it supplied mechanistic evidence unavailable in humans. Because selection was not systematic, the possibility of citation bias towards positive findings cannot be excluded, and this review should be read as an interpretive synthesis rather than an exhaustive account.

Structure. Section 2 sets out terminology, the evidence-grading scheme and the confounding framework; Section 3 asks which microbial features of older age are reproducible; Section 4 examines mechanistic pathways from microbial function to immune phenotype; Section 5 separates shared from disease-specific mechanisms; Section 6 appraises interventions and safety; Section 7 states the field's limitations and a research agenda.

2. Definitions, Evidence Grading, and the Confounding Framework

2.1 Terminology of aging

Inconsistent terminology is a substantive obstacle in this literature, because studies that use "aging" to mean chronological age, biological age, frailty or the presence of age-related disease are frequently pooled as if they addressed the same question. We use the following definitions throughout.

Chronological age is time since birth. *Biological aging* denotes the progressive, multi-system accumulation of molecular and cellular damage that increases vulnerability to disease and death; it is estimated, imperfectly, by composite biomarker or epigenetic clocks and is only partly predicted by chronological age [5]. *Healthy aging*, following the World Health Organization's framing, is the process of developing and maintaining the functional ability that enables wellbeing in older age, encompassing mobility, cognition, psychological wellbeing, sensory function, continence and the capacity to meet basic needs and maintain relationships; it is defined by preserved function rather than by absence of disease. *Healthspan* is the period of life spent free of clinically significant chronic disease and of disability and is operationalised in cohort studies as time to first major age-related morbidity or to incident disability. *Frailty* is a state of reduced physiological reserve and diminished resistance to stressors, quantified either as a phenotype (weight loss, exhaustion, weakness, slow gait, low activity) or as a deficit-accumulation index. *Healthy longevity* refers to survival to extreme age with preserved function; the phenotype studied in centenarian cohorts. *Disease-associated dysbiosis* denotes microbial configurations that accompany a defined pathological state and that may be consequence rather than cause.

These distinctions matter empirically. Cohorts of frail, institutionalised older adults and cohorts of community-dwelling centenarians differ from young controls in opposite directions on several microbial

indices: the former show contraction of fibre-fermenting taxa and expansion of Enterobacteriaceae, whereas the latter show enrichment of *Akkermansia*, *Christensenellaceae* and *Bifidobacterium* and increased inter-individual uniqueness [6, 8]. Treating both as "the aging microbiome" produces a composite phenotype that describes neither.

2.2 Terminology of immune aging

The manuscript literature often uses "inflammaging" as a synonym for immune aging. We distinguish four related but separately measurable constructs, summarised in Table 1.

Table 1. Terminology and the evidence-grading scheme used in this review. Definitions are given because studies that use “aging” to mean chronological age, biological age, frailty or age-related disease are frequently pooled as though they addressed the same question.

Term or code	Definition as used here	Why the distinction matters
Chronological age	Time since birth.	The exposure is most often reported, and the least mechanistically informative.
Biological aging	Progressive multi-system accumulation of molecular and cellular damage increasing vulnerability to disease and death; estimated by composite or epigenetic clocks.	Two people of the same chronological age may differ markedly; microbial correlates of biological age are largely unstudied.
Healthy aging	Development and maintenance of the functional ability that enables wellbeing in older age (mobility, cognition, mood, sensory function, continence, ability to meet basic needs and maintain relationships).	Defined by preserved function, not by absence of disease; requires functional rather than compositional endpoints.
Healthspan	Period of life free of clinically significant chronic disease and of disability; operationalised as time to first major age-related morbidity or to incident disability.	The outcome that microbiota-targeted interventions are claimed to extend, and that no trial has yet measured.
Frailty	Reduced physiological reserve and diminished resistance to stressors; measured as a phenotype or as a deficit-accumulation index.	Correlates with microbial composition more strongly than chronological age; a composite exposure, not a nuisance covariate.
Healthy longevity	Survival to extreme age with preserved function; the phenotype of centenarian cohorts.	Microbial features diverge from, and sometimes oppose, those of frail elderly cohorts.
Disease-associated dysbiosis	Microbial configuration accompanying a defined pathological state.	May be consequence rather than cause; conflation with age-associated change is the field’s commonest inferential error.
Immunosenescence	Age-associated remodelling of adaptive and innate immunity: thymic involution and naive T-cell contraction, memory and CD28-null accumulation, restricted B-cell repertoire, myeloid skewing, impaired antigen presentation, altered mucosal IgA, reduced vaccine responsiveness.	Not equivalent to inflammation; an intervention lowering IL-6 has not been shown to restore thymic output or vaccine response.
Inflammaging	Chronic, low-grade, sterile elevation of circulating inflammatory mediators (IL-6, TNF, CRP, soluble TNF receptors) predictive of disability and mortality.	The most frequently measured outcome, and only one component of immune aging.
Immune exhaustion	Antigen-driven, potentially reversible T-cell hypofunction with sustained inhibitory receptor expression (PD-1, TIM-3, LAG-3) and distinct transcriptional and epigenetic programmes.	Mechanistically distinct from senescence although co-occurring; evidence in aging outside tumour models is minimal.
Cellular senescence and SASP	Stable cell-cycle arrest with apoptosis resistance; the senescence-associated secretory phenotype is the accompanying secretion of cytokines, chemokines, proteases and growth factors.	Whether microbiota-targeted intervention alters senescent-cell burden in humans is unknown.
H-CS	Human cross-sectional or case–control association.	Cannot establish direction; vulnerable to the confounders in Section 2.4.
H-LONG	Human longitudinal or prospective cohort.	Establishes temporality but not causation.
H-RCT	Randomised controlled trial in humans.	The design required for intervention claims; rare with functional endpoints in older adults.

H-MR	Mendelian randomisation or other genetic instrumental-variable analysis in humans.	Addresses causality observationally; instrument strength for microbial traits is limited.
A-TRANSFER	Microbiota transfer, gnotobiotic colonisation or germ-free comparison in animals.	The principal basis for causal claims in this field; translational relevance to human healthspan unresolved.
A-OBS	Observational or interventional animal study without transfer.	Mechanistically useful; does not establish that the microbiota is the operative variable.
IV	In-vitro, ex-vivo, organoid or cell-based work.	Concentration and context frequently non-physiological; see Section 4.2.
Confidence: high	Consistent findings from more than one human interventional or longitudinal study in an age-relevant population, with supporting mechanism.	In our appraisal, no claim linking a microbial feature causally to human healthspan currently reaches this grade.
Confidence: moderate	Consistent human observational evidence with supporting animal-transfer data but no adequate trial.	Applies to most metabolite–disease associations reviewed here.
Confidence: low	Limited or discordant human evidence, or mechanism established only in animals or in vitro.	Applies to most immune-aging-specific claims.
Confidence: very low	Single studies, non-age-relevant models, or evidence confined to disease or toxicological models.	Applies to claims derived from acute-injury and toxicology models.

Immunosenescence is the remodelling of adaptive and innate immunity with age. Its principal components are thymic involution with contraction of the naïve T-cell pool; accumulation of differentiated effector-memory and CD28-null T cells with oligoclonal expansion; restriction of the B-cell repertoire with reduced class-switch recombination and lower-affinity antibody; myeloid skewing of haematopoiesis; impaired dendritic-cell antigen presentation and reduced co-stimulatory capacity; diminished mucosal secretory IgA quantity and specificity; and reduced responsiveness to vaccination, which is the most clinically consequential functional read-out [2,9]. *Inflammaging* is the chronic, low-grade, sterile elevation of circulating inflammatory mediators—most consistently IL-6, TNF, CRP and soluble TNF receptors—observed in older populations and predictive of disability and mortality [3,10,11]. *Immune exhaustion* is an antigen-driven, reversible hypofunctional T-cell state characterised by sustained inhibitory receptor expression (PD-1, TIM-3, LAG-3) and defined transcriptional and epigenetic programmes; it is mechanistically distinct from senescence, although the two co-occur. *Cellular senescence* is stable cell-cycle arrest with resistance to apoptosis; the *senescence-associated secretory phenotype* (SASP) is the accompanying secretion of cytokines, chemokines, proteases and growth factors that propagate inflammation locally and, in part, systemically [12,13].

Conflating these constructs has practical consequences. An intervention that lowers circulating IL-6 has not necessarily improved naïve T-cell output or vaccine responsiveness; an intervention that increases faecal butyrate has not necessarily reduced senescent-cell burden. Wherever possible we therefore state which feature of immune aging a given microbial exposure has been shown to affect and note where the link is assumed rather than measured.

2.3 An evidence-grading scheme

We assign each major claim in this review a design descriptor and a confidence grade (Table 1). Design descriptors are: *H-CS,* human cross-sectional or case–control association; *H-LONG*, human longitudinal or prospective cohort; *H-RCT*, randomised controlled trial in humans; *H-MR*, Mendelian randomisation or other genetic instrumental-variable analysis; *A-TRANSFER*, microbiota transfer, gnotobiotic colonisation or germ-free comparison in animals; *A-OBS*, observational or interventional animal study without transfer; *IV*, in-vitro, ex-vivo or organoid work. Confidence grades are: *high* , consistent findings from more than one human interventional or longitudinal study in an age-relevant population, supported by mechanistic evidence; *moderate* , consistent human observational evidence with supporting animal-transfer data but no adequate trial; *low* , limited or discordant human evidence, or mechanism established only in animals or in vitro; *very low* , single studies, non-age-relevant models, or evidence confined to disease or toxicological models. Applying this scheme to the field yields a sobering distribution: the great majority of mechanistic claims linking specific microbial features to human healthspan currently attract low or moderate confidence, and we consider none to reach high confidence for a causal effect on human healthspan endpoints.

We have applied two additional rules. General toxicology and acute disease models—heavy-metal exposure, perfluorinated compounds, cardiopulmonary bypass, heat stroke, sleep deprivation, chemically induced colitis—are cited only where they establish a molecular mechanism that is otherwise inaccessible and are explicitly not treated as evidence about healthy aging, because the magnitude, kinetics and context of barrier injury in these models differ qualitatively from age-

associated change. And because bidirectionality is the norm rather than the exception, we describe relationships as bidirectional unless directionality has been established experimentally: disease, drug exposure, altered dietary intake, reduced mobility and systemic inflammation each reshape the microbiota, so an association observed in patients cannot be read as microbial causation of their disease.

2.4 Determinants of the older-adult microbiome: an interpretive framework

Any statement of the form "aging is associated with X" should be read as shorthand for "cohorts of older adults differ from cohorts of younger adults in X, for reasons that include but are not limited to age". The principal alternative and mediating explanations are as follows, and we return to them throughout.

Frailty and functional status. In older-adult cohorts, frailty indices correlate with microbial composition more strongly than chronological age does, and several taxa initially reported as age-associated track frailty instead [14,15] (H-CS, moderate). Because frailty is also associated with reduced dietary variety, lower fibre intake, reduced mobility and higher drug burden, it should be treated as a composite exposure rather than a covariate to be adjusted away.

Residential setting. Long-term residential care is accompanied by markedly less diverse diets and by microbial profiles distinguishable from those of community-dwelling older adults of the same age, with intermediate profiles in rehabilitation and day-hospital settings [16]. Studies that recruit from institutions and compare with community-dwelling young controls confound age with residence.

Medication. Older adults are exposed to proton-pump inhibitors, metformin, antibiotics, laxatives, opioids, antidepressants, statins and antipsychotics, several of which have effect sizes on microbial composition comparable to or larger than that of age; antibiotic courses in hospitalised older adults produce measurable and sometimes persistent compositional change [17] (H-LONG, moderate). Polypharmacy count is itself associated with compositional variation and should be reported.

Habitual diet and dietary variety. Fibre quantity and diversity, protein source, polyphenol intake and overall dietary variety are strong and modifiable determinants of both composition and fermentation output; dietary diversity is associated with microbial evenness and with SCFA-producer abundance in older adults, although the association attenuates in the oldest groups [18] (H-CS, moderate).

Physical activity and body composition. Habitual activity and cardiorespiratory fitness are associated with SCFA-producer abundance in older adults, with the clearest signals in those with excess adiposity [19,20] (H-CS/H-RCT, low to moderate).

Bowel transit and stool consistency. Transit time is among the strongest single correlates of faecal microbial composition and of the faecal metabolome; constipation is common in older adults, and slower transit shifts fermentation from saccharolytic towards proteolytic pathways, raising faecal branched-chain fatty acids, ammonia and phenolic compounds while lowering butyrate. Failure to record stool form or transit is a recurrent limitation of aging-microbiome studies.

Geography, urbanisation and socioeconomic position. In large population studies, geography explains more compositional variance than diet or ethnicity, urbanisation is associated with reduced intra-individual and increased inter-individual diversity, and socioeconomic position is independently associated with composition [21-26] (H-CS, moderate). Comparisons across cohorts recruited on different continents are therefore poorly interpretable as evidence about age.

Comorbidity and organ function. Renal impairment, heart failure, hepatic dysfunction, diabetes and malignancy alter both microbial composition and the circulating concentrations of microbial metabolites through changes in substrate delivery and, critically, in clearance. This is the dominant confounder in the TMAO literature (Section 4.5).

Two conclusions follow. First, studies of the aging microbiome should report frailty status, residence, medication list, dietary intake, physical activity, stool form and renal function as a minimum, and analyses that omit them should be interpreted accordingly. Second, the appropriate contrast for geroscience questions is usually not young versus old but, within older adults, robust versus frail, or high versus low functional trajectory, ideally longitudinally.

3. Microbial Features of Older Age: What Is Reproducible

3.1 Diversity, composition and heterogeneity

Alpha diversity. Reduced faecal alpha diversity in older adults is the most frequently reported finding, and it is less robust than its ubiquity suggests. Many cohorts report lower Shannon or Chao1 indices in older than younger participants [27-29] (H-CS, moderate), but the effect is inconsistent across cohorts, is attenuated or abolished by adjustment for frailty, medication and diet, and is reversed in some healthy-longevity cohorts, in which evenness is preserved or increased [6, 8]. Longitudinal and cross-

sectional data in non-human primates and other mammals similarly show that diversity does not decline uniformly with age; the more consistent age signal is increased inter-individual divergence and reduced temporal stability [30,31] (A-OBS, moderate). The most defensible summary is that older age is associated with increased heterogeneity and reduced within-person stability, and only variably with lower diversity.

Composition. Recurrent, though not universal, compositional differences include a lower relative abundance of *Bifidobacterium* and of *Faecalibacterium*, contraction of butyrate-producing Lachnospiraceae and Ruminococcaceae including *Roseburia* and *Coprococcus*, and expansion of facultative anaerobes of the Enterobacteriaceae, including *Escherichia* and *Klebsiella* [28,29,32-34] (H-CS, moderate). Two qualifications are important. Enterobacteriaceae expansion is a non-specific consequence of increased luminal oxygen and nitrate availability during mucosal inflammation and occurs in many acute and chronic conditions unrelated to age; its presence in older cohorts is therefore weak evidence for an age-specific process. And relative-abundance data cannot distinguish a true increase in one taxon from a decrease in others, an issue we return to in Section 3.2.

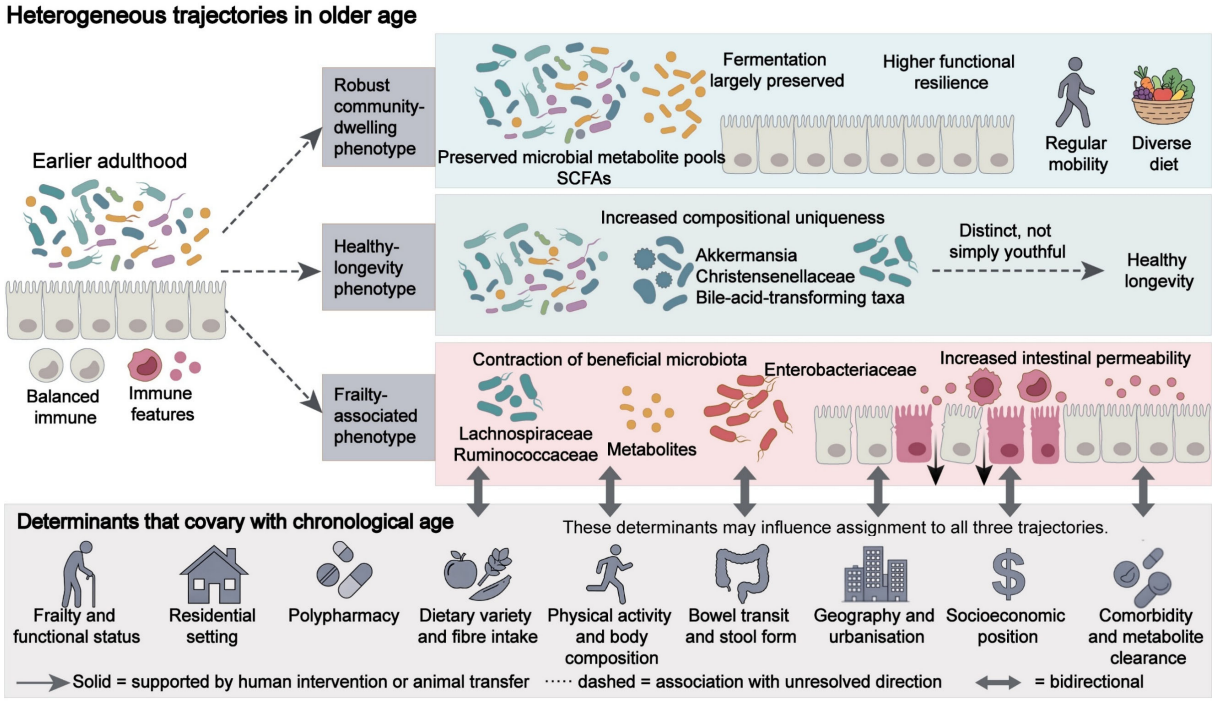


Figure 1. Heterogeneous trajectories of the intestinal microbiota in older age, and their principal determinants. The microbiota of older adults does not converge on a single dysbiotic configuration. At least three distinguishable trajectories are described in the literature: a robust, community-dwelling phenotype in which fibre-fermenting taxa and fermentation capacity are largely preserved; a healthy-longevity phenotype (centenarian and long-lived cohorts) characterised by increased compositional uniqueness and enrichment of Akkermansia, Christensenellaceae and bile-acid-transforming taxa; and a frailty-associated phenotype with contraction of Lachnospiraceae and Ruminococcaceae and expansion of facultative anaerobes including Enterobacteriaceae. The lower panel lists the determinants that covary with chronological age and that account for a substantial and often unquantified fraction of reported age differences: frailty and functional status, residential setting, polypharmacy, habitual dietary variety and fibre intake, physical activity, bowel transit time and stool form, geography and urbanisation, socioeconomic position, and comorbidity with its effects on metabolite clearance. Arrow convention: solid arrows denote relationships supported by human interventional or animal transfer experiments; dashed arrows denote associations for which directionality has not been established. Figure drawn to scale of evidence, not of effect size.

Healthy longevity as a distinct phenotype. Centenarian and long-lived cohorts show enrichment of *Akkermansia muciniphila*, *Christensenellaceae*, *Bifidobacterium* and bile-acid-transforming taxa, and increased compositional uniqueness relative to younger adults [6, 8] (H-CS, moderate). Whether these features contribute to longevity, are consequences of the physiology that permits it, or reflect selection of the fittest survivors cannot be resolved cross-sectionally. Their existence nonetheless refutes any model in which a single dysbiotic configuration is the necessary correlate of advanced age (Fig. 1).

Regional heterogeneity within the gut. Faecal samples represent the distal colonic lumen and are a poor

proxy for mucosa-adherent communities and for the small intestine, where age-associated changes in the duodenal and jejunal microbiota have been described that are not detectable in stool [32] (H-CS, low). Because barrier interactions, immune sampling and much of the physiologically relevant metabolite exposure occur at the mucosal surface and in the proximal gut, inference from faecal composition to mucosal immunology is indirect.

Beyond bacteria. The fungal, viral and archaeal components of the ecosystem, and bacterial strain-level and functional-gene variation, are addressed by few aging studies. Because functionally decisive traits—bile-salt hydrolase activity, butyryl-CoA:acetate CoA-transferase, TMA lyase (*cutC/D*), mucin-degrading glycoside hydrolases—are strain- rather than genus-level properties, taxonomic summaries at genus level may misrepresent functional capacity in either direction.

3.2 Methodological determinants of reported microbial signatures

A substantial fraction of the inconsistency in this literature is technical, and any proposed "aging microbial signature" should be evaluated against the following sources of variation before biological interpretation.

Sampling. Stool versus mucosal biopsy, luminal versus mucus-associated fractions, single versus repeated sampling, and time since last defecation all affect measured composition; within-person day-to-day variation can approach between-person variation for some taxa [35,36] (H-LONG, moderate). Storage temperature, freeze–thaw cycles, use of preservative buffers and transit time to the laboratory introduce further systematic differences.

Nucleic-acid extraction and amplification. Extraction chemistry and the efficiency of lysis of Gram-positive cell walls are among the largest single contributors to inter-laboratory disagreement, systematically biasing Firmicutes-to-Bacteroidetes ratios. In amplicon sequencing, primer choice and the hypervariable region targeted (V1–V2, V3–V4, V4) alter apparent abundances and, in particular, systematically under-detect *Bifidobacterium* with commonly used V3–V4 primer sets—directly relevant to the widely reported age-associated decline in this genus. Copy-number variation of the 16S rRNA gene further distorts relative abundance.

Sequencing approach and annotation. 16S rRNA amplicon sequencing provides genus-level resolution and no direct functional information; functional profiles inferred by predictive tools are not equivalent to measured gene content. Shotgun metagenomics provides species- and gene-level resolution but depends on read depth, host-DNA depletion and reference-database completeness; taxonomic assignments change with database version, so studies using different releases are not strictly comparable. Metatranscriptomics, metaproteomics and enzyme-activity assays address expression rather than potential, and are rarely applied in aging cohorts.

Relative versus absolute abundance. Sequencing yields compositional data constrained to a constant sum. Analysis with methods that ignore this constraint generates spurious negative correlations and inflated false-positive rates; appropriate approaches use log-ratio transformations or explicit compositional models. Quantitative approaches using flow cytometry or spike-in standards show that changes in total microbial load can invert the direction of apparent taxon-level change, and absolute quantification is almost never reported in aging studies.

Metabolite measurement. Targeted and untargeted platforms differ in coverage, sensitivity and quantitative accuracy; SCFAs are volatile and require appropriate derivatisation, acidification and headspace handling, and faecal SCFA concentration reflects the difference between production and colonic absorption rather than production rate. Absorption exceeds 90–95% of produced SCFAs, so faecal concentrations may correlate poorly or even inversely with fermentation rate. Stable-isotope infusion or arteriovenous sampling is required to estimate flux, and has been applied in only a handful of human studies. Circulating metabolite concentrations additionally reflect hepatic first-pass extraction and renal clearance.

Analysis and reporting. High-dimensional microbiome data with modest sample sizes invite multiplicity and overfitting. Studies frequently report nominally significant taxa without adequate false-discovery control, and machine-learning classifiers are often reported without external validation, so apparent diagnostic performance is optimistically biased. Batch effects arising from sequencing run, extraction batch or recruitment site are commonly unmodelled and can align with the exposure of interest when cases and controls are recruited at different times or sites. Heterogeneity in protocols and incomplete metadata reporting have been documented as barriers to meta-analysis [37] (moderate).

Two implications follow for the interpretation of Sections 4 and 5. Compositional "signatures" of aging reported without absolute quantification, transit-time data, external validation and compositional-aware statistics should be regarded as hypothesis-generating. And because the same technical factors affect metabolite quantification, inferences that move from faecal metabolite concentration to systemic host exposure require independent measurement of circulating or tissue concentrations.

4. Mechanistic Pathways from Microbial Function to Immune Aging

This section is organised by microbial function rather than by taxon, because function is what the host senses. For each pathway we state the proposed mechanism, the evidence types available, and the principal uncertainties.

4.1 Barrier function and microbial translocation

The proposed pathway is straightforward: reduced fermentative substrate delivery to colonocytes, thinning of the mucus layer and altered tight-junction protein expression increase paracellular permeability, permitting translocation of lipopolysaccharide (LPS), flagellin, peptidoglycan and bacterial DNA to the portal and systemic circulation, where they engage TLR4–MyD88–NF-κB and inflammasome signalling and sustain low-grade cytokine production [38,39] (IV/A-OBS, moderate for the molecular steps).

In humans, the evidence that this pathway operates as an age-related process is weaker than usually presented. Circulating markers used as proxies for permeability—LPS-binding protein, soluble CD14, zonulin, intestinal fatty-acid-binding protein—are indirect, and zonulin assays in particular have documented specificity problems, with commercial kits detecting proteins other than pre-haptoglobin-2. Direct permeability measurement by differential sugar absorption is rarely performed in aging cohorts. Studies reporting higher permeability markers and higher IL-6 or CRP in older adults are cross-sectional [40-42] (H-CS, low to moderate), and because systemic inflammation itself increases permeability, the direction of the association is not established. A sex difference in epithelial permeability and associated inflammation has been reported in mice [40] (A-OBS, very low as a basis for human sex-specific claims).

Mechanistic support for the pathway comes largely from injury models—cadmium and perfluoro-octanesulfonic acid exposure, cardiopulmonary bypass, heat stroke, sleep deprivation, chemically induced colitis [43-50] (A-OBS/IV). These establish that barrier disruption can drive systemic inflammation, but they involve acute, high-magnitude injury and are not models of healthy aging; we cite them for molecular mechanism only. Age-relevant support is provided by studies in aged mice showing reduced barrier integrity that is modifiable by monocyte-directed intervention [40,42] (A-OBS, low), and by small human studies of intestinal alkaline phosphatase and fibre supplementation reporting reduced endotoxaemia markers [51,52] (H-RCT small/IV, low).

Terminology: we use "increased intestinal permeability" as the primary term and retain "leaky gut" only once, as an explanatory gloss, because the popular term implies a discrete pathological entity that has not been defined clinically.

4.2 Short-chain fatty acids: a context-dependent signal

SCFAs are saturated aliphatic organic acids with fewer than six carbon atoms produced by anaerobic fermentation of non-digestible carbohydrate; acetate (C2), propionate (C3) and butyrate (C4) account for the large majority, with valerate (C5) and the branched-chain isomers isobutyrate and isovalerate—products of amino-acid rather than carbohydrate fermentation—present at lower concentrations [53-55]. We note that definitions in the literature vary between "fewer than six" and "two to six" carbons, and that formate (C1) is sometimes included; we use the fewer-than-six-carbon definition and specify chain length where it matters. Fermentation occurs predominantly in the proximal colon, where substrate availability is highest, with the caecum and ascending colon the principal sites and progressively lower concentrations distally; the small distal intestine contributes minimally in health. Acetate is produced by many taxa via the acetyl-CoA and Wood–Ljungdahl pathways, propionate via the succinate, acrylate and propanediol pathways in distinct lineages, and butyrate mainly via butyryl-CoA:acetate CoA-transferase and, less commonly, butyrate kinase, in Lachnospiraceae and Ruminococcaceae [56] (IV/genomic, high for pathway assignment).

Local versus systemic exposure. Luminal colonic SCFA concentrations are in the 10–100 mmol/L range; portal concentrations are two to three orders of magnitude lower, and peripheral venous concentrations lower again, typically low micromolar for butyrate because of colonocyte oxidation and hepatic extraction. This gradient is decisive for interpretation, because most in-vitro immunological studies apply 0.5–5 mmol/L SCFA to immune cells—concentrations attainable in the colonic lumen and in mucosal tissue but not in the systemic circulation. Effects demonstrated at millimolar concentrations should therefore be attributed to the gut mucosa and gut-draining lymphoid tissue, not to systemic immunity, unless systemic concentrations have been measured.

Receptor- and concentration-dependence. SCFAs signal through FFAR2 (GPR43), FFAR3 (GPR41) and HCAR2 (GPR109A), which differ in ligand preference, potency and cellular distribution: FFAR2 responds to acetate and propionate at low micromolar to millimolar concentrations, FFAR3 to propionate and butyrate, and HCAR2 to butyrate at higher concentrations [57] (IV, high). Because receptor expression differs across neutrophils, macrophages, dendritic cells, T-cell subsets, enteroendocrine cells and enteric neurons, and because

HDAC inhibition by butyrate requires concentrations that are physiological in the lumen but not systemically, the same metabolite can be pro- or anti-inflammatory depending on cell type, concentration and receptor availability.

Immunological effects are not uniformly beneficial. Butyrate and propionate promote peripheral regulatory T-cell generation and enhance FOXP3 expression through HDAC inhibition in mice and in human in-vitro systems [57-59] (A-TRANSFER/IV, moderate). But SCFAs also impair neutrophil antimicrobial and antiviral function in an age-dependent manner [60,61] (A-OBS/IV, low), can suppress protective effector responses in the context of infection, and in specific settings promote rather than restrain inflammatory signalling. Butyrate is a preferred energy substrate for normal colonocytes yet inhibits proliferation of transformed colonocytes that have shifted to glycolysis—the "butyrate paradox"—so its net effect depends on host cell metabolic state. In germ-free and antibiotic-treated animals, SCFA supplementation can worsen as well as improve outcomes depending on model and timing. The claim that higher SCFA is uniformly better is therefore not supportable.

Abundance, activity and concentration are not interchangeable. The abundance of taxa carrying butyrate-synthesis genes predicts neither expression nor flux; faecal butyrate concentration reflects production minus absorption; and because absorption is near-complete, low faecal butyrate may indicate either low production or efficient uptake. Studies that infer "reduced butyrate production in aging" from lower relative abundance of *Roseburia* constitute weak evidence.

Barrier and metabolic effects. Butyrate supports tight-junction assembly, mucin expression and hypoxia-inducible-factor-dependent epithelial barrier maintenance, and SCFAs influence appetite, incretin secretion and hepatic gluconeogenesis [62-64] (IV/A-OBS, moderate; H-RCT, low). Human trials of fibre or SCFA supplementation in older adults with functional endpoints remain few and small.

4.3 Bile acids, tryptophan derivatives and polyamines

Bile acids. Microbial bile-salt hydrolases deconjugate primary bile acids, and 7α-dehydroxylating taxa generate deoxycholic and lithocholic acid; further microbial epimerisation yields isoallolithocholic and 3-oxolithocholic acid, which modulate the Treg–Th17 balance through distinct receptor-independent and RORγt-dependent mechanisms, while FXR and TGR5 mediate broader metabolic and immune effects [65,66] (A-TRANSFER/IV, moderate). Human data in older adults are sparse, and secondary bile acids have both immunoregulatory and, at higher colonic concentrations, genotoxic potential, so directionality is context-dependent.

Tryptophan derivatives. Microbial tryptophan metabolism generates indole, indole-3-aldehyde, indole-3-propionate and indole-3-acetate, which activate the aryl hydrocarbon receptor on intestinal epithelial cells, group 3 innate lymphoid cells, T cells and dendritic cells, supporting IL-22-dependent barrier maintenance and antimicrobial peptide production [67-70] (A-TRANSFER/IV, moderate). Competing host pathways matter: with age and inflammation, IDO1-dependent kynurenine production increases and the circulating kynurenine-to-tryptophan ratio rises, which is associated with frailty and mortality; because IDO1 and microbial indole pathways compete for the same substrate, tryptophan metabolite profiles reflect host as well as microbial activity, and cannot be attributed to the microbiota without isotope tracing or germ-free comparison.

Polyamines. Spermidine and spermine, produced by both host and microbiota, induce autophagy and have been associated with reduced mortality in observational cohorts and with lifespan extension in model organisms [71] (H-CS/A-OBS, low). The proportion of circulating polyamine derived from microbial rather than host or dietary sources in humans is unquantified, and human interventional data with functional endpoints are limited to small trials.

Polyphenol-derived metabolites. Because most dietary polyphenols are poorly absorbed intact, their biological activity is largely attributable to microbial catabolites—phenolic acids, urolithins from ellagitannins, equol from daidzein, valerolactones from flavan-3-ols—whose formation depends on possession of specific microbial pathways [72-76]. This generates discrete metabotypes: only a minority of individuals produce equol, and urolithin-producing capacity is similarly stratified, so identical intake yields markedly different systemic exposure. This is a mechanistic argument for stratified rather than population-level dietary recommendations, and it also means that trials of polyphenol supplementation that do not stratify by metabotype are likely to be underpowered for true effects [77-81] (H-RCT, low; IV/A-OBS, moderate).

4.4 Interfaces between microbial function and discrete features of immune aging

Aligning microbial functions with specific immune-aging features, rather than with inflammation generally, exposes where the evidence is thin (Table 2).

Mucosal IgA. Commensal-driven IgA induction is well established in mice, and both IgA quantity and antigen-specificity change with age, with reduced coating

of specific taxa reported in aged animals (A-OBS, moderate). Human data on mucosal IgA in older adults, and on whether microbial intervention modifies it, are limited (H-CS, low).

Table 2. Evidence appraisal for the principal microbial functions and metabolites proposed to affect immune aging. Design codes and confidence grades are defined in Table 1. Sample sizes are reported for representative human studies where a single study is cited; for multi-experiment animal and in-vitro evidence, n varies by experiment and is reported in the cited primary articles.

Microbial function / metabolite	Population or model (n)	Design	Exposure measured	Immune-aging feature or outcome	Principal finding	Major limitation	Confidence
Saccharolytic fermentation to butyrate (Lachnospiraceae, Ruminococcaceae; but-CoA transferase)	ELDERMET cohort: 178 older adults across community, day-hospital, rehabilitation and long-term residential settings; younger comparison participants were analysed separately	H-CS	Relative abundance of butyrate-synthesis taxa or genes; faecal SCFA concentration	Circulating IL-6, TNF, CRP; permeability markers	Lower abundance of butyrate producers and lower faecal butyrate reported in older, particularly frail, cohorts	Relative abundance does not measure flux; faecal concentration reflects production minus near-complete absorption; frailty, diet and transit unadjusted in most studies	Moderate for association; low for causation
Butyrate: regulatory T-cell induction	Murine colonic Treg experiments and human naive CD4 T-cell cultures from healthy donors; n varied by experiment in the cited primary studies	A-TRANSFER; IV	0.5–5 mmol/L butyrate or propionate	FOXP3 induction, histone acetylation, suppressive function	HDAC-dependent enhancement of peripheral Treg generation	Concentrations physiological in colonic lumen but not systemically; no demonstration in older humans	Moderate (mechanism); very low (systemic relevance in older adults)
SCFAs: innate effector function	Aged versus young mice and neutrophils from younger and older adult donors; n varied by assay in the cited primary studies	A-OBS; IV	Millimolar SCFA exposure	Neutrophil migration, antimicrobial peptide release, antiviral capacity	Age-dependent impairment of neutrophil antimicrobial and antiviral function	Direction of effect opposite to the "SCFA is beneficial" framing; small studies, model-dependent	Low
Secondary and microbially epimerised bile acids (BSH, 7alpha-dehydroxylation)	Gnotobiotic and antibiotic-treated mouse experiments; limited human cohorts; n varied across the cited studies	A-TRANSFER; IV	Deoxycholate, lithocholate, 3-oxoLCA, isoalloLCA	Treg/Th17 balance; FXR- and TGR5-dependent signalling	Distinct bile-acid species shift Treg–Th17 balance in opposite directions	Almost no data in older adults; higher colonic concentrations are genotoxic, so directionality is dose-dependent	Moderate (mechanism); very low (human aging)
Tryptophan-to-indole conversion (AhR ligands)	Murine ILC3 and epithelial experiments plus human association studies; n varied across	A-TRANSFER; IV; H-CS	Indole-3-aldehyde, indole-3-propionate, indole-3-acetate	AhR-dependent IL-22, antimicrobial peptides, barrier integrity	Microbial indoles support barrier and mucosal immunity; kynurenine:tryptophan	Host IDO1 competes for the same substrate, so metabolite profiles are not microbe-specific without tracing or germ-free comparison	Moderate (mechanism); low (attribution to microbiota in humans)

	the cited studies				ratio rises with age and frailty		
Polyamines (spermidine, spermine)	Bruneck prospective cohort (n = 829) plus model-organism experiments with study-specific n	H-CS; A-OBS	Dietary and circulating polyamine	Autophagy induction; mortality association	Higher intake associated with lower mortality; lifespan extension in models	Microbial versus host and dietary contribution unquantified in humans; no adequately powered trial	Low
Polyphenol catabolites (urolithins, equol, valerolactones)	Representative older-adult urolithin A intervention (n = 60) plus smaller metabotype-stratified studies	H-RCT; IV	Ellagitannin, isoflavone and flavan-3-ol intake with metabolite measurement	Inflammatory markers; mitophagy; muscle endpoints in early trials	Systemic exposure depends on possession of specific microbial pathways, generating discrete producer and non-producer metabotypes	Unstratified trials dilute true effects; older-adult data limited to small trials	Low to moderate
cutC/D-dependent TMA generation and hepatic FMO3 oxidation to TMAO	Prospective cardiovascular cohort (n = 4,007) plus atherosclerosis-prone mouse experiments with study-specific n	H-LONG; A-TRANSFER; H-MR	Plasma TMAO; dietary choline and carnitine	Incident major adverse cardiovascular events; platelet reactivity; NLRP3 activation	Higher plasma TMAO associated with cardiovascular events; TMA-lyase inhibition reduces atherosclerosis in mice	Renal clearance dominates plasma concentration and is rarely measured directly; FMO3 varies by genotype and sex; fish contributes preformed TMAO; Mendelian randomisation inconsistent; no outcome trial	Moderate for association; low for causation in humans
Microbial products and barrier permeability (LPS, flagellin, peptidoglycan)	Multiple small human aging cohorts plus aged-mouse and acute-injury experiments; n varied across studies	H-CS; A-OBS; IV	LPS-binding protein, soluble CD14, zonulin, I-FABP; rarely direct sugar-permeability testing	Monocyte activation; IL-6, TNF, CRP	Higher permeability markers and inflammatory markers in older, especially frail, cohorts	Surrogate markers are indirect and zonulin assays have specificity problems; inflammation itself increases permeability, so direction is unresolved; mechanistic support comes largely from acute toxicological models	Low to moderate
Commensal-driven mucosal IgA	Germ-free and colonised mouse experiments plus limited human cohorts; n varied across studies	A-TRANSFER; H-CS	Colonisation status; IgA coating of taxa	Secretory IgA quantity and antigen specificity	Commensals induce IgA; coating specificity changes with age in animals	Human mucosal IgA data in older adults sparse; no intervention data	Moderate (animals); low (humans)
Microbiota and thymic output / naive T-cell pool	Germ-free and antibiotic-treated animal experiments; n varied by experiment	A-TRANSFER	Colonisation or depletion	Thymic and peripheral T-cell development	Altered T-cell development in the absence of a microbiota	No human evidence that microbiota-targeted intervention alters thymic output or naive T-cell frequency in older adults	Very low (human aging)

Microbiota and vaccine responsiveness	Antibiotic-perturbation influenza-vaccine trial (n = 22) plus probiotic and prebiotic trials in older adults (typically n = 20-80)	H-RCT	Antibiotic depletion; defined strains or prebiotic substrates	Vaccine-specific antibody titre and cellular response	Microbiota depletion reduces influenza vaccine responses in those with low pre-existing immunity; some trials in older adults report modest titre increases	Inconsistent replication, heterogeneous strains and endpoints, small samples; the most tractable endpoint and the one most in need of adequately powered trials	Low to moderate
Dysbiosis, cGAS–STING signalling and senescence	Murine bone-loss and senescent-cell experiments; n varied by experiment	A-OBS; IV	Microbial perturbation; DNA sensing	Osteoclastogenesis; SASP amplification	Microbial perturbation linked to macrophage cGAS–STING activation and bone loss	No human evidence that microbiota modulation reduces senescent-cell burden in any tissue	Low

Naïve T-cell pool and thymic output. Germ-free and antibiotic-treated animals show altered thymic and peripheral T-cell development (A-TRANSFER, moderate), but no human evidence indicates that microbiota-targeted intervention alters thymic output or naïve T-cell frequency in older adults (very low). Statements linking dysbiosis to immunosenescence should be read as referring to the effector and inflammatory compartments, not to thymic involution.

Vaccine responsiveness. This is the most tractable functional endpoint. Antibiotic-induced microbiota depletion reduces influenza vaccine antibody responses in humans with low pre-existing immunity, and some probiotic and prebiotic trials in older adults report modest increases in vaccine-specific antibody titres, with inconsistent replication and heterogeneous endpoints (H-RCT, low to moderate). We regard vaccine response as the priority endpoint for near-term trials because it is standardised, clinically meaningful and mechanistically informative.

Myeloid compartment and trained immunity. Microbial products and metabolites influence haematopoietic output and monocyte functional programming (A-TRANSFER/IV, moderate); age-relevant human evidence is largely limited to associations between permeability markers and monocyte activation phenotypes [40] (H-CS, low).

Cellular senescence and SASP. Dysbiosis-associated activation of macrophage cGAS–STING signalling has been linked to osteoclastogenesis and bone loss, and senescent cells amplify inflammatory signalling [13,82] (A-OBS/IV, low to moderate). Whether microbiota-targeted intervention reduces senescent-cell burden in any human tissue is unknown (very low).

Immune exhaustion. Microbial metabolites influence T-cell exhaustion programmes and ferroptosis susceptibility in tumour models [83,84] (A-OBS/IV, low). Extrapolation from tumour microenvironments to immune aging in non-malignant tissue is not currently justified.

4.5 TMAO: a case study in confounding

TMAO illustrates why metabolite-level causal claims require caution. Dietary choline, phosphatidylcholine, betaine and L-carnitine are converted by microbial cutC/D and cntA/B enzymes to trimethylamine, which is absorbed and oxidised by hepatic flavin-containing monooxygenase 3 (FMO3) to TMAO [85,86]. Higher circulating TMAO is associated with incident major adverse cardiovascular events in multiple cohorts [87,88] (H-LONG, moderate), and mechanistic work in mice shows impaired reverse cholesterol transport, enhanced platelet reactivity via altered calcium signalling, and NLRP3 inflammasome activation, with reduction of atherosclerosis by dietary modification or TMA-lyase inhibition [89-93] (A-TRANSFER, moderate).

Four considerations limit the inference to older humans. First, renal clearance dominates plasma TMAO: concentrations rise several-fold with declining glomerular filtration rate, and chronic kidney disease is highly prevalent in older adults. Much of the association between TMAO and cardiovascular outcomes may therefore be confounded by, or mediated through, renal function, and studies that do not adjust for measured GFR—or that adjust only for creatinine—cannot separate these. Second, hepatic FMO3 activity varies with genotype, sex and hormonal status, so the same TMA production yields

different TMAO exposure between individuals. Third, dietary sources of TMAO precursors include fish, which contributes preformed TMAO directly and is associated with favourable rather than adverse cardiovascular outcomes; single measurements therefore conflate distinct exposures. Fourth, Mendelian randomisation analyses have not consistently supported a causal effect of circulating TMAO on cardiovascular disease (H-MR, low), and no trial has shown that lowering TMAO improves clinical outcomes in humans. We accordingly describe TMAO as a metabolite associated with cardiovascular risk whose causal contribution in humans is unresolved (Fig. 2).

The symmetrical point applies to SCFAs: neither metabolite class should be labelled beneficial or harmful without specifying concentration, site, host clearance and measurement method. Table 2 records these qualifications for each metabolite class.

5. Age-Related Disease: Shared Versus Disease-Specific Mechanisms

Rather than summarise each disease separately, this section first identifies what is common across domains and then states what is specific to each. Table 3 provides the comparative appraisal, including the most consistently reported microbial features, key metabolites, strength of human evidence, evidence bearing on causality, intervention data, principal confounders and the outstanding question for each domain.

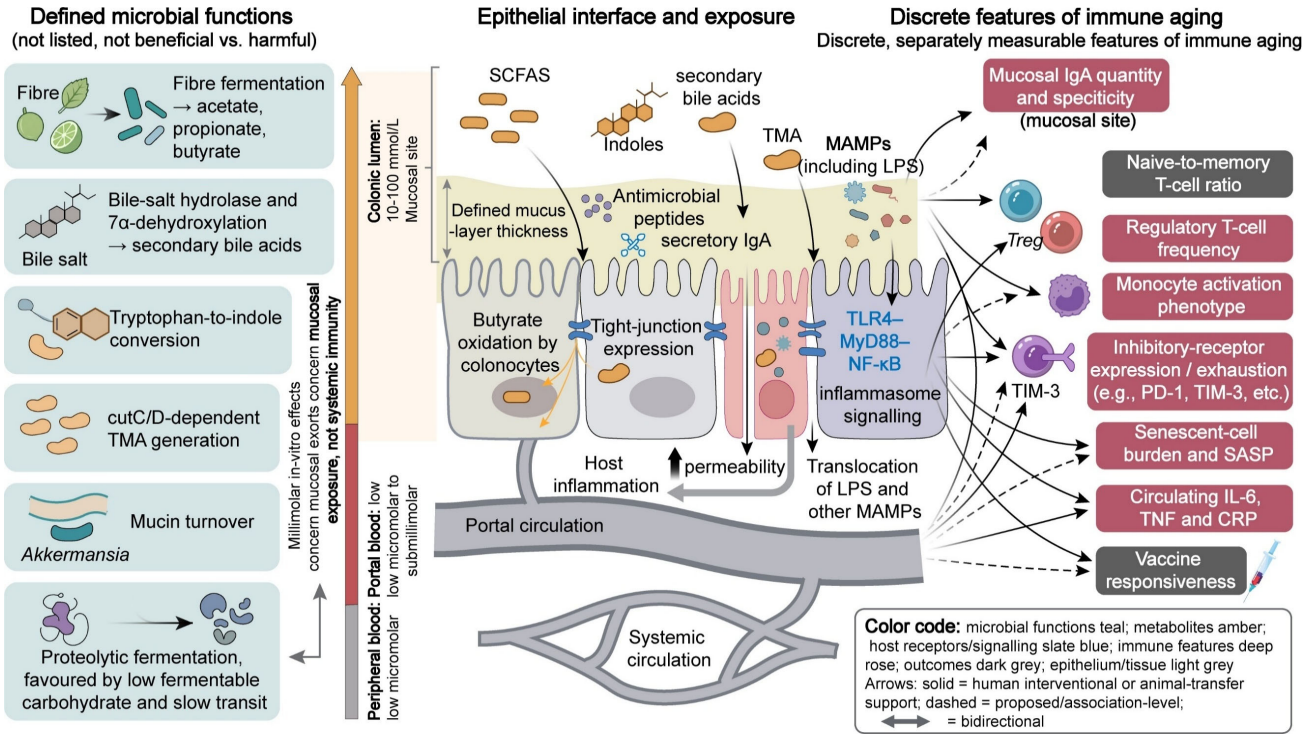


Figure 2. Microbial function, barrier integrity and discrete features of immune aging. Left, microbial functions rather than taxa: saccharolytic fermentation to acetate, propionate and butyrate; bile-salt hydrolase activity and 7alpha-dehydroxylation to secondary bile acids; tryptophan conversion to indole derivatives; cutC/D-dependent trimethylamine generation; and proteolytic fermentation, which predominates when transit is slow and fermentable carbohydrate is scarce. Centre, the epithelial interface: butyrate oxidation by colonocytes, mucin and tight-junction protein expression, antimicrobial peptide and secretory IgA output, and the consequences of increased permeability for translocation of lipopolysaccharide and other microbial-associated molecular patterns with TLR4-MyD88-NF-kappaB and inflammasome signalling. Right, discrete and separately measurable features of immune aging: mucosal IgA quantity and specificity, naive-to-memory T-cell ratio, regulatory T-cell frequency, monocyte activation phenotype, inhibitory-receptor expression denoting exhaustion, senescent-cell burden with its secretory phenotype, circulating IL-6, TNF and CRP, and vaccine responsiveness as the integrative functional read-out. A concentration scale is shown for short-chain fatty acids (colonic lumen, 10-100 mmol/L; portal blood, low micromolar to submillimolar; peripheral blood, low micromolar) to indicate which effects are attributable to mucosal and which to systemic exposure. Solid arrows, relationships supported by human interventional or animal transfer evidence; dashed arrows, proposed or association-level relationships; grey arrows, bidirectional relationships in which host inflammation also modifies the microbiota.

5.1 Three shared mechanisms

Reduced saccharolytic fermentation capacity. Contraction of fibre-fermenting, butyrate-producing taxa is the single most frequently reported microbial correlate across cardiometabolic, neurodegenerative, hepatic, musculoskeletal and oncological outcomes [28,29,94,95]. Its very ubiquity limits its specificity: a feature common to most chronic disease states, and shared with reduced fibre intake, immobility and slowed transit, functions poorly as a disease-specific mechanism and may be a marker of general physiological decline.

Increased translocation of microbial products with innate immune activation. The barrier–endotoxaemia–TLR4–NF-κB sequence recurs in metabolic disease, hepatic disease, neuroinflammation and vascular disease [39,96-99]. It is best supported in metabolic and hepatic disease, where portal delivery of microbial products to the liver is anatomically direct, and weakest for the central nervous system, where an additional barrier must be crossed.

Shifted metabolite signalling at host receptors. A limited set of receptors—FFAR2/3, HCAR2, AhR, FXR, TGR5, PXR—transduces microbial metabolite signals in all these tissues, which explains why interventions altering fermentation produce pleiotropic effects and why disease-specific targeting is difficult.

Against these commonalities, the disease-specific elements are as follows.

5.2 Cardiovascular disease

Two metabolite pathways are specific to this domain. TMAO generation is a discrete microbial enzymatic function with a defined mechanistic account and prospective human associations, subject to the confounding discussed in Section 4.5 [85-90,92,93]. SCFA-dependent blood-pressure regulation operates partly through FFAR3 and olfactory receptor 78 in the renal vasculature and through sympathetic modulation, and is supported by animal work and by associations between fibre intake, SCFA production and blood pressure [100-104] (A-TRANSFER, moderate; H-RCT, low). Fibre and Mediterranean-pattern intervention trials show improvements in blood pressure and lipid profile, but attribution of these effects to the microbiota specifically, rather than to the diets themselves, requires mediation analysis that has rarely been performed [103,104]. Butyrate reduces monocyte–endothelial adhesion in vitro and in animal models [105] (IV/A-OBS, moderate). Key confounders are renal function, diuretic and statin exposure, and dietary fish intake.

Table 3. Comparative appraisal across age-related disease domains. Design codes and confidence grades are defined in Table 1. The final column states the question whose answer would most change interpretation in that domain.

Domain	Most consistently reported microbial features	Key metabolites	Strength of human evidence	Evidence bearing on causality	Intervention data in humans	Principal confounders	Unresolved question
Cardiovascular	Higher TMA-producing capacity (cutC/D); lower abundance of butyrate producers	TMAO; acetate, propionate, butyrate	H-LONG: multiple prospective cohorts link plasma TMAO to major adverse cardiovascular events (moderate)	A-TRANSFER: TMA-lyase inhibition and dietary modification reduce atherosclerosis in mice; H-MR inconsistent (low for human causality)	Fibre and Mediterranean-pattern trials improve blood pressure and lipids without microbiota-specific mediation analysis; no TMAO-lowering outcome trial	Renal function (dominant), dietary fish, statins and diuretics, FMO3 genotype and sex	Does lowering TMAO by a microbiota-specific mechanism reduce events independently of renal function?
Metabolic (insulin resistance, type 2 diabetes)	Lower Faecalibacterium and Akkermansia; reduced fermentation capacity; higher permeability markers	SCFAs; secondary bile acids; branched-chain amino acids; LPS	H-CS and H-LONG associations with insulin resistance (moderate)	H-RCT: lean-donor faecal transfer transiently improves peripheral insulin sensitivity, dependent on baseline	Prebiotic and fibre trials: small, inconsistent improvements in insulin sensitivity and inflammatory markers; no	Metformin (substantial microbiome effect, partly microbiota-dependent action), adiposity, energy intake	Is the transient effect of transfer extendable and clinically meaningful in older adults?

				microbiota; A-TRANSFER supportive (moderate — the strongest causal evidence in the field)	data on incident diabetes or disability		
Hepatic (MASLD, alcohol-related liver disease, encephalopathy)	Reduced diversity; expansion of Enterobacteriaceae; altered bile-acid-transforming taxa; ethanol-producing taxa	LPS via portal circulation; primary and secondary bile acids; microbial ethanol	H-CS with strong anatomical rationale (moderate)	A-TRANSFER supportive; small H-RCT of faecal transfer in encephalopathy and alcohol-related liver disease (low to moderate)	Small randomised transfer trials with favourable safety and surrogate outcomes; no hard-outcome data	Portal hypertension, cholestasis, alcohol intake, lactulose and rifaximin exposure	Do transfer-based approaches change decompensation or survival rather than surrogates?
Neurodegenerative (Alzheimer's, Parkinson's)	Lower Prevotellaceae and butyrate producers; higher Akkermansia in Parkinson's disease (direction disputed)	SCFAs; indoles; bile acids; microbial extracellular vesicles	H-CS associations, frequently discordant between cohorts (low)	A-TRANSFER: patient-derived microbiota exacerbates motor deficits in alpha-synuclein-overexpressing mice; observational human support from prodromal constipation and vagotomy registry data (moderate in animals, low in humans)	Small, short, heterogeneous probiotic and prebiotic trials with effects mainly on constipation; faecal transfer limited to case series	Reverse causation via prodromal constipation, reduced intake and mobility; dopaminergic and anticholinergic drugs	Does intervening before motor or cognitive onset alter progression, or is the association a consequence of prodromal disease?
Musculoskeletal (sarcopenia, osteoporosis)	Lower fermentation capacity; associations with grip strength and gait speed	SCFAs; amino acids; secondary bile acids	H-CS associations with muscle mass and function (low)	A-TRANSFER: germ-free and antibiotic-treated animals show reduced muscle mass, partly reversible by colonisation or SCFA; Th17–IL-17 and cGAS–STING pathways in bone loss (moderate in animals)	One postbiotic preparation with preclinical and small clinical sarcopenia data; no fracture or incident-sarcopenia trial	Protein and energy intake, vitamin D, physical activity, glucocorticoids, sex steroids	Does any microbiota-targeted intervention alter muscle function or fracture rate in older adults?
Cancer and cancer therapy	Fusobacterium nucleatum, colibactin-producing E. coli, enterotoxigenic Bacteroides	Secondary bile acids; genotoxins; SCFAs	H-CS for carcinogenesis and for immunotherapy response (moderate)	A-TRANSFER for carcinogenesis; phase I human transfer	Phase I faecal transfer in melanoma; antibiotic avoidance around	Antibiotic exposure, tumour stage, prior therapy; trial populations frequently	Does modulation improve outcomes in older, frailer patients, in whom

	fragilis; responder-associated taxa in immunotherapy			restores checkpoint-inhibitor responsiveness (moderate — best-evidenced clinical application)	immunotherapy	younger than geroscience target populations	immunotherapy tolerance differs?

5.3 Metabolic disease and insulin resistance

This is the domain with the strongest causal evidence, because transfer experiments have been performed in humans as well as animals. Faecal transfer from lean donors transiently improves peripheral insulin sensitivity in men with metabolic syndrome, with effects that wane by 18 weeks and depend on recipient baseline microbiota (H-RCT, moderate) [106,107]. Mechanisms include metabolic endotoxaemia and TLR4-dependent inflammation, SCFA effects on incretin secretion and hepatic gluconeogenesis, microbial modification of bile-acid signalling through FXR and TGR5, and microbial branched-chain amino-acid metabolism [108-114] (A-TRANSFER/IV, moderate). Metformin is a major confounder: it alters microbial composition substantially, and part of its glucose-lowering effect appears microbiota-dependent, so studies of type 2 diabetes that do not stratify by metformin exposure are difficult to interpret. Prebiotic and SCFA-directed interventions improve insulin sensitivity and inflammatory markers inconsistently and with small effect sizes; effects on incident diabetes or on disability are untested [108,109,111,115-117] (H-RCT, low).

5.4 Neurodegenerative disease and the gut–brain axis

The domain-specific elements are the enteric nervous system and vagal route, and the blood–brain barrier. In Parkinson's disease, α-synuclein pathology can be detected in enteric neurons before motor onset, constipation frequently precedes diagnosis by years, and truncal vagotomy has been associated with reduced subsequent risk in registry analyses—the strongest available human support for gut-origin propagation, though it remains observational [118-120]. Rodent work shows that microbiota from patients with Parkinson's disease exacerbates motor deficits in α-synuclein-overexpressing mice, and that SCFAs modulate microglial maturation and function [121-123] (A-TRANSFER, moderate). In Alzheimer's disease, associations between microbial composition, amyloid burden and cognition are reported, and rodent transfer experiments show effects on amyloid and tau pathology [99,124-127] (H-CS, low; A-TRANSFER, moderate).

Two cautions are specific to this domain. Reverse causation is particularly plausible: prodromal neurodegeneration causes constipation, reduced appetite, dietary change and reduced mobility, each of which alters the microbiota, and dopaminergic and anticholinergic drugs further modify it. And the human intervention evidence is weak: probiotic and prebiotic trials in Alzheimer's and Parkinson's disease are small, short and heterogeneous in endpoint, with the more consistent effects on constipation rather than on cognition or motor progression, and faecal transplantation data are limited to small series and case reports [128-134] (H-RCT, low; case series, very low).

5.5 Hepatic disease

The gut–liver axis is anatomically privileged: portal blood delivers microbial products directly to Kupffer cells and hepatocytes, and hepatic first-pass extraction determines systemic exposure to most microbial metabolites. In metabolic dysfunction-associated steatotic liver disease and in alcohol-related liver disease, increased permeability, endotoxaemia and altered bile-acid signalling through hepatic and intestinal FXR are documented, and microbial ethanol production by specific taxa has been proposed as a mechanism in steatohepatitis (H-CS, moderate; A-TRANSFER, moderate) [135-137]. Faecal transplantation has been evaluated in hepatic encephalopathy and in alcohol-related liver disease in small randomised trials with encouraging safety and surrogate outcomes [138,139] (H-RCT small, low). Because liver disease itself alters bile-acid pools, transit and portal pressure, bidirectionality is strong, and this domain illustrates why hepatic clearance must be considered when interpreting circulating metabolite concentrations in any other domain.

5.6 Musculoskeletal aging: sarcopenia and bone loss

The proposed "gut–muscle" and "gut–bone" axes rest on amino-acid and SCFA availability, insulin sensitivity, and inflammatory tone. Cross-sectional studies report associations between microbial composition and muscle mass, grip strength or gait speed in older adults [140] (H-CS, low), and germ-free and antibiotic-treated animals show reduced muscle mass and altered neuromuscular

junction gene expression that is partly reversible by colonisation or SCFA supplementation (A-TRANSFER, moderate). For bone, microbiota influences osteoclastogenesis through Th17/IL-17 signalling, sex-steroid-dependent pathways and macrophage cGAS–STING activation [82,141,142] (A-OBS/IV, low to moderate). A postbiotic preparation has been reported to affect sarcopenia-related outcomes in preclinical and small clinical work [143] (low). Confounding by protein and energy intake, vitamin D status, physical activity and glucocorticoid exposure is severe, and no trial has shown that a microbiota-targeted intervention prevents fracture or incident sarcopenia.

5.7 Cancer and cancer therapy in older adults

Two distinct questions should be separated. Regarding carcinogenesis, specific microbial functions have defined mechanistic roles—colibactin-producing *Escherichia coli*, *Bacteroides fragilis* toxin, *Fusobacterium nucleatum* adhesion and immune evasion in colorectal cancer, and secondary bile acids in hepatobiliary and gastrointestinal malignancy—with human association data and animal support [144-147] (H-CS, moderate; A-TRANSFER, moderate). Regarding therapy, microbial composition is associated with response to immune checkpoint blockade, and faecal transplantation from responders has restored responsiveness in small phase I trials in melanoma (H-RCT/phase I, moderate) [148-150]. This is currently the best-evidenced clinical application of microbiota modulation, but it concerns treatment response in cancer rather than healthspan in the general older population, and the ages of participants in these trials are frequently below those of the populations to which geroscience conclusions are extended. Antibiotic exposure around the time of immunotherapy is associated with worse outcomes (H-CS, moderate), which is mechanistically informative and clinically actionable (Fig. 3).

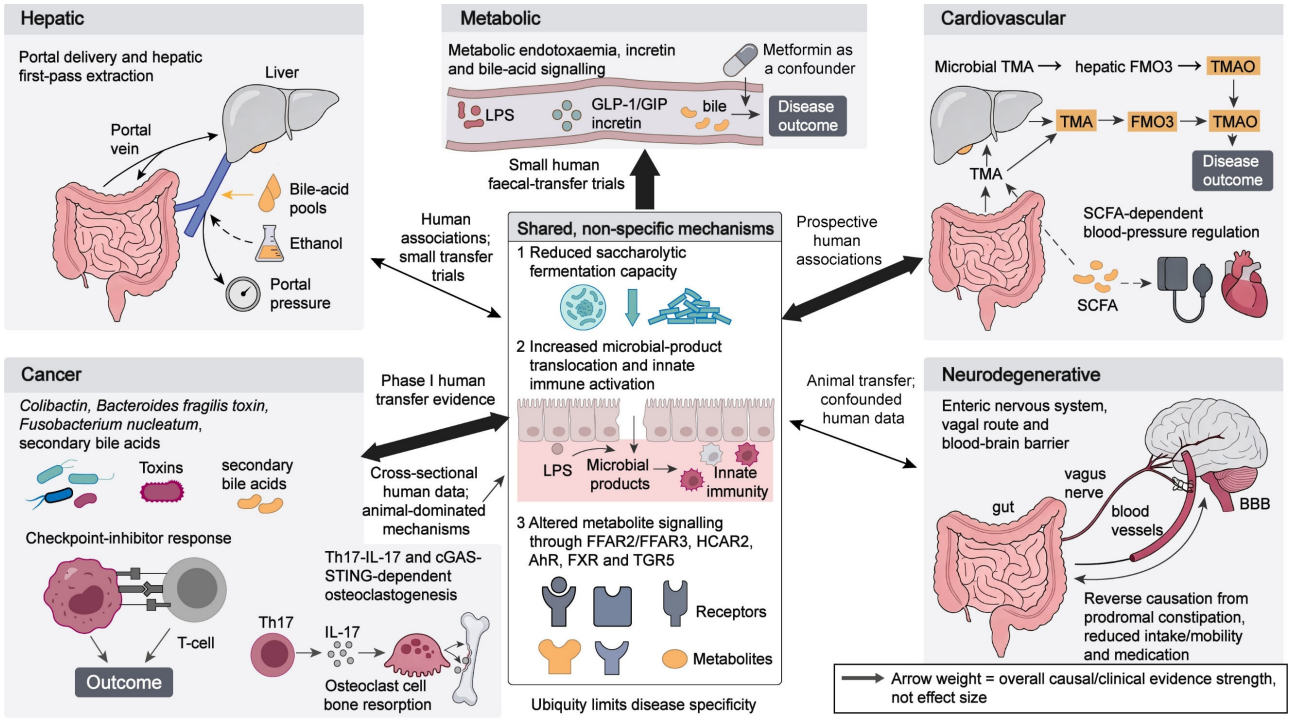


Figure 3. Convergent and divergent mechanisms across age-related disease domains. The central column shows the three mechanisms shared across domains: reduced saccharolytic fermentation capacity, increased translocation of microbial products with innate immune activation, and altered metabolite signalling at a limited set of host receptors (FFAR2/FFAR3, HCAR2, AhR, FXR, TGR5). Radiating panels show the elements specific to each domain, with the highest available level of human evidence indicated: cardiovascular disease (TMAO generation; SCFA-dependent blood-pressure regulation; prospective cohort associations); metabolic disease (metabolic endotoxaemia, incretin and bile-acid signalling; human faecal-transfer trials); hepatic disease (portal delivery and hepatic first-pass extraction; small randomised transfer trials); neurodegenerative disease (enteric nervous system and vagal route, blood-brain barrier; animal transfer with confounded human data); musculoskeletal aging (amino-acid and SCFA availability, Th17-IL-17 and cGAS-STING-dependent osteoclastogenesis; cross-sectional human data); and cancer (colibactin, Bacteroides fragilis toxin, Fusobacterium nucleatum, secondary bile acids; phase I transfer trials in checkpoint-inhibitor response). Arrow weight is proportional to the strength of human evidence, not to presumed effect size. Solid arrows established causal relationships; dashed arrows, proposed associations. *Endpoint badges: C, compositional; B, biomarker; F, functional.*

5.8 Synthesis

Across domains, the ranking of causal evidence is: metabolic disease and cancer immunotherapy response (human transfer or human intervention data available) > cardiovascular and hepatic disease (prospective human associations with animal transfer support) > neurodegenerative disease (animal transfer with weak and confounded human data) > musculoskeletal aging (predominantly animal and cross-sectional). No domain has evidence that a microbiota-targeted intervention alters a hard geroscience endpoint—incident multimorbidity, incident disability, or mortality—in older adults.

6. Microbiota-Targeted Interventions: Evidence, Specificity and Safety

We preface this section with the interpretive rule we apply throughout: a demonstrated change in microbiome composition or in a faecal metabolite is a pharmacodynamic response, not a health outcome. Composition-only endpoints establish that an intervention engages its target; they do not establish benefit. Because most trials in older adults report composition, faecal SCFA or circulating inflammatory markers rather than function, the evidence base speaks mainly to target engagement. Table 4 summarises intervention type, evidence level, reported effects, replication status and safety considerations.

6.1 Dietary patterns, fibre and polyphenols

Whole-diet intervention has the strongest rationale and the least specificity. Mediterranean-pattern diets are associated with higher alpha diversity, greater abundance of fibre-degrading taxa, higher faecal SCFA and lower frailty and inflammatory markers in older adults, and dietary intervention over 12 months has been reported to shift composition in older Europeans with parallel improvements in frailty and cognition markers [151-154] (H-RCT, moderate for composition and inflammatory markers; low for functional endpoints) **[155]**. Fibre type matters: inulin-type fructans, resistant starches, β-glucan and arabinoxylan differ in fermentation site, kinetics and metabolite profile, and are not interchangeable. Fibre and polyphenols interact, with fibre affecting colonic delivery of polyphenols and polyphenols modifying fermentation dynamics [80,156-160] (IV/H-RCT, low to moderate). Practical limits in older adults include reduced appetite, dentition, dysphagia, cost, food access and gastrointestinal tolerance—flatulence and bloating are common with rapid fibre escalation and are a frequent cause of non-adherence. Because effects are mediated by many non-microbial routes, mediation analysis is required before attributing dietary benefit to microbiota.

6.2 Prebiotics

Prebiotics are substrates selectively utilised by host microorganisms conferring a health benefit; the definition requires demonstrated benefit, not merely a bifidogenic effect. Structure determines selectivity: degree of polymerisation, monomer composition and linkage type determine which taxa can utilise a given substrate, and β-galacto-oligosaccharides of different chain length differ in bifidogenic activity and physiological effect [161-166] (IV/H-RCT, moderate for compositional effects). In older adults, prebiotic supplementation has been reported to increase *Bifidobacterium* abundance and to modify inflammatory and immune markers, with small trials also reporting improvements in aspects of immune function and, in some cohorts, in frailty-associated inflammation [167-174] (H-RCT, low to moderate). Reported effects on metabolic and cognitive endpoints are inconsistent, trials are typically 4–12 weeks with 20–80 participants, endpoints are unstandardised, and there is little evidence of maintained benefit after cessation [175-182] (H-RCT, low). Dose is the main determinant of gastrointestinal tolerance, and doses above roughly 10 g/day of inulin-type fructans are frequently symptomatic.

Table 4. Microbiota-targeted interventions in older adults: evidence, specificity and safety. A demonstrated change in composition or in a faecal metabolite indicates target engagement, not health benefit. Design codes and confidence grades are defined in Table 1.

Intervention	Best available evidence in older adults	Endpoints reported	Replication	Specificity determinants	Safety in frail, immunocompromised or catheterised older adults	Confidence
Dietary pattern (Mediterranean-type) and dietary fibre	H-RCT of 12-month dietary intervention in older adults with compositional,	Composition, faecal SCFA, inflammatory markers, frailty and cognitive markers	Compositional effects reproducible; functional effects less so	Fibre type (inulin-type fructans, resistant starch, beta-glucan, arabinoxylan	Generally safe; limits are appetite, dentition, dysphagia, cost and food access; rapid fibre escalation	Moderate for composition and inflammatory markers;

	inflammatory and frailty outcomes			differ in fermentation site and kinetics); baseline intake; polyphenol co-exposure	causes bloating and non-adherence	low for function
Prebiotics (inulin-type fructans, GOS, resistant starch)	Multiple small H-RCTs, typically 4–12 weeks, 20–80 participants	Bifidobacterium abundance, faecal SCFA, inflammatory and some immune markers	Bifidogenic effect replicable; metabolic and cognitive effects inconsistent	Degree of polymerisation, monomer composition and linkage determine which taxa can utilise the substrate; dose	Dose-limiting flatulence and bloating above roughly 10 g/day of fructans; no serious safety signal	Low to moderate
Probiotics (defined strains)	Numerous small H-RCTs; effects on respiratory and gastrointestinal infection, glycaemic and inflammatory markers, periodontal indices, mood	Mainly biomarker and symptom endpoints; few functional endpoints	Limited independent replication; several well-conducted null trials	Strain, not species or genus; dose (commonly 10^8–10^11 CFU/day, dose–response rarely characterised); viability through shelf life and gastric transit; formulation and matrix; single versus multi-strain; duration; recipient baseline microbiota and medication; documented label–content discrepancies in commercial products	Case reports of Lactobacillus and Bifidobacterium bacteraemia, Saccharomyces fungaemia with central venous catheters, and endocarditis, predominantly with immunosuppression, indwelling catheters, mucosal barrier injury or critical illness; excess mortality in a randomised trial in predicted severe acute pancreatitis; transferable antimicrobial-resistance determinants, with particular caution for Enterococcus preparations; D-lactate production; environmental contamination with fungal preparations	Low to moderate, strain-dependent
Postbiotics (ISAPP definition: preparation of inanimate microorganisms and/or their components conferring a health benefit)	Few H-RCTs in older adults; one preparation with sarcopenia-related outcomes	Mainly biomarker and preclinical endpoints	Insufficient for appraisal	Inactivation method (heat, high pressure, ultraviolet, sonication) determines which structures and effects are retained; active component (peptidoglycan, lipoteichoic acid, exopolysaccharide, extracellular vesicles) often uncharacterised; quantification not standardised	No translocation or bacteraemia risk, which makes this class mechanistically attractive for frail and immunosuppressed populations; long-term data absent	Low
Fermented foods	H-RCT in healthy younger adults showing	Composition, diversity, inflammatory proteins	Single well-conducted trial, not yet	Live organism content varies by orders of magnitude	Sodium content of traditional preparations; tyramine with	Moderate in younger adults; low

	increased diversity and reduced inflammatory proteins; small studies in older adults		replicated in older adults	between products; many commercial products are pasteurised after fermentation and contain no live organisms	monoamine oxidase inhibitors; histamine; listeriosis risk from unpasteurised dairy in immunosenescent individuals; rare adverse events reported with kombucha	in older adults
Faecal microbiota transplantation	Established efficacy only in recurrent C. difficile infection; small trials and case series in metabolic syndrome, hepatic encephalopathy, alcohol-related liver disease and checkpoint-inhibitor response	Surrogate and, in C. difficile, clinical cure	Reproducible for C. difficile; not for aging-relevant indications	Donor selection remains empirical and "super-donor" effects are inconsistently replicated; preparation, dose, route and pretreatment unstandardised	Donor screening must exclude transmissible infection, and protocols have been revised after adverse events: transmission of extended-spectrum beta-lactamase-producing E. coli caused bacteraemia and one death in immunocompromised recipients, with other reports of enteropathogenic and Shiga-toxin-producing E. coli, prompting regulatory alerts and addition of multidrug-resistant organism and SARS-CoV-2 screening; procedural risk of aspiration (upper route) or perforation and sedation events (colonoscopic route), of higher consequence in frail patients; unquantified long-term risk of transmitting metabolic, immune or neoplastic traits not detected by infectious screening; engraftment persistence and delayed effects uncharacterised beyond a few years	Low for aging-relevant indications
Defined consortia and single-strain live biotherapeutic products	Phase III data for recurrent C. difficile infection; none for aging endpoints	Clinical recurrence	Reproducible in the licensed indication	Defined composition and dose, which is the principal advantage over donor material	Preferable to undefined donor material on both safety and reproducibility grounds; long-term data still limited	Moderate in the licensed indication; very low for aging
Personalised nutrition informed by microbiome features	Cohort and small H-RCT evidence for prediction of postprandial glycaemic response and dietary tailoring	Glycaemic response; composition	Partially replicated; not in older adults	Model transportability across populations; feature availability; adherence	Low physical risk; equity and cost concerns are the principal issue	Low to moderate

Engineered microorganisms, biosensors, synthetic consortia	Preclinical only	Preclinical	Not applicable	Biocontainment, genetic stability in vivo, durable colonisation	Not evaluable; regulatory pathway for live genetically modified therapeutics undefined	Very low

6.3 Probiotics

Probiotic effects are strain-specific, and evidence for one strain does not transfer to another strain of the same species, let alone to the genus. This is not a caveat but the central interpretive fact of the field, and it is frequently violated in both the literature and in product marketing. Commonly studied genera include *Lactobacillus* and its reclassified descendants, *Bifidobacterium, Bacillus* and *Saccharomyces* [188-190]. In older adults, specific strains have been reported to reduce respiratory and gastrointestinal infection incidence or duration, to modestly improve glycaemic and inflammatory markers in type 2 diabetes, to influence periodontal indices, and in a few small trials to affect mood and cognitive measures [191-205] (H-RCT, low to moderate; heterogeneous). Effects are typically small, replication across independent groups is limited, and several well-conducted trials have been null.

Beyond strain identity, outcomes depend on dose (commonly 10^8–10^{11} CFU/day, with dose–response rarely characterised), viability and stability through shelf life and gastric transit, formulation and matrix, single versus multi-strain composition, duration of administration, and recipient characteristics including baseline microbiota, diet and medication. Product variability is a documented problem: independent analyses have found discrepancies between labelled and actual strain content and viable counts in commercial products, which limits reproducibility across trials using nominally identical preparations.

Safety in older and vulnerable adults requires explicit statement rather than a closing sentence. Case reports and series document *Lactobacillus* and *Bifidobacterium* bacteraemia, *Saccharomyces* fungaemia associated with central venous catheters, and endocarditis attributable to probiotic strains, predominantly in patients with immunosuppression, indwelling catheters, mucosal barrier injury, short bowel or critical illness [206]. A randomised trial of a probiotic–prebiotic preparation in predicted severe acute pancreatitis reported excess mortality in the intervention arm, which remains the strongest available signal that live biotherapeutics are not risk-free in acutely ill patients. Additional considerations are transferable antimicrobial-resistance determinants in probiotic strains— *Enterococcus* preparations warrant particular caution, and resistance-gene screening should be a condition of use—D-lactate production and, in the case of fungal preparations, environmental contamination of hospital surfaces. Probiotics should therefore not be recommended indiscriminately to frail, immunocompromised, catheterised or critically ill older adults, and trials in these populations require independent safety monitoring.

6.4 Postbiotics

We adopt the ISAPP consensus definition: a postbiotic is a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host. Two consequences follow, and both are frequently ignored. The definition requires the presence of inactivated microbial cells or cell components, so purified single metabolites—butyrate salts, urolithin A, isolated exopolysaccharides—are not postbiotics but microbial metabolites or microbial-derived compounds and should be described as such; and the definition requires demonstrated benefit, so an inactivated preparation without efficacy data is not a postbiotic. We follow this distinction throughout [207-210].

Postbiotics offer practical advantages relevant to older adults—stability without cold chain, absence of translocation and bacteraemia risk, compatibility with immunosuppression—which makes them mechanistically attractive for frail populations. Evidence is nonetheless earlier-stage than for probiotics: inactivation method (heat, high pressure, ultraviolet, sonication) alters which molecular structures are preserved and therefore which effects are retained [211,212], the active component—peptidoglycan, lipoteichoic acid, exopolysaccharide, extracellular vesicles or metabolites—is often uncharacterised [213,214], quantification and batch consistency are poorly standardised [215], and few randomised trials in older adults with functional endpoints exist [143,216-218] (H-RCT small, low).

6.5 Fermented foods

Fermented foods including yoghurt, kefir, kimchi and kombucha increase the intake of live microorganisms and of fermentation metabolites, and a randomised comparison of high-fermented-food versus high-fibre diets in healthy adults reported increased diversity and decreased inflammatory proteins with fermented foods (H-RCT, moderate, in younger adults) [219]. Data in older adults are limited to small studies with compositional endpoints [220,221] (H-RCT small, low). Product-level microbial content varies by orders of magnitude and many

commercial products are pasteurised after fermentation and contain no live organisms. Specific cautions in older adults include high sodium content in several traditional preparations, tyramine content with monoamine oxidase inhibitors, histamine, and, for unpasteurised dairy, listeriosis risk in immunosenescent individuals. Kombucha in particular has been associated with rare adverse events and lacks controlled data in older populations (Fig. 4).

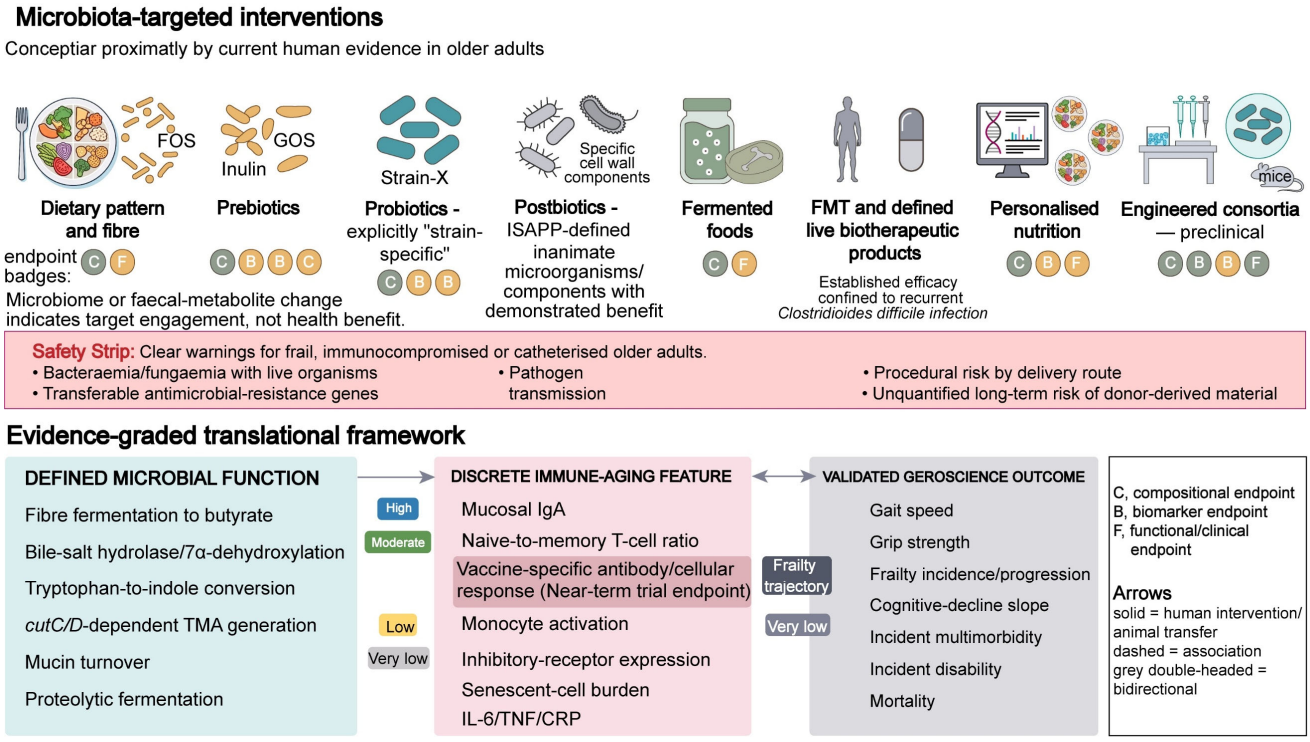


Figure 4. Microbiota-targeted interventions, level of evidence, and the translational agenda. Upper panel, interventions ordered by the level of human evidence currently available in older adults rather than by mechanistic appeal: dietary pattern and fibre; prebiotics; probiotics (strain-specific); postbiotics as defined by ISAPP consensus; fermented foods; faecal microbiota transplantation and defined live biotherapeutic products; personalised nutrition; and engineered consortia, which remain preclinical. For each, the reported endpoints are marked as compositional (target engagement), biomarker, or functional, to make explicit that most evidence is compositional. Safety considerations specific to frail, immunocompromised or catheterised older adults are shown alongside: bacteraemia and fungaemia with live organisms, transferable antimicrobial-resistance determinants, pathogen transmission and unquantified long-term risk with donor-derived material, and procedural risk by delivery route. Lower panel, the proposed evidence-graded framework: defined microbial function, to discrete feature of immune aging, to validated geroscience outcome, with an evidence grade attached to each link and empty cells indicating the studies the field still requires. The three columns represent candidate, evidence-graded mappings and should not be interpreted as one-to-one or established causal relationships.

6.6 Faecal microbiota transplantation and defined live biotherapeutics

FMT has established efficacy in recurrent *Clostridioides difficile* infection and this indication accounts for nearly all of its favourable safety record. Outside it, evidence in aging-relevant indications is limited to small trials and case series: transient improvement in insulin sensitivity in metabolic syndrome [106,107], surrogate and cognitive outcomes in hepatic encephalopathy [138], alcohol craving in alcohol-related liver disease [139], and restoration of checkpoint-inhibitor responsiveness in melanoma [148-150] (H-RCT small/phase I, low to moderate).

Safety requires proportionate emphasis. Donor screening must exclude transmissible infection, and screening protocols have been repeatedly revised after adverse events: transmission of extended-spectrum β-lactamase-producing *Escherichia coli* caused bacteraemia and one death in immunocompromised recipients, and enteropathogenic *E. coli* and Shiga-toxin-producing organisms have been transmitted in other instances, prompting regulatory safety alerts and the addition of multidrug-resistant organism and, subsequently, SARS-CoV-2 screening. Procedural risks

depend on delivery route and include aspiration with upper-gastrointestinal administration and perforation or sedation-related events with colonoscopic delivery, both of higher consequence in frail older adults. Longer-term uncertainties are substantive and unresolved: donor microbiota may transmit traits related to adiposity, metabolic or immune phenotype, or cancer risk that would not be detected by infectious screening; engraftment persistence and the possibility of delayed adverse effects are not characterised beyond a few years; and no framework exists for long-term follow-up of recipients. Donor selection criteria remain empirical, "super-donor" effects are inconsistently replicated, and preparation, dose, route and pretreatment are not standardised [223]. Defined consortia and single-strain live biotherapeutic products, some of which have completed phase III evaluation for recurrent *C. difficile* infection, are preferable to undefined donor material on both safety and reproducibility grounds, and we regard them as the appropriate direction for aging-related indications.

6.7 Personalised nutrition and engineered approaches

Prediction of individual postprandial glycaemic responses from microbiome and clinical features, with subsequent dietary tailoring, remains among the more convincing applications of microbiome-informed intervention [224] (H-RCT, low to moderate), but independent replication in older adults and evidence that tailored diets outperform standard advice on functional endpoints are lacking. Gene-edited and engineered microorganisms, biosensor consortia and synthetic ecosystems remain preclinical, the obstacles being biocontainment, in-vivo genetic stability, durable colonisation and the regulatory pathway for live genetically modified therapeutics; we describe them as prospects rather than near-term options [225-227].

6.8 Access, equity and ethics

Socioeconomic position is associated with both microbial composition and access to microbiota-targeted interventions, so a field that advances mainly through commercial supplements and personalised services risks widening rather than narrowing health inequity in older populations [25,26,228]. Cost-effectiveness analyses are essentially absent. Ethical requirements specific to older participants include capacity-sensitive consent for FMT and other invasive procedures, protection of microbiome data, which is individually identifying and health-informative, and equitable recruitment across socioeconomic, geographic and ethnic groups so that findings generalise to the populations that carry most of the disease burden [229-231].

7. Limitations of the Field and a Research Agenda

Limitations of this review. Search and selection were not systematic; no risk-of-bias tool was applied; we did not quantify effect sizes or perform meta-analysis; and our confidence grades are consensus judgements of the authors rather than the output of a validated instrument. Positive-result citation bias is likely. Coverage of fungal, viral and archaeal components, and of strain-level variation, is limited by the primary literature. Non-English studies were generally excluded, which is a particular limitation given the strong geographic structuring of the microbiome.

What the field lacks. Longitudinal human data of adequate duration, since almost all inference about "the aging microbiome" derives from age-stratified cross-sectional comparison rather than within-person change; quantitative rather than compositional measurement, with flux estimation in place of faecal metabolite concentration; trials with functional endpoints; standard reporting of frailty, medication, transit, renal function and residence; causal-inference designs suited to observational data, including Mendelian randomisation with microbial instruments, target-trial emulation and mediation analysis within existing dietary trials; and an explicit account of which feature of immune aging a given intervention is expected to change.

A proposed framework. We suggest that progress requires replacing the taxon-to-disease association with a three-layer mapping in which each link carries an evidence grade: a defined *microbial function* (fibre fermentation to butyrate; bile-salt hydrolase and 7α-dehydroxylation; tryptophan-to-indole conversion; cutC/D-dependent TMA generation; mucin turnover; proteolytic fermentation) → a *discrete, separately measurable feature of immune aging* (mucosal IgA quantity and specificity; naïve-to-memory T-cell ratio; vaccine-specific antibody and cellular response; monocyte activation phenotype; inhibitory-receptor expression; senescent-cell burden; circulating IL-6/TNF/CRP) → a *validated geroscience outcome* (gait speed, grip strength, frailty index trajectory, cognitive decline slope, incident multimorbidity, incident disability, mortality). Populating this matrix reveals that the great majority of cells are currently empty or occupied by animal-only evidence, and it identifies the specific studies that would fill them. Table 2 is a first attempt at the middle layer, and Table 3 at the third.

Priority studies. In our view the highest-yield near-term designs are: randomised fibre or defined-consortium intervention in community-dwelling older adults with vaccine response as the primary endpoint and quantitative microbial and flux measurements as mediators; longitudinal cohorts of older adults sampled at least

annually with concurrent frailty, medication, transit and renal phenotyping, powered for within-person trajectory analysis; mediation analysis within completed dietary trials that retained biospecimens; and stratified trials in polyphenol metabotypes. Each is feasible with existing methods and would produce evidence of the kind the field currently lacks.

8. Conclusion

The intestinal microbiota is a plausible and attractive modifiable contributor to healthy aging, and the mechanistic pathways connecting microbial fermentation, barrier function and innate immune activation are well described at a molecular level. The human evidence, however, remains predominantly associative, and the field's central claims are more provisional than their frequent restatement implies. The microbiota of older adults is heterogeneous rather than uniformly dysbiotic, and much of the difference between older and younger cohorts is attributable to frailty, medication, diet, mobility, transit and geography; the principal microbial metabolites are context-dependent rather than categorically beneficial or harmful, with SCFA effects varying by concentration and site and TMAO associations substantially confounded by renal function; and a large fraction of reported microbial signatures of aging is sensitive to sampling, extraction, sequencing and analytical choices. Interventions engage their target reliably and improve clinical function inconsistently: effects are strain-, dose- and formulation-specific, replication is limited, follow-up is short, and safety in frail and immunocompromised older adults—bacteraemia with live organisms, pathogen transmission and unquantified long-term risk with faecal transplantation—requires more emphasis than it usually receives.

We therefore conclude not that the microbiota is unimportant in aging, but that establishing its importance requires a different kind of evidence than the field has so far generated. The framework we propose—defined microbial functions mapped to discrete features of immune aging and then to validated geroscience outcomes, with an explicit evidence grade on each link—is intended to make the gaps visible and the priorities concrete. Until studies of that design are available, changes in microbiome composition should be reported as what they are: evidence that an intervention has engaged its target, and not evidence that healthspan has been extended.

Author contributions

Dong-Sheng He: Conceptualization, investigation, visualization, and writing - original draft. Fei Liao: Conceptualization, investigation, and writing - original draft. Hai-Lin Chen and Qi Zhang: Investigation, data curation, and writing - review and editing. Min-Xue Wei and Dong-Ming Guo: Conceptualization, supervision, and writing - review and editing. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

References

[1] Gyriki D, Nikolaidis CG, Bezirtzoglou E, Voidarou C, Stavropoulou E, Tsigalou C (2025). The gut microbiota and aging: interactions, implications, and interventions. Front Aging, 6:1452917.

[2] Conway J, Duggal NA (2021). Ageing of the gut microbiome: Potential influences on immune senescence and inflammageing. Ageing Res Rev, 68:101323.

[3] Shintouo CM, Mets T, Beckwee D, Bautmans I, Ghogomu SM, Souopgui J, et al. (2020). Is inflammageing influenced by the microbiota in the aged gut? A systematic review. Exp Gerontol, 141:111079.

[4] Liu W, Wang L, Ou J, Peng D, Zhang Y, Chen W, et al. (2025). Gut Microbiota Metabolites and Chronic Diseases: Interactions, Mechanisms, and Therapeutic Strategies. Int J Mol Sci, 26:3752.

[5] Kočar E, Šket R, Vasle AH, Avguštin G, Benedik E, Seljak BK, et al. (2026). Measuring biological age: Insights from omics studies. Ageing Res Rev, 114:102988.

[6] Deng M, Chen W, Wang X (2025). Characteristics of gut microbiota in longevity populations in China and its relationship with healthy aging. Medicine (Baltimore), 104:e43633.

[7] McCormick C, Degregori S, Johnson GC, Blumstein DT, Barber PH (2025). Marmot gut microbiomes are stable against dietary variation. Oecologia, 208:15.

[8] Ai X, Liu Y, Shi J, Xie X, Li L, Duan R, et al. (2024). Structural characteristics of gut microbiota in longevity from Changshou town, Hubei, China. Appl Microbiol Biotechnol, 108:300.

[9] Ullah H (2025). Gut-vitamin D interplay: key to mitigating immunosenescence and promoting healthy ageing. Immun Ageing, 22:20.

[10] Spray L, Richardson G, Haendeler J, Altschmied J, Rumampouw V, Wallis SB, et al. (2025). Cardiovascular inflammaging: Mechanisms, consequences, and therapeutic perspectives. Cell Rep Med, 6:102264.

[11] Aranda JF, Ramírez CM, Mittelbrunn M (2025). Inflammageing, a targetable pathway for preventing cardiovascular diseases. Cardiovasc Res, 121:1537-1550.

[12] Lagoumtzi SM, Chondrogianni N (2021). Senolytics and senomorphics: Natural and synthetic therapeutics in the treatment of aging and chronic diseases. Free Radic Biol Med, 171:169-190.

[13] De Luca F, Camporeale V, Leccese G, Cuttano R, Troise D, Infante B, et al. (2025). From Senescent Cells to Systemic Inflammation: The Role of Inflammaging in Age-Related Diseases and Kidney Dysfunction. Cells, 14:1831.
[14] Chen R, Zhu D, Yang R, Wu Z, Xu N, Chen F, et al. (2022). Gut microbiota diversity in middle-aged and elderly patients with end-stage diabetic kidney disease. Ann Transl Med, 10:750.
[15] Bedzhanyan AL, Kovalskaya YV, Petrenko KN, Frolova YV (2025). [Role of gut microbiota in aging processes]. Khirurgiia (Mosk). doi: 10.17116/hirurgia202511267.
[16] Claesson MJ, Jeffery IB, Conde S, et al (2012). Gut microbiota composition correlates with diet and health in the elderly. Nature, 488:178-184.
[17] Xue L, Ding Y, Qin Q, Liu L, Ding X, Zhou Y, et al. (2022). Assessment of the impact of intravenous antibiotics treatment on gut microbiota in patients: Clinical data from pre-and post-cardiac surgery. Front Cell Infect Microbiol, 12:1043971.
[18] Amamoto R, Shimamoto K, Suwa T, Park S, Matsumoto H, Shimizu K, et al. (2022). Relationships between dietary diversity and gut microbial diversity in the elderly. Benef Microbes, 13:453-464.
[19] Zhu Q, Jiang S, Du G (2020). Effects of exercise frequency on the gut microbiota in elderly individuals. Microbiologyopen, 9:e1053.
[20] Cancino Ramírez J, González Rojas L, Magne F, Gotteland M (2025). Modulation of gut microbiota through physical activity in individuals with obesity-a systematic review. Appl Physiol Nutr Metab, 50:1-11.
[21] Ahn J, Kwak S, Usyk M, Beggs D, Choi H, Ahdoot D, et al. (2023). Sociobiome - Individual and neighborhood socioeconomic status influence the gut microbiome in a multi-ethnic population in the US. Res Sq. doi: 10.21203/rs.3.rs-2733916/v1.
[22] Lu J, Zhang L, Zhai Q, Zhao J, Zhang H, Lee YK, et al. (2021). Chinese gut microbiota and its associations with staple food type, ethnicity, and urbanization. NPJ Biofilms Microbiomes, 7:71.
[23] Sun S, Wang H, Tsilimigras MC, Howard AG, Sha W, Zhang J, et al. (2020). Does geographical variation confound the relationship between host factors and the human gut microbiota: a population-based study in China. BMJ Open, 10:e038163.
[24] Ren Y, Wu J, Wang Y, Zhang L, Ren J, Zhang Z, et al. (2023). Lifestyle patterns influence the composition of the gut microbiome in a healthy Chinese population. Sci Rep, 13:14425.
[25] Lewis CR, Bonham KS, McCann SH, Volpe AR, D'Sa V, Naymik M, et al. (2021). Family SES Is Associated with the Gut Microbiome in Infants and Children. Microorganisms, 9:1608.
[26] Tamayo M, Olivares M, Ruas-Madiedo P, Margolles A, Espín JC, Medina I, et al. (2024). How Diet and Lifestyle Can Fine-Tune Gut Microbiomes for Healthy Aging. Annu Rev Food Sci Technol, 15:283-305.
[27] Madison AA, Burd CE, Andridge R, Wilson SJ, Bailey MT, Belury MA, et al. (2024). Gut Microbiota Richness and Diversity Track With T Cell Aging in Healthy Adults. J Gerontol A Biol Sci Med Sci, 79:glad276.
[28] Sang J, Zhuang D, Zhang T, Wu Q, Yu J, Zhang Z (2022). Convergent and Divergent Age Patterning of Gut Microbiota Diversity in Humans and Nonhuman Primates. mSystems, 7:e0151221.
[29] Ma T, Yao C, Shen X, Jin H, Guo Z, Zhai Q, et al. (2021). The diversity and composition of the human gut lactic acid bacteria and bifidobacterial microbiota vary depending on age. Appl Microbiol Biotechnol, 105:8427-8440.
[30] Sadoughi B, Schneider D, Daniel R, Schülke O, Ostner J (2022). Aging gut microbiota of wild macaques are equally diverse, less stable, but progressively personalized. Microbiome, 10:95.
[31] Reese AT, Phillips SR, Owens LA, Venable EM, Langergraber KE, Machanda ZP, et al. (2021). Age Patterning in Wild Chimpanzee Gut Microbiota Diversity Reveals Differences from Humans in Early Life. Curr Biol, 31:613-620.e3.
[32] Leite G, Pimentel M, Barlow GM, Mathur R (2022). The small bowel microbiome changes significantly with age and aspects of the ageing process. Microb Cell, 9:21-23.
[33] Yang B, Yan S, Chen Y, Ross RP, Stanton C, Zhao J, et al. (2020). Diversity of Gut Microbiota and Bifidobacterial Community of Chinese Subjects of Different Ages and from Different Regions. Microorganisms, 8:1108.
[34] Ruiz-Rico M, Ye H, O'Callaghan TF, O'Toole PW, McCarthy EK (2025). Iron-saturated bovine lactoferrin preserves microbiota diversity and healthy ageing-associated taxa in an in vitro colon model of elderly gut microbiota (Iron-saturated bovine lactoferrin impact on elderly gut microbiota). PLoS One, 20:e0332631.
[35] Kruger K, Myeonghyun Y, van der Wielen N, Kok DE, Hooiveld GJ, Keshtkar S, et al. (2024). Evaluation of inter- and intra-variability in gut health markers in healthy adults using an optimised faecal sampling and processing method. Sci Rep, 14:24580.
[36] Piancone E, Fosso B, Marzano M, De Robertis M, Notario E, Oranger A, et al. (2022). Natural and after colon washing fecal samples: the two sides of the coin for investigating the human gut microbiome. Sci Rep, 12:17909.
[37] Kachroo N, Lange D, Penniston KL, Stern J, Tasian G, Bajic P, et al. (2021). Meta-analysis of Clinical Microbiome Studies in Urolithiasis Reveal Age, Stone Composition, and Study Location as the Predominant Factors in Urolithiasis-Associated Microbiome Composition. mBio, 12:e0200721.
[38] Okumura R, Takeda K (2024). The role of the mucosal barrier system in maintaining gut symbiosis to prevent intestinal inflammation. Semin Immunopathol, 47:2.
[39] Zhao Y, Wang B, Wei X, Liu D, Wang R, Xie S, et al. (2025). Impact of gut microbiota dysbiosis on intestinal barrier integrity and systemic inflammation in a pre-eclampsia mouse model. Microb Pathog, 209:108053.
[40] Quin C, Breznik JA, Kennedy AE, DeJong EN, Andary CM, Ermolina S, et al. (2024). Monocyte-driven

inflamm-aging reduces intestinal barrier function in females. Immun Ageing, 21:65.

[41] Gámez-Macías PE, Félix-Soriano E, Samblas M, Sáinz N, Moreno-Aliaga MJ, González-Muniesa P (2024). Intestinal Permeability, Gut Inflammation, and Gut Immune System Response Are Linked to Aging-Related Changes in Gut Microbiota Composition: A Study in Female Mice. J Gerontol A Biol Sci Med Sci, 79:glae045.

[42] Conway J, De Jong EN, White AJ, Dugan B, Rees NP, Parnell SM, et al. (2025). Age-related loss of intestinal barrier integrity plays an integral role in thymic involution and T cell ageing. Aging Cell, 24:e14401.

[43] Verma M, Garg M, Khan AS, Yadav P, Rahman SS, Ali A, et al. (2024). Cadmium modulates intestinal Wnt/β-catenin signaling ensuing intestinal barrier disruption and systemic inflammation. Ecotoxicol Environ Saf, 277:116337.

[44] Diaz OE, Sorini C, Morales RA, Luo X, Frede A, Krais AM, et al. (2021). Perfluorooctanesulfonic acid modulates barrier function and systemic T-cell homeostasis during intestinal inflammation. Dis Model Mech, 14:dmm049104.

[45] Salomon JD, Qiu H, Feng D, Owens J, Khailova L, Osorio Lujan S, et al. (2023). Piglet cardiopulmonary bypass induces intestinal dysbiosis and barrier dysfunction associated with systemic inflammation. Dis Model Mech, 16:dmm049742.

[46] Wei Z, Shen H, Wang F, Huang W, Li X, Xu H, et al. (2024). Melatonin mediates intestinal barrier dysfunction and systemic inflammation in moderate-severe OSA patients. Ann Med, 56:2361825.

[47] Wu Z, Liu L, Li L, Cao X, Jia W, Liao X, et al. (2023). Oral nano-antioxidants improve sleep by restoring intestinal barrier integrity and preventing systemic inflammation. Natl Sci Rev, 10:nwad309.

[48] Du L, Jiang W, Zhu X, Zhu L, Fan Y, Jiang W (2024). Rifaximin alleviates intestinal barrier disruption and systemic inflammation via the PXR/NFκB/MLCK pathway and modulates intestinal Lachnospiraceae abundance in heat-stroke mice. Int Immunopharmacol, 143:113462.

[49] Zhang X, Zuo L, Geng Z, Song X, Li J, Ge S, et al. (2022). Vindoline ameliorates intestinal barrier damage in Crohn's disease mice through MAPK signaling pathway. FASEB J, 36:e22589.

[50] Chen M, Wei S, Wu X, Xiang Z, Li X, He H, et al. (2024). 2'-Hydroxycinnamaldehyde Alleviates Intestinal Inflammation by Attenuating Intestinal Mucosal Barrier Damage Via Directly Inhibiting STAT3. Inflamm Bowel Dis, 30:992-1008.

[51] Kühn F, Adiliaghdam F, Cavallaro PM, Hamarneh SR, Tsurumi A, Hoda RS, et al. (2020). Intestinal alkaline phosphatase targets the gut barrier to prevent aging. JCI Insight, 5:e134049.

[52] Kono G, Yoshida K, Kokubo E, Ikeda M, Matsubara T, Koyama T, et al. (2023). Fermentation Supernatant of Elderly Feces with Inulin and Partially Hydrolyzed Guar Gum Maintains the Barrier of Inflammation-Induced Caco-2/HT29-MTX-E12 Co-Cultured Cells. J Agric Food Chem, 71:1510-1517.

[53] Lymperopoulos A, Suster MS, Borges JI (2022). Short-Chain Fatty Acid Receptors and Cardiovascular Function. Int J Mol Sci, 23:3303.

[54] Kim CH (2021). Control of lymphocyte functions by gut microbiota-derived short-chain fatty acids. Cell Mol Immunol, 18:1161-1171.

[55] Frampton J, Murphy KG, Frost G, Chambers ES (2020). Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. Nat Metab, 2:840-848.

[56] Frolova MS, Suvorova IA, Iablokov SN, Petrov SN, Rodionov DA (2022). Genomic reconstruction of short-chain fatty acid production by the human gut microbiota. Front Mol Biosci, 9:949563.

[57] Bolognini D, Dedeo D, Milligan G (2021). Metabolic and inflammatory functions of short-chain fatty acid receptors. Curr Opin Endocr Metab Res, 16:1-9.

[58] Hu M, Alashkar Alhamwe B, Santner-Nanan B, Miethe S, Harb H, Renz H, et al. (2022). Short-Chain Fatty Acids Augment Differentiation and Function of Human Induced Regulatory T Cells. Int J Mol Sci, 23:5740.

[59] Qu S, Gao Y, Ma J, Yan Q (2023). Microbiota-derived short-chain fatty acids functions in the biology of B lymphocytes: From differentiation to antibody formation. Biomed Pharmacother, 168:115773.

[60] Carrillo-Salinas FJ, Parthasarathy S, Moreno de Lara L, Borchers A, Ochsenbauer C, Panda A, et al. (2022). Short-Chain Fatty Acids Impair Neutrophil Antiviral Function in an Age-Dependent Manner. Cells, 11:2515.

[61] Miller A, Fantone KM, Tucker SL, Gokanapudi N, Goldberg JB, Rada B (2023). Short chain fatty acids reduce the respiratory burst of human neutrophils in response to cystic fibrosis isolates of Staphylococcus aureus. J Cyst Fibros, 22:756-762.

[62] Liu L, Li Q, Yang Y, Guo A (2021). Biological Function of Short-Chain Fatty Acids and Its Regulation on Intestinal Health of Poultry. Front Vet Sci, 8:736739.

[63] Chen N, Wu J, Wang J, Piri N, Chen F, Xiao T, et al. (2021). Short chain fatty acids inhibit endotoxin-induced uveitis and inflammatory responses of retinal astrocytes. Exp Eye Res, 206:108520.

[64] Rosli NSA, Abd Gani S, Khayat ME, Zaidan UH, Ismail A, Abdul Rahim MBH (2023). Short-chain fatty acids: possible regulators of insulin secretion. Mol Cell Biochem, 478:517-530.

[65] Yoon JH, Do JS, Velankanni P, Lee CG, Kwon HK (2023). Gut Microbial Metabolites on Host Immune Responses in Health and Disease. Immune Netw, 23:e6.

[66] Yu S, Zhang M, Dou Z, Tian B, Lu J (2025). Gut microbiota metabolites in the immunoregulation of enteritis: research progress. Front Immunol, 16:1706472.

[67] Song Y, Li M, Liu J, Wang J, Zhou A, Cao Y, et al. (2024). Screening study of hydroxytyrosol metabolites from in vitro fecal fermentation and their interaction with intestinal barrier repair receptor AhR. J Food Sci, 89:10134-10151.

[68] Chen HC, Cheng YC, Hsieh ML, Lin PJ, Wissel EF, Steward T, et al. (2025). Gut microbiota modulation in cardiac cell therapy with immunosuppression in a

nonhuman primate ischemia/reperfusion model. NPJ Regen Med, 10:2.
[69] Pei T, Li W, Zhou Z, Zhang Q, Yu G, Yin S, et al. (2025). The relationship between tryptophan metabolism and gut microbiota: Interaction mechanism and potential effects in infection treatment. Microbiol Res, 298:128211.
[70] Lun H, Li P, Li J, Liu F (2024). The effect of intestinal flora metabolites on macrophage polarization. Heliyon, 10:e35755.
[71] Yu L, Pan J, Guo M, Duan H, Zhang H, Narbad A, et al. (2024). Gut microbiota and anti-aging: Focusing on spermidine. Crit Rev Food Sci Nutr, 64:10419-10437.
[72] Mithul Aravind S, Wichienchot S, Tsao R, Ramakrishnan S, Chakkaravarthi S (2021). Role of dietary polyphenols on gut microbiota, their metabolites and health benefits. Food Res Int, 142:110189.
[73] Man AWC, Zhou Y, Xia N, Li H (2020). Involvement of Gut Microbiota, Microbial Metabolites and Interaction with Polyphenol in Host Immunometabolism. Nutrients, 12:3054.
[74] Li H, Christman LM, Li R, Gu L (2020). Synergic interactions between polyphenols and gut microbiota in mitigating inflammatory bowel diseases. Food Funct, 11:4878-4891.
[75] Kiriyama Y, Tokumaru H, Sadamoto H, Kobayashi S, Nochi H (2024). Effects of Phenolic Acids Produced from Food-Derived Flavonoids and Amino Acids by the Gut Microbiota on Health and Disease. Molecules, 29:5102.
[76] Wang X, Qi Y, Zheng H (2022). Dietary Polyphenol, Gut Microbiota, and Health Benefits. Antioxidants (Basel), 11:1212.
[77] Zhou N, Gu X, Zhuang T, Xu Y, Yang L, Zhou M (2020). Gut Microbiota: A Pivotal Hub for Polyphenols as Antidepressants. J Agric Food Chem, 68:6007-6020.
[78] Zhang L, Lu J (2025). Combating depression with rosemary (Rosmarinus officinalis L.)-derived polyphenols: Major phytochemicals, functional properties, and potential. Fitoterapia, 183:106563.
[79] Bianchi F, Cappella A, Gagliano N, Sfondrini L, Stacchiotti A (2022). Polyphenols-Gut-Heart: An Impactful Relationship to Improve Cardiovascular Diseases. Antioxidants (Basel), 11:1700.
[80] Cheng B, Feng H, Li C, Jia F, Zhang X (2025). The mutual effect of dietary fiber and polyphenol on gut microbiota: Implications for the metabolic and microbial modulation and associated health benefits. Carbohydr Polym, 358:123541.
[81] Rocchetti G, Luisa Callegari M, Senizza A, Giuberti G, Ruzzolini J, Romani A, et al. (2022). Oleuropein from olive leaf extracts and extra-virgin olive oil provides distinctive phenolic profiles and modulation of microbiota in the large intestine. Food Chem, 380:132187.
[82] Wang Z, Zhang W, Liu Z, Huang D, Kang H, Wang J, et al. (2025). Gut microbiota dysbiosis involved in decabromodiphenyl ether-induced bone homeostasis disorder through inflammaging. Environ Pollut, 368:125710.
[83] Tran M, Huh JR, Devlin AS (2025). The role of gut microbial metabolites in the T cell lifecycle. Nat Immunol, 26:1246-1257.
[84] Liu R, Wang J, Liu Y, Gao Y, Yang R (2024). Regulation of gut microbiota on immune cell ferroptosis: A novel insight for immunotherapy against tumor. Cancer Lett, 598:217115.
[85] Witkowski M, Weeks TL, Hazen SL (2020). Gut Microbiota and Cardiovascular Disease. Circ Res, 127:553-570.
[86] Zhen J, Zhou Z, He M, Han HX, Lv EH, Wen PB, et al. (2023). The gut microbial metabolite trimethylamine N-oxide and cardiovascular diseases. Front Endocrinol (Lausanne), 14:1085041.
[87] Cao H, Zhu Y, Hu G, Zhang Q, Zheng L (2023). Gut microbiome and metabolites, the future direction of diagnosis and treatment of atherosclerosis?. Pharmacol Res, 187:106586.
[88] Nemet I, Saha PP, Gupta N, Zhu W, Romano KA, Skye SM, et al. (2020). A Cardiovascular Disease-Linked Gut Microbial Metabolite Acts via Adrenergic Receptors. Cell, 180:862-877.e22.
[89] Dicks LMT (2024). Cardiovascular Disease May Be Triggered by Gut Microbiota, Microbial Metabolites, Gut Wall Reactions, and Inflammation. Int J Mol Sci, 25:10634.
[90] Zhu J, Lyu J, Zhao R, Liu G, Wang S (2023). Gut macrobiotic and its metabolic pathways modulate cardiovascular disease. Front Microbiol, 14:1272479.
[91] Anselmi G, Gagliardi L, Egidi G, Leone S, Gasbarrini A, Miggiano GAD, et al. (2021). Gut Microbiota and Cardiovascular Diseases: A Critical Review. Cardiol Rev, 29:195-204.
[92] Jiang Y, Pang S, Liu X, Wang L, Liu Y (2024). The Gut Microbiome Affects Atherosclerosis by Regulating Reverse Cholesterol Transport. J Cardiovasc Transl Res, 17:624-637.
[93] Zhu Y, Shui X, Liang Z, Huang Z, Qi Y, He Y, et al. (2020). Gut microbiota metabolites as integral mediators in cardiovascular diseases (Review). Int J Mol Med, 46:936-948.
[94] Yu Y, Ding Y, Wang S, Jiang L (2025). Gut Microbiota Dysbiosis and Its Impact on Type 2 Diabetes: From Pathogenesis to Therapeutic Strategies. Metabolites, 15:397.
[95] Novakovic M, Rout A, Kingsley T, Kirchoff R, Singh A, Verma V, et al. (2020). Role of gut microbiota in cardiovascular diseases. World J Cardiol, 12:110-122.
[96] Li J, Lv JL, Cao XY, Zhang HP, Tan YJ, Chu T, et al. (2022). Gut microbiota dysbiosis as an inflammaging condition that regulates obesity-related retinopathy and nephropathy. Front Microbiol, 13:1040846.
[97] Floyd JL, Prasad R, Dupont MD, Adu-Rutledge Y, Anshumali S, Paul S, et al. (2025). Intestinal neutrophil extracellular traps promote gut barrier damage exacerbating endotoxaemia, systemic inflammation and progression of diabetic retinopathy in type 2 diabetes. Diabetologia, 68:866-889.
[98] Botía-Sánchez M, Galicia G, Albaladejo-Marico L, Toro-Domínguez D, Morell M, Marcos-Fernández R, et

al. (2023). Gut epithelial barrier dysfunction in lupus triggers a differential humoral response against gut commensals. Front Immunol, 14:1200769.
[99] Wu ML, Yang XQ, Xue L, Duan W, Du JR (2021). Age-related cognitive decline is associated with microbiota-gut-brain axis disorders and neuroinflammation in mice. Behav Brain Res, 402:113125.
[100] Hsu CN, Yu HR, Lin IC, Tiao MM, Huang LT, Hou CY, et al. (2022). Sodium butyrate modulates blood pressure and gut microbiota in maternal tryptophan-free diet-induced hypertension rat offspring. J Nutr Biochem, 108:109090.
[101] Ronen D, Rokach Y, Abedat S, Qadan A, Daana S, Amir O, et al. (2024). Human Gut Microbiota in Cardiovascular Disease. Compr Physiol, 14:5449-5490.
[102] Chen X, Zhang H, Ren S, Ding Y, Remex NS, Bhuiyan MS, et al. (2023). Gut microbiota and microbiota-derived metabolites in cardiovascular diseases. Chin Med J (Engl), 136:2269-2284.
[103] Abrignani V, Salvo A, Pacinella G, Tuttolomondo A (2024). The Mediterranean Diet, Its Microbiome Connections, and Cardiovascular Health: A Narrative Review. Int J Mol Sci, 25:4942.
[104] García-Gavilán JF, Atzeni A, Babio N, Liang L, Belzer C, Vioque J, et al. (2024). Effect of 1-year lifestyle intervention with energy-reduced Mediterranean diet and physical activity promotion on the gut metabolome and microbiota: a randomized clinical trial. Am J Clin Nutr, 119:1143-1154.
[105] Yang Y, Karampoor S, Mirzaei R, Borozdkin L, Zhu P (2023). The interplay between microbial metabolites and macrophages in cardiovascular diseases: A comprehensive review. Int Immunopharmacol, 121:110546.
[106] Vrieze A, Van Nood E, Holleman F, et al (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology, 143:913-916.e7.
[107] Kootte RS, Levin E, Salojarvi J, et al (2017). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. Cell Metab, 26:611-619.e6.
[108] Napolitano M, Covasa M (2020). Microbiota Transplant in the Treatment of Obesity and Diabetes: Current and Future Perspectives. Front Microbiol, 11:590370.
[109] Wang H, Zhang H, Gao Z, Zhang Q, Gu C (2022). The mechanism of berberine alleviating metabolic disorder based on gut microbiome. Front Cell Infect Microbiol, 12:854885.
[110] Fan Y, Pedersen O (2021). Gut microbiota in human metabolic health and disease. Nat Rev Microbiol, 19:55-71.
[111] Zhao Q, Wu J, Ding Y, Pang Y, Jiang C (2023). Gut microbiota, immunity, and bile acid metabolism: decoding metabolic disease interactions. Life Metab, 2:load032.
[112] Ye J, Wu Z, Zhao Y, Zhang S, Liu W, Su Y (2022). Role of gut microbiota in the pathogenesis and treatment of diabetes mullites: Advanced research-based review. Front Microbiol, 13:1029890.
[113] Yan X, Shi L, Zhu X, Zhao Y, Luo J, Li Q, et al. (2025). From Microbial Homeostasis to Systemic Pathogenesis: A Narrative Review on Gut Flora's Role in Neuropsychiatric, Metabolic, and Cancer Disorders. J Inflamm Res, 18:8851-8873.
[114] Zhong H, Yu Y, Abdullah, Zhang H, Du J, Sun J, et al. (2025). Lactiplantibacillus plantarum N1 derived lipoteichoic acid alleviates insulin resistance in association with modulation of the gut microbiota and amino acid metabolism. Food Funct, 16:1371-1388.
[115] Wang JS, Liu JC (2022). Intestinal microbiota in the treatment of metabolically associated fatty liver disease. World J Clin Cases, 10:11240-11251.
[116] Barber TM, Hanson P, Weickert MO (2023). Metabolic-Associated Fatty Liver Disease and the Gut Microbiota. Endocrinol Metab Clin North Am, 52:485-496.
[117] Basnet TB, Gc S, Basnet R, Fatima S, Safdar M, Sehar B, et al. (2023). Interaction between gut microbiota metabolites and dietary components in lipid metabolism and metabolic diseases. Access Microbiol, 5:acmi000403.
[118] Wang W, Jiang S, Xu C, Tang L, Liang Y, Zhao Y, et al. (2022). Interactions between gut microbiota and Parkinson's disease: The role of microbiota-derived amino acid metabolism. Front Aging Neurosci, 14:976316.
[119] Nie T, You L, Tang F, Duan Y, Nepovimova E, Kuca K, et al. (2025). Microbiota-Gut-Brain Axis in Age-Related Neurodegenerative Diseases. Curr Neuropharmacol, 23:524-546.
[120] Loh JS, Mak WQ, Tan LKS, Ng CX, Chan HH, Yeow SH, et al. (2024). Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. Signal Transduct Target Ther, 9:37.
[121] Chen C, Wang GQ, Li DD, Zhang F (2025). Microbiota-gut-brain axis in neurodegenerative diseases: molecular mechanisms and therapeutic targets. Mol Biomed, 6:64.
[122] Cheng Y, Chen C, Zhang F (2023). Immunity orchestrates a bridge in gut-brain axis of neurodegenerative diseases. Ageing Res Rev, 85:101857.
[123] Wachamo S, Gaultier A (2025). The emerging role of microbiota derived SCFAs in neurodegenerative disorders. Brain Behav Immun Health, 46:101012.
[124] Solanki R, Karande A, Ranganathan P (2023). Emerging role of gut microbiota dysbiosis in neuroinflammation and neurodegeneration. Front Neurol, 14:1149618.
[125] Olejnik P, Golenia A, Małyszko J (2025). The Potential Role of Microbiota in Age-Related Cognitive Decline: A Narrative Review of the Underlying Molecular Mechanisms. Int J Mol Sci, 26:1590.
[126] Bonaz B (2024). The gut-brain axis in Parkinson's disease. Rev Neurol (Paris), 180:65-78.
[127] Tuigunov D, Sinyavskiy Y, Nurgozhin T, Zholdassova Z, Smagul G, Omarov Y, et al. (2025). Precision Nutrition and Gut-Brain Axis Modulation in the Prevention of Neurodegenerative Diseases. Nutrients, 17:3068.
[128] Ren J, Wang Q, Hong H, Tang C (2025). Fecal Microbiota Transplantation in Alzheimer's Disease:

Mechanistic Insights Through the Microbiota-Gut-Brain Axis and Therapeutic Prospects. Microorganisms, 13:1956.
[129] Munteanu C, Onose G, Rotariu M, Poștaru M, Turnea M, Galaction AI (2024). Role of Microbiota-Derived Hydrogen Sulfide (H2S) in Modulating the Gut-Brain Axis: Implications for Alzheimer's and Parkinson's Disease Pathogenesis. Biomedicines, 12:2670.
[130] Kim NY, Lee HY, Choi YY, Mo SJ, Jeon S, Ha JH, et al. (2024). Effect of gut microbiota-derived metabolites and extracellular vesicles on neurodegenerative disease in a gut-brain axis chip. Nano Converg, 11:7.
[131] Gong Y, Chen A, Zhang G, Shen Q, Zou L, Li J, et al. (2023). Cracking Brain Diseases from Gut Microbes-Mediated Metabolites for Precise Treatment. Int J Biol Sci, 19:2974-2998.
[132] Kurhaluk N, Kamiński P, Bilski R, Kołodziejska R, Woźniak A, Tkaczenko H (2025). Role of Antioxidants in Modulating the Microbiota-Gut-Brain Axis and Their Impact on Neurodegenerative Diseases. Int J Mol Sci, 26:3658.
[133] Uceda S, Echeverry-Alzate V, Reiriz-Rojas M, Martínez-Miguel E, Pérez-Curiel A, Gómez-Senent S, et al. (2023). Gut Microbial Metabolome and Dysbiosis in Neurodegenerative Diseases: Psychobiotics and Fecal Microbiota Transplantation as a Therapeutic Approach-A Comprehensive Narrative Review. Int J Mol Sci, 24:13294.
[134] Raber J, Sharpton TJ (2023). Gastrointestinal Dysfunction in Neurological and Neurodegenerative Disorders. Semin Neurol, 43:634-644.
[135] Loomba R, Seguritan V, Li W, et al (2017). Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab, 25:1054-1062.e5.
[136] Yuan J, Chen C, Cui J, et al (2019). Fatty liver disease caused by high-alcohol-producing Klebsiella pneumoniae. Cell Metab, 30:675-688.e7.
[137] Duan Y, Llorente C, Lang S, et al (2019). Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature, 575:505-511.
[138] Bajaj JS, Kassam Z, Fagan A, et al (2017). Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. Hepatology, 66:1727-1738.
[139] Bajaj JS, Gavis EA, Fagan A, et al (2021). A randomized clinical trial of fecal microbiota transplant for alcohol use disorder. Hepatology, 73:1688-1700.
[140] Zhou J, Liu J, Lin Q, Shi L, Zeng Z, Guan L, et al. (2023). Characteristics of the gut microbiome and metabolic profile in elderly patients with sarcopenia. Front Pharmacol, 14:1279448.
[141] Lian WS, Wang FS, Chen YS, Tsai MH, Chao HR, Jahr H, et al. (2022). Gut Microbiota Ecosystem Governance of Host Inflammation, Mitochondrial Respiration and Skeletal Homeostasis. Biomedicines, 10:860.
[142] You X, Dadwal UC, Lenburg ME, Kacena MA, Charles JF (2022). Murine Gut Microbiome Meta-analysis Reveals Alterations in Carbohydrate Metabolism in Response to Aging. mSystems, 7:e0124821.
[143] Lee K, Park SD, Kim JY, Shim JJ, Lee JH (2025). Postbiotic Intervention in Sarcopenia: The Role of Lactiplantibacillus plantarum HY7715 and Its Extracellular Vesicles. Life (Basel), 15:1101.
[144] Xu J, Zheng Z, Yang L, Li R, Ma X, Zhang J, et al. (2022). A novel promising diagnosis model for colorectal advanced adenoma and carcinoma based on the progressive gut microbiota gene biomarkers. Cell Biosci, 12:208.
[145] Liu C, Wang L, Huai J, He S, Su Q, Min Q, et al. (2025). TMOD2 and DOCK4 as Novel Gut Microbiota-Associated Biomarkers for Colorectal Adenoma: Integrated Transcriptomic Analysis and Therapeutic Target Identification. Mediators Inflamm, 2025:6267309.
[146] Peng Y, Gu J, Liu F, Wang P, Wang X, Si C, et al. (2024). Integrated analysis of microbiota and gut microbial metabolites in blood for breast cancer. mSystems, 9:e0064324.
[147] Li MY, Wu XQ, Lee TKW (2026). Harnessing the microbiome: unveiling the influence of the gut microbiota on hepatobiliary cancer therapeutic strategies. Clin Mol Hepatol, 32:91-126.
[148] Jiang SS, Kang ZR, Chen YX, Fang JY (2025). The gut microbiome modulate response to immunotherapy in cancer. Sci China Life Sci, 68:381-396.
[149] Lucas A, Reale M, Wolf YI, Duong B, Zhang Y, Wickramasinghe J, et al. (2025). Taxonomy-free fecal microbiome profiles enable robust prediction of immunotherapy response and toxicity in melanoma. bioRxiv. doi: 10.1101/2025.11.06.686285.
[150] Silva CAC, Fidelle M, Almonte AA, Derosa L, Zitvogel L (2025). Gut Microbiota-Related Biomarkers in Immuno-Oncology. Annu Rev Pharmacol Toxicol, 65:333-354.
[151] Merra G, Noce A, Marrone G, Cintoni M, Tarsitano MG, Capacci A, et al. (2020). Influence of Mediterranean Diet on Human Gut Microbiota. Nutrients, 13:7.
[152] Shah S, Mu C, Shen-Tu G, Rohmann N, Schlicht K, Laudes M, et al. (2025). Influence of dietary components on the gut microbiota of middle-aged adults: the gut-Mediterranean connection. BMC Microbiol, 25:573.
[153] Meiners F, Ortega-Matienzo A, Fuellen G, Barrantes I (2025). Gut microbiome-mediated health effects of fiber and polyphenol-rich dietary interventions. Front Nutr, 12:1647740.
[154] Craig WJ, Mangels AR, Fresán U, Marsh K, Miles FL, Saunders AV, et al. (2021). The Safe and Effective Use of Plant-Based Diets with Guidelines for Health Professionals. Nutrients, 13:4144.
[155] Ghosh TS, Rampelli S, Jeffery IB, et al (2020). Mediterranean diet intervention alters the gut microbiome in older people, reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. Gut, 69:1218-1228.
[156] Nemzer BV, Al-Taher F, Kalita D, Yashin AY, Yashin YI (2025). Health-Improving Effects of Polyphenols on

the Human Intestinal Microbiota: A Review. Int J Mol Sci, 26:1335.
[157] Xie J, Sun N, Huang H, Xie J, Chen Y, Hu X, et al. (2022). Catabolism of polyphenols released from mung bean coat and its effects on gut microbiota during in vitro simulated digestion and colonic fermentation. Food Chem, 396:133719.
[158] Ao T, Huang H, Zheng B, Chen Y, Xie J, Hu X, et al. (2024). Ameliorative effect of bound polyphenols in mung bean coat dietary fiber on DSS-induced ulcerative colitis in mice: the intestinal barrier and intestinal flora. Food Funct, 15:4154-4169.
[159] Karl JP, Fagnant HS, Radcliffe PN, Wilson M, Karis AJ, Sayers B, et al. (2025). Gut microbiota-targeted dietary supplementation with fermentable fibers and polyphenols prevents hypobaric hypoxia-induced increases in intestinal permeability. Am J Physiol Regul Integr Comp Physiol, 329:R378-R399.
[160] Song Z, Jiang H, Dai H, Bo L, Song C, Liu X, et al. (2025). Corn Bran IDF and Polyphenol-Reduced IDF Alleviate Low-Dose 3-MCPD-Induced Toxicity by Modulating Gut Microbiota and Intestinal Barrier. Foods, 14:4253.
[161] Basavaiah R, Gurudutt PS (2021). Prebiotic Carbohydrates for Therapeutics. Endocr Metab Immune Disord Drug Targets, 21:230-245.
[162] Ambrogi V, Bottacini F, Cao L, Kuipers B, Schoterman M, van Sinderen D (2023). Galacto-oligosaccharides as infant prebiotics: production, application, bioactive activities and future perspectives. Crit Rev Food Sci Nutr, 63:753-766.
[163] Zeng M, van Pijkeren JP, Pan X (2023). Gluco-oligosaccharides as potential prebiotics: Synthesis, purification, structural characterization, and evaluation of prebiotic effect. Compr Rev Food Sci Food Saf, 22:2611-2651.
[164] Ryabtseva SA, Shpack MA, Anisimov GS, Sazanova SN, Skorokhodova MV, Romakhova VY (2025). [Prebiotic properties and application of galactooligosaccharides]. Vopr Pitan, 94:6-23.
[165] Hamdi A, Viera-Alcaide I, Guillén-Bejarano R, Rodríguez-Arcos R, Muñoz MJ, Monje Moreno JM, et al. (2022). Asparagus Fructans as Emerging Prebiotics. Foods, 12:81.
[166] Zhang N, Zhang C, Zhang Y, Ma Z, Li L, Liu W (2024). Distinct prebiotic effects of polysaccharide fractions from Polygonatum kingianum on gut microbiota. Int J Biol Macromol, 279:135568.
[167] Peredo-Lovillo A, Romero-Luna HE, Jiménez-Fernández M (2020). Health promoting microbial metabolites produced by gut microbiota after prebiotics metabolism. Food Res Int, 136:109473.
[168] Villoslada-Blanco P, Pérez-Matute P, Oteo JA (2021). Lights and Shadows of Microbiota Modulation and Cardiovascular Risk in HIV Patients. Int J Environ Res Public Health, 18:6837.
[169] Yoo S, Jung SC, Kwak K, Kim JS (2024). The Role of Prebiotics in Modulating Gut Microbiota: Implications for Human Health. Int J Mol Sci, 25:4834.
[170] Rousseaux A, Brosseau C, Bodinier M (2023). Immunomodulation of B Lymphocytes by Prebiotics, Probiotics and Synbiotics: Application in Pathologies. Nutrients, 15:269.
[171] Shahbazi R, Yasavoli-Sharahi H, Mallet JF, Sharifzad F, Alsadi N, Cuenin C, et al. (2023). Novel Probiotic Bacterium Rouxiella badensis subsp. acadiensis (Canan SV-53) Modulates Gut Immunity through Epigenetic Mechanisms. Microorganisms, 11:2456.
[172] Satoh T (2023). New prebiotics by ketone donation. Trends Endocrinol Metab, 34:414-425.
[173] Yang J, Hou L, Wang A, Shang L, Jia X, Xu R, et al. (2024). Prebiotics improve frailty status in community-dwelling older individuals in a double-blind, randomized, controlled trial. J Clin Invest, 134:e176507.
[174] Balthazar CF, Guimarães JF, Coutinho NM, Pimentel TC, Ranadheera CS, Santillo A, et al. (2022). The future of functional food: Emerging technologies application on prebiotics, probiotics and postbiotics. Compr Rev Food Sci Food Saf, 21:2560-2586.
[175] Chu X, Xing H, Chao M, Xie P, Jiang L (2025). Gut Microbiota Modulation in Osteoporosis: Probiotics, Prebiotics, and Natural Compounds. Metabolites, 15:301.
[176] Mahdavi M, Laforest-Lapointe I, Massé E (2021). Preventing Colorectal Cancer through Prebiotics. Microorganisms, 9:1325.
[177] Rout SK, Rhyner D, Riek R, Greenwald J (2022). Prebiotically Plausible Autocatalytic Peptide Amyloids. Chemistry, 28:e202103841.
[178] Rodriguez J, Delzenne NM (2021). Modulation of the gut microbiota-adipose tissue-muscle interactions by prebiotics. J Endocrinol, 249:R1-R23.
[179] Liu Y, Zeng Y, Liu Y, Wang X, Chen Y, Lepp D, et al. (2022). Regulatory Effect of Isomaltodextrin on a High-Fat Diet Mouse Model with LPS-Induced Low-Grade Chronic Inflammation. J Agric Food Chem, 70:11258-11273.
[180] Yang Y, Zhou B, Zhang S, Si L, Liu X, Li F (2023). Prebiotics for depression: how does the gut microbiota play a role?. Front Nutr, 10:1206468.
[181] Dalile B, Boyle NB, Ruiz FT, Chakrabarti A, Respondek F, Dodd GF, et al. (2025). Targeting Cognitive Resilience through Prebiotics: A Focused Perspective. Adv Nutr, 16:100343.
[182] Kong Y, Cao P, Wu J, Ye X (2026). Prebiotics as adjunctive treatment ameliorates DSS-induced colitis and gut microbiota. Microbiol Spectr, 14:e0150225.
[183] Ricke SC (2021). Prebiotics and alternative poultry production. Poult Sci, 100:101174.
[184] Richards PJ, Almutrafy A, Liang L, Flaujac Lafontaine GM, King E, Fish NM, et al. (2024). Prebiotic galactooligosaccharide feed modifies the chicken gut microbiota to efficiently clear Salmonella. mSystems, 9:e0075424.
[185] Zeng M, Li Y, Cheng J, Wang J, Liu Q (2025). Prebiotic Oligosaccharides in Skin Health: Benefits, Mechanisms, and Cosmetic Applications. Antioxidants (Basel), 14:754.

[186] Shanuke DS, Ranasinghage NBDP, Illippangama AU, Kulathunga J, Bandara MD (2025). Co-Encapsulation of Probiotics and Prebiotics: Techniques and Applications in Food Fortification. Food Sci Nutr, 13:e70426.
[187] Kvakova M, Bertkova I, Stofilova J, Savidge TC (2021). Co-Encapsulated Synbiotics and Immobilized Probiotics in Human Health and Gut Microbiota Modulation. Foods, 10:1297.
[188] Mafe AN, Iruoghene Edo G, Akpoghelie PO, Gaaz TS, Yousif E, Zainulabdeen K, et al. (2025). Probiotics and Food Bioactives: Unraveling Their Impact on Gut Microbiome, Inflammation, and Metabolic Health. Probiotics Antimicrob Proteins, 17:1851-1892.
[189] Ghadaksaz A, Nodoushan SM, Sedighian H, Behzadi E, Fooladi AAI (2022). Evaluation of the Role of Probiotics As a New Strategy to Eliminate Microbial Toxins: a Review. Probiotics Antimicrob Proteins, 14:224-237.
[190] Hmar EBL, Paul S, Sharma HK (2024). An Insight into the Combination of Probiotics and their Implications for Human Health. Endocr Metab Immune Disord Drug Targets, 24:1-12.
[191] Tas GG, Sati L (2024). Probiotic Lactobacillus rhamnosus species: considerations for female reproduction and offspring health. J Assist Reprod Genet, 41:2585-2605.
[192] Du Q, Li Q, Liu C, Liao G, Li J, Yang J, et al. (2024). Probiotics/prebiotics/synbiotics and human neuropsychiatric outcomes: an umbrella review. Benef Microbes, 15:589-608.
[193] Szczepankowska AK, Cukrowska B, Aleksandrzak-Piekarczyk T (2025). Complete genome sequence of Bifidobacterium animalis subsp. lactis BI040, a probiotic strain with multiple health benefits from the NORDBIOTIC collection. Microbiol Resour Announc, 14:e0111724.
[194] Ghelardi E, Abreu Y Abreu AT, Marzet CB, Álvarez Calatayud G, Perez M, Moschione Castro AP (2022). Current Progress and Future Perspectives on the Use of Bacillus clausii. Microorganisms, 10:1246.
[195] Kwoji ID, Aiyegoro OA, Okpeku M, Adeleke MA (2021). Multi-Strain Probiotics: Synergy among Isolates Enhances Biological Activities. Biology (Basel), 10:322.
[196] Dailey Z, Sanders ME, Merenstein D (2020). Retail refrigerated probiotic foods and their association with evidence of health benefits. Benef Microbes, 11:131-133.
[197] Shehata HR, Ragupathy S, Allen S, Leyer G, Newmaster SG (2021). Real-Time PCR Assays for the Specific Identification of Probiotic Strains Lactobacillus gasseri BNR17 and Lactobacillus reuteri LRC (NCIMB 30242). Probiotics Antimicrob Proteins, 13:837-846.
[198] Zhang Y, Wei X, Sun Q, Qian W, Liu X, Li J, et al. (2021). Different Types and Functional Effects of Probiotics on Human Health through Regulating Glucose Homeostasis. J Agric Food Chem, 69:14781-14791.
[199] Li X, Xia Y, Song X, Xiong Z, Ai L, Wang G (2025). Probiotics intervention for type 2 diabetes mellitus therapy: a review from proposed mechanisms to future prospects. Crit Rev Food Sci Nutr, 65:4457-4475.
[200] Kesika P, Sivamaruthi BS, Chaiyasut C (2019). Do Probiotics Improve the Health Status of Individuals with Diabetes Mellitus? A Review on Outcomes of Clinical Trials. Biomed Res Int, 2019:1531567.
[201] Thoda C, Touraki M (2025). Molecular Mechanisms of Probiotic Action Against Gastrointestinal Cancers. Int J Mol Sci, 26:7857.
[202] Thapa D, Kumar V, Naik B, Kumar V, Gupta AK, Mohanta YK, et al. (2024). Harnessing probiotic foods: managing cancer through gut health. Food Sci Biotechnol, 33:2141-2160.
[203] Farias da Cruz M, Baraúna Magno M, Alves Jural L, Pimentel TC, Masterson Tavares Pereira Ferreira D, Almeida Esmerino E, et al. (2022). Probiotics and dairy products in dentistry: A bibliometric and critical review of randomized clinical trials. Food Res Int, 157:111228.
[204] Baddouri L, Hannig M (2024). Probiotics as an adjunctive therapy in periodontitis treatment-reality or illusion-a clinical perspective. NPJ Biofilms Microbiomes, 10:148.
[205] Sharma R, Mokhtari S, Jafari SM, Sharma S (2022). Barley-based probiotic food mixture: health effects and future prospects. Crit Rev Food Sci Nutr, 62:7961-7975.
[206] Mikucka A, Deptuła A, Bogiel T, Chmielarczyk A, Nurczyńska E, Gospodarek-Komkowska E (2022). Bacteraemia Caused by Probiotic Strains of Lacticaseibacillus rhamnosus-Case Studies Highlighting the Need for Careful Thought before Using Microbes for Health Benefits. Pathogens, 11:977.
[207] Vinderola G, Sanders ME, Salminen S (2022). The Concept of Postbiotics. Foods, 11:1077.
[208] Amobonye A, Pillay B, Hlope F, Asong ST, Pillai S (2025). Postbiotics: an insightful review of the latest category in functional biotics. World J Microbiol Biotechnol, 41:293.
[209] Pandey K, Gosai H, Patel RB (2025). An updated review on advantages, disadvantages and uncertainties associated with probiotics. J Dairy Res. doi: 10.1017/S0022029925101155.
[210] Scott E, De Paepe K, Van de Wiele T (2022). Postbiotics and Their Health Modulatory Biomolecules. Biomolecules, 12:1640.
[211] Thirumdas R, Mudgil P (2025). Emerging Nonthermal Technologies for the Production of Postbiotics. Compr Rev Food Sci Food Saf, 24:e70335.
[212] Zhang T, Zhang W, Feng C, Kwok LY, He Q, Sun Z (2022). Stronger gut microbiome modulatory effects by postbiotics than probiotics in a mouse colitis model. NPJ Sci Food, 6:53.
[213] Wang G, Wang Y, Sheng K, Wang Y (2025). Effect of probiotic extracellular vesicles and their applications on health and disease. J Sci Food Agric, 105:3539-3549.
[214] Wang P, Wang S, Wang D, Li Y, Yip RCS, Chen H (2024). Postbiotics-peptidoglycan, lipoteichoic acid, exopolysaccharides, surface layer protein and pili proteins-Structure, activity in wounds and their delivery systems. Int J Biol Macromol, 274:133195.
[215] Vinderola G, Benkowski A, Bernardeau M, Chenoll E, Collado MC, Cronin U, et al. (2025). Postbiotics: a

perspective on their quantification. Front Nutr, 12:1582733.
[216] Mandaliya DK, Patel S, Seshadri S (2025). Postbiotic potential of SCFAs on metaflammation and gut microbiota alteration in diabetes. J Biosci, 50:57.
[217] Zhou G, Wang R, Fu C, Du Y, Li X, Xia Q, et al. (2026). Postbiotics: emerging regulatory mechanisms and therapeutic applications in obesity and its complications. Int J Obes (Lond), 50:976-987.
[218] Stelmach V, Stavrou G, Theodorou I, Semertzidou E, Tzikos G, Menni AE, et al. (2024). Postbiotics: Mapping the Trend. Nutrients, 16:3077.
[219] Wastyk HC, Fragiadakis GK, Perelman D, et al (2021). Gut-microbiota-targeted diets modulate human immune status. Cell, 184:4137-4153.e14.
[220] Choi Y, Keum GB, Kang J, Doo H, Kwak J, Kim H, et al. (2025). Evaluation of kefir consumption on gut microbial diversity in a healthy young population using full-length 16S rRNA sequencing. Front Microbiol, 16:1587831.
[221] Ariestine DA, Sari DK, Siahaan L, Widyawati T, Rusda M, Sari MD, et al. (2025). Impact of Areca Catechu Kombucha on Inflammatory Markers and Gut Microbiota - a Review. Med Arch, 79:411-416.
[222] Hemachandra S, Rathnayake SN, Jayamaha AA, Francis BS, Welmillage D, Kaur DN, et al. (2025). Fecal Microbiota Transplantation as an Alternative Method in the Treatment of Obesity. Cureus, 17:e76858.
[223] Muralitharan RR, Snelson M, Meric G, Coughlan MT, Marques FZ (2023). Guidelines for microbiome studies in renal physiology. Am J Physiol Renal Physiol, 325:F345-F362.
[224] Liu Z, de Vries B, Gerritsen J, Smidt H, Zoetendal EG (2020). Microbiome-based stratification to guide dietary interventions to improve human health. Nutr Res, 82:1-10.
[225] Beltrán-Velasco AI, Clemente-Suárez VJ (2025). Harnessing Gut Microbiota for Biomimetic Innovations in Health and Biotechnology. Biomimetics (Basel), 10:73.
[226] Yang W, Jin Q, Xiao D, Li X, Huang D (2025). Interaction mechanism and intervention strategy between metabolic dysfunction-associated steatotic liver disease and intestinal microbiota. Front Microbiol, 16:1597995.
[227] Tin CM, Cordazzo Vargas B, Abbott DA, Lohar AR, Taylor TC, Valishev IA, et al. (2025). Single-cell quantification of the microbiota by flow cytometry: MicFLY. bioRxiv. doi: 10.1101/2025.09.18.677130.
[228] Lee SH, You HS, Kang HG, Kang SS, Hyun SH (2020). Association between Altered Blood Parameters and Gut Microbiota after Synbiotic Intake in Healthy, Elderly Korean Women. Nutrients, 12:3112.
[229] Liu H, Yin X, Li J, Cao Y, Wang Y, Mu W, et al. (2022). Preoperative intestinal microbiome and metabolome in elderly patients with delayed neurocognitive recovery. Anaesth Crit Care Pain Med, 41:101140.
[230] Korf JM, Ganesh BP, McCullough LD (2022). Gut dysbiosis and age-related neurological diseases in females. Neurobiol Dis, 168:105695.
[231] Wei CR, Basharat Z, Osama M, Mah K, Waheed Y, Hassan SS (2025). An Overview of the Association of the Urinary Tract Microbiome with Various Diseases and Implications for Therapeutics. Mini Rev Med Chem, 25:1420-1443.