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From mouth to muscle: mechanistic and interventional perspectives on the tongue-coating microbiome in sarcopenia

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Abstract

Background Sarcopenia, the progressive loss of skeletal muscle mass and function, needs upstream, low-burden tools for early detection and high-frequency monitoring, especially in older adults. Conventional assessments such as handgrip strength and gait speed mainly capture downstream impairment and may miss early physiological change. The tongue-coating microbiome is an emerging, measurable niche on the oral–gut–muscle axis that may provide proximal signals of metabolic, inflammatory, and circadian status.

Methods We performed a narrative summary of recent evidence on tongue–gut coupling, mapped plausible mechanisms to muscle regulation, and evaluated the feasibility of tongue-based measurement. We propose a minimal methods set (fixed pre-breakfast sampling, strict low-biomass quality control, AI-assisted standardized tongue imaging, saliva assays integrated with multi-omics) and a three-tier metric structure aligned to the minimal clinically important difference (MCID) for functional endpoints.

Results Evidence supports links across three axes: metabolic (microbial metabolites such as short-chain fatty acids and niacin that modulate mitochondrial energetics and anabolism), inflammatory (oral dysbiosis and barrier disruption amplifying systemic inflammation via lipopolysaccharide, Toll-like receptor 4, and NF-κB signaling), and circadian (microbiome rhythms coupled to eating and sleep timing). The tongue coating forms a stable niche suitable for frequent follow-up. An upstream–midstream–downstream metric stack enables MCID-anchored interpretation. Current data are limited and heterogeneous, so tongue-derived metrics should complement stool testing and functional standards.

Conclusions Tongue-based monitoring is a practical adjunct for earlier risk signaling and community-level follow-up. Priorities are multicenter validation, interpretable and device agnostic models, and axis-stratified trials to define when and for whom tongue-derived signals add MCID-level clinical value. Because direct longitudinal human evidence linking tongue-coating signals to clinically meaningful sarcopenia outcomes remains limited, we frame the tongue-coating microbiome primarily as a hypothesis-driven, upstream monitoring niche and outline testable priorities for validation and translation.

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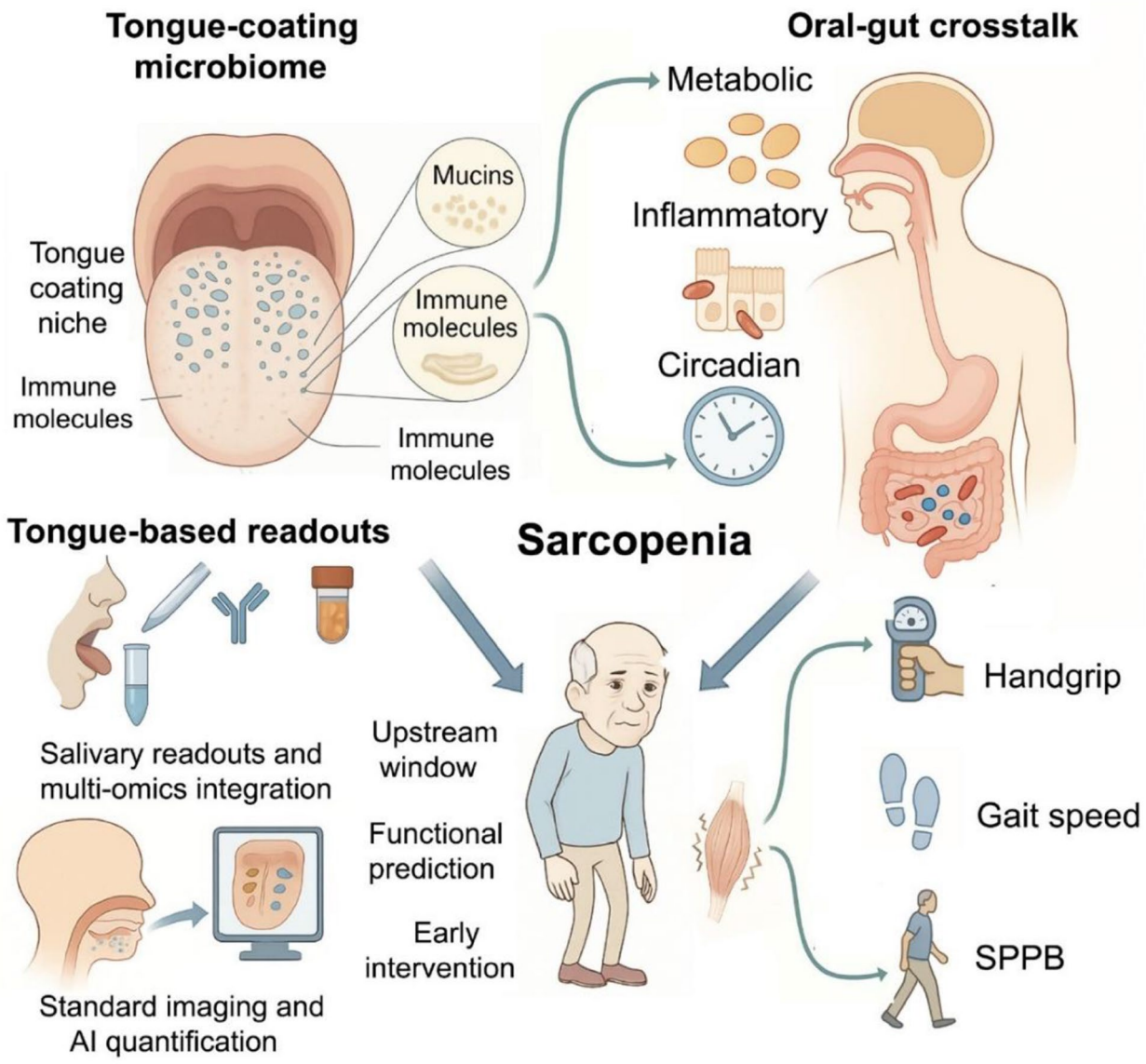
Highlights

- Tongue-coating microbiome offers upstream, low-burden tracking in sarcopenia.
- Mechanisms span metabolic, inflammatory, and circadian regulatory pathways.
- Standardized tongue imaging and saliva assays support longitudinal follow-up.
- Framework aligns microbiome signals with minimal clinically important differences.

Keywords Sarcopenia, Tongue-coating microbiome, Oral–gut–muscle axis, Salivary biomarkers, Short-chain fatty acids

Graphical Abstract



Introduction

Sarcopenia is a major driver of disability, falls, and mortality in ageing societies, yet existing screening and follow-up tools rarely enable early, high-frequency,

low-burden phenotyping that can be deployed in community or home settings [1–4]. International consensus increasingly centers muscle strength as the core criterion, with muscle quantity and functional performance—Short Physical Performance Battery (SPPB) scores and gait speed, for example—used to grade severity; however, these endpoints are largely downstream and respond slowly to changes in diet, inflammation, or lifestyle, limiting their value for timely optimization of care [5–8]. Consequently, there is an urgent need for an upstream, repeatable biological window that links short-term behavioral or nutritional changes to quantifiable signals and, ultimately, to functional outcomes.

Recent microbiota research has also underscored that microbial ecosystems can influence host physiology well beyond local niches, spanning neuropsychiatric and neuroinflammatory conditions as well as cancers, and has begun to move from association toward intervention. For example, gut–brain axis mechanisms have been linked to psychological conditions and neuroinflammation, and microbiota-modulating strategies such as probiotics/live biotherapeutic products and fecal microbiota transplantation are increasingly explored in clinical contexts [9–11]. Similarly, reviews highlight microbiome-driven pathways in gastrointestinal cancers and other neurological conditions [12, 13]. These broader advances motivate the general premise of this Perspective: accessible microbial niches may provide upstream, modifiable signals relevant to systemic outcomes.

Current phenotyping approaches—such as dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), opportunistic computed tomography/magnetic resonance imaging (CT/MRI), and tests of handgrip strength and gait speed—are more sensitive later in the disease course and are constrained by cost, setting, and patient adherence [14–16]. The gut–muscle axis is implicated in energy metabolism, inflammation, and protein synthesis; however, the sampling burden and temporal latency of stool-based assays limit high-frequency monitoring and the creation of a closed intervention loop [17–20]. These limitations motivate a shift upstream along the oral–gut continuum to ask whether more accessible oral ecological signals can capture subtle, near-term changes in metabolic and immune status, thereby aligning assessment with real-world management scenarios. In this context, tongue-coating imaging and saliva-based readouts emerge as promising candidates for upstream, low-burden monitoring—an idea we develop in the sections that follow.

Importantly, tongue-coating assessment is not intended to replace either stool-based gut microbiome profiling or standard functional tests; rather, it aims to fill a practical gap in upstream, high-frequency monitoring. Compared with stool sampling, standardized tongue imaging and small-volume saliva/tongue swabs can be performed repeatedly in clinic or at home with low participant burden, facilitating short-timescale and circadian-aware tracking while still allowing deeper gut sequencing when needed [21–23]. Compared with grip strength or gait speed, tongue-coating features may shift earlier along the oral–gut–muscle continuum, providing complementary risk signals before overt functional decline.

The tongue coating is a stable, visible biofilm niche in the oral cavity that supports both standardized imaging and saliva-based biomarker assessment. On the imaging side, tongue features—thickness, coverage, colour, and texture—can be reliably quantified with deep learning; on the biochemical side, saliva offers proximal functional readouts, including short-chain fatty acids (SCFAs), inflammatory mediators, and circadian rhythm–related indices, all obtainable with low sampling burden and at high frequency [24–27]. In parallel, the swallowing pathway provides a biological conduit through which oral microbiota and their metabolites may influence intestinal homeostasis, forming a putative tongue–gut–muscle information route. Together, these properties position tongue-based measures as an upstream window that could detect earlier and more sensitive shifts in risk relevant to muscle function [21, 28–30]. Building on this rationale, the following sections examine (i) oral–gut coupling mechanisms, (ii) convergent microbial pathways linked to muscle health, and (iii) the inferential—yet potentially actionable—value of tongue-derived measurements for stratification and follow-up.

Despite the biological rationale and early signals, the evidence directly linking tongue- or saliva-derived measures to clinically meaningful outcomes in sarcopenia remains limited. Longitudinal causal associations with handgrip strength, gait speed, and the SPPB are underpowered, and multicenter cohorts with adequate follow-up are needed to define thresholds aligned to the minimal clinically important difference (MCID). Methodological heterogeneity persists for low-biomass sampling and timing (density of negative controls, decontamination and batch correction; fasted versus post-prandial; morning/evening pairing), while intervention studies (probiotics/synbiotics, polyphenols, time-restricted eating with or without resistance training) show variable effects on functional endpoints [31–34]. Together, these factors argue for positioning tongue-based metrics as complementary rather than substitutive and for adopting a harmonized minimal-methods set—fixed pre-breakfast window with optional AM/PM pairing, mandated controls and decontamination, explicit batch correction—paired with MCID-aligned longitudinal tracking to establish added clinical utility. Accordingly, we explicitly distinguish established evidence from proposed

mechanisms, and we emphasize knowledge gaps that must be addressed before clinical deployment.

Against this backdrop, we move from the oral–gut continuum to summarize coupling between tongue-coating and gut ecosystems, integrate recent evidence on metabolic, inflammatory, and circadian pathways linked to muscle function, and appraise the feasibility of tongue-based, upstream measurement—covering fixed sampling windows, low-biomass quality control, standardized imaging with AI quantification, and saliva-based functional readouts—before outlining a three-stage translational roadmap from causal validation to biomarker modeling and real-world deployment. Three questions frame the agenda: (i) can tongue/salivary signals anticipate clinically meaningful change (minimal clinically important difference) beyond conventional metrics? (ii) what harmonized "minimal methods set" ensures reproducibility and cross-center portability? (iii) which stratification schemas (metabolic vs. inflammatory vs. circadian) maximize trial responsiveness and added clinical utility (e.g., decision-curve and reclassification analyses)? Addressing these questions could shift tongue-coating microbiome measures from plausible proxies to complementary, actionable tools that enable earlier risk signaling, pragmatic, high-frequency follow-up, and more precise intervention sequencing in community and home settings, thereby advancing prevention and care for sarcopenia [23, 35–37].

The tongue-coating microbiome as a potential biomarker and target in sarcopenia

Composition and spatial organization of the tongue-coating microbiome

The tongue coating is one of the most stable and structurally complex biofilm niches in the oral cavity, with pronounced spatial heterogeneity across the dorsum [38]. Lower oxygen tension on the posterior tongue favors anaerobes and creates a major microbial hotspot. At the phylum level, communities are typically dominated by Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, while core genera commonly include *Streptococcus*, *Prevotella*, *Neisseria*, and *Veillonella* (Fig. 1A). These taxa help maintain local ecological homeostasis and may influence host physiology through their metabolic products, providing biologically plausible links to intestinal and systemic processes [39]. Despite inter-individual variability, the tongue-coating microbiome exhibits high intra-individual temporal stability, supporting its use in longitudinal monitoring, stratification, and intervention studies.

Research techniques and challenges for the tongue-coating microbiome

With rapid advances in microbiome science, a systematic workflow has emerged that spans sampling, nucleic acid extraction, multi-omics profiling, and multimodal integration. Sampling typically uses sterile swabs targeted to the mid-to-posterior tongue dorsum to maximize capture of anaerobic communities [40]. Nucleic acid extraction generally requires high-energy mechanical disruption (e.g., bead-beating) with optimized chemistries to improve cell-wall lysis and downstream sequencing quality (Fig. 1B) [41]. Analytical layers commonly include 16 S ribosomal RNA (rRNA) gene sequencing, shotgun metagenomics, metabolomics, and proteomics, together building a multidimensional microbiome–function–metabolism map [42]. Increasingly, artificial intelligence (AI)–driven analysis of standardized tongue images is integrated with omics data to achieve image–microbiome–function fusion, improving taxonomic resolution and phenotype prediction [43].

Despite this progress, standardization remains incomplete across key steps, which can materially affect downstream signals. Examples include (i) sampling timing (fasted vs. postprandial; pre- vs. post-brushing), (ii) anatomical site choice (dorsal midline vs. posterior dorsum vs. lateral margins) and swab pressure/duration, (iii) specimen type and handling (tongue swab vs. scraping vs. saliva; transport buffer; storage temperature and freeze–thaw), (iv) low-biomass contamination control and decontamination workflows, and (v) sequencing/imaging harmonization (16 S region/platform, batch correction, and color/lighting calibration for tongue imaging) [22, 23, 36, 44, 45]. Therefore, a minimal, harmonized operating procedure spanning both wet-lab and imaging steps is urgently needed to enable cross-cohort comparability and clinical translation.

Mechanisms of interaction between the tongue-coating and gut microbiomes

Although anatomically distinct, the tongue-coating and gut microbiomes are physiologically coupled, forming a coherent oral–gut microbial axis in autism children [46]. Via the swallowing route, oral bacteria can enter the gastrointestinal tract, influence patterns of intestinal colonization, and modulate community diversity and ecological balance [29, 47]. As the first immune barrier, the oral mucosa shapes downstream responses through cytokines and signaling pathways, thereby tuning intestinal mucosal immunity and systemic inflammatory tone (Fig. 1C) [25, 48]. Metabolically, the two ecosystems exhibit functional homology in amino acid pathways, SCFA production, and regulation of inflammatory mediators [49–51].

Cross-disease observations in conditions such as autism spectrum disorder and metabolic syndrome

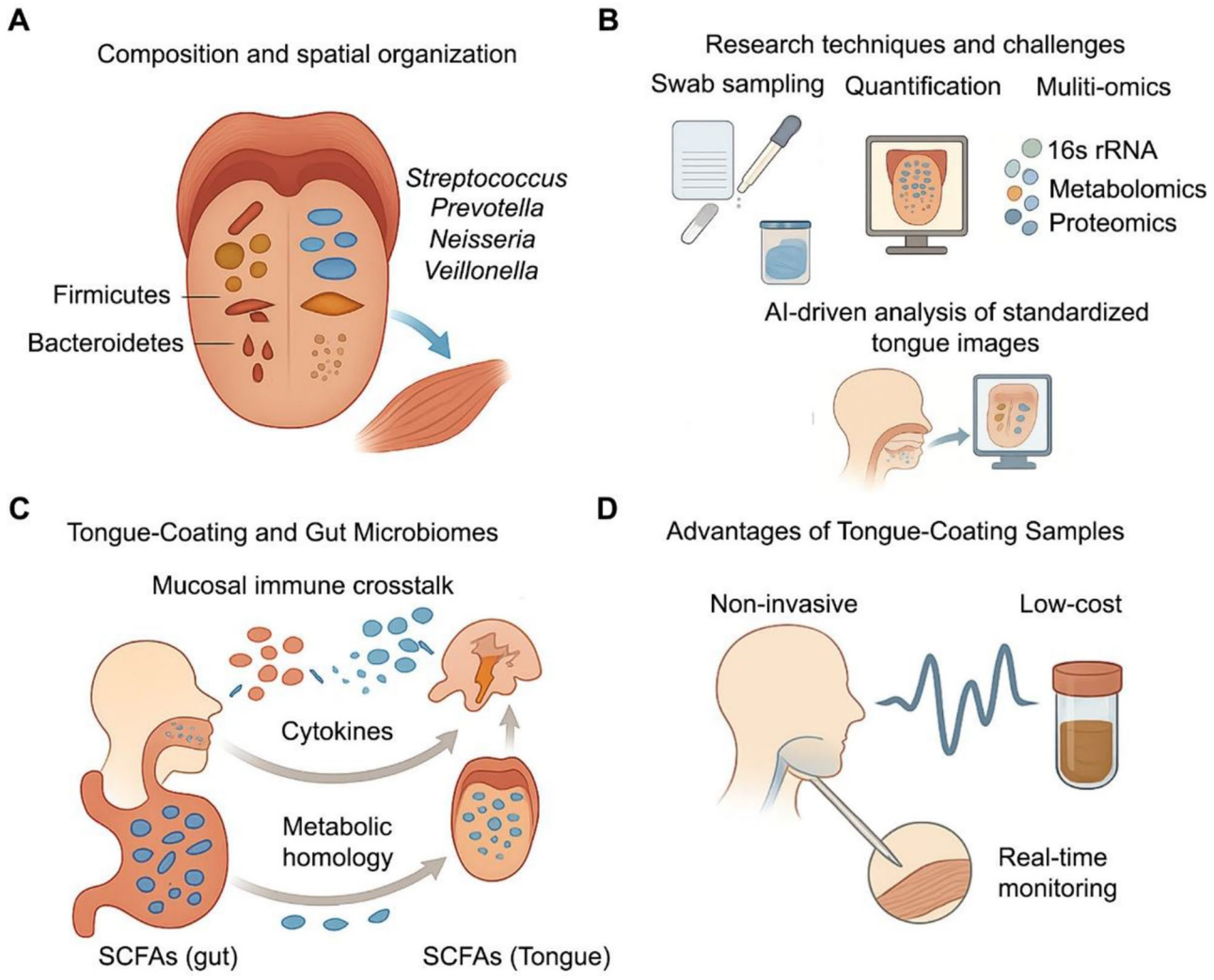


Fig. 1 Tongue-coating microbiome as a low-burden biomarker: composition, research workflow, host–gut interactions, and comparative advantages. (**A**) The tongue dorsum hosts a spatially structured microbiome with distinct zones (e.g., anaerobic posterior tongue), comprising core taxa such as *Streptococcus*, *Prevotella*, *Veillonella*, and *Neisseria*. These microbial communities generate local metabolites and immune signals with potential effects on muscle physiology. (**B**) Research workflows involve swab-based sampling, DNA extraction, and multi-omics profiling (16S rRNA sequencing, metagenomics, metabolomics, proteomics), increasingly paired with AI-driven tongue imaging to integrate visual and molecular data. (**C**) The tongue and gut microbiomes interact through swallowing-mediated bacterial transfer, mucosal immune crosstalk, and metabolic homology (e.g., shared SCFA pathways, inflammatory mediators), suggesting coordinated regulation across the oral–gut axis. (**D**) Compared to stool samples, tongue-coating samples offer practical advantages: they are non-invasive, more acceptable to participants, and better suited for capturing short-term dynamics, making them ideal for high-frequency longitudinal monitoring in sarcopenia and related conditions

reveal an "oral–gut dysbiosis" signature—relative increases in pro-inflammatory taxa with reductions in beneficial commensals across both sites [46, 48]. Together, these findings support the notion that the tongue-coating microbiome may act as an early sentinel of intestinal imbalance, offering a practical entry point for earlier detection and more precise intervention.

Advantages of tongue-coating samples over stool samples

In microbiome research and clinical applications, tongue-coating monitoring offers three practical advantages: (i) non-invasive, low-burden repeat sampling via standardized imaging plus saliva/tongue swabs, (ii) proximity to diet, oral health, and circadian cues that enables higher temporal resolution than intermittent stool sampling, and (iii) scalability for community settings through smartphone-assisted imaging and simple collection workflows. Large-scale oral/saliva microbiome studies and standardized saliva sampling protocols support feasibility [21, 52], and established reporting and low-biomass quality-control standards can be adapted to oral samples [23, 36]. Importantly, because stool captures distal gut composition more directly, tongue-coating assessment should be viewed as complementary, prioritizing

convenience, adherence, and dynamic responsiveness (Fig. 1D) (Table 1).

Taken together, these attributes position the tongue coating as a frontline, low-burden matrix for the "microbiome–muscle" intervention pathway, combining technical feasibility with practical deploy ability. The tongue-coating microbiome shows good temporal stability yet remains responsive to near-term perturbations, and its coupling with the gut and host metabolic/immune signaling provides a biologically coherent basis for upstream monitoring within the tongue–gut–muscle framework.

Microbial mechanisms in sarcopenia: commonalities and coordinated regulation from gut to tongue

Core mechanisms by which the gut microbiome modulates muscle function

The gut microbiome exerts broad control over host metabolism and immunity and is closely linked to the onset and progression of sarcopenia. A first, metabolic axis centers on SCFAs—notably butyrate and propionate (with acetate often predominant)—which support mitochondrial efficiency, enhance insulin sensitivity, and promote muscle protein anabolism. Mechanistically, SCFAs signal through G-protein–coupled receptors (GPR41/FFAR3, GPR43/FFAR2), activate AMP-activated protein kinase (AMPK), and inhibit histone deacetylases (HDACs), thereby fostering oxidative metabolism and translational capacity in myocytes (Fig. 2A). Lower SCFA availability has been frequently observed in sarcopenia and may contribute to impaired energetic status and reduced recovery potential [53–55].

A second signaling axis, involving amino acid and anabolic, is mediated by branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine—whose availability and handling are shaped by microbial metabolism. Adequate BCAA flux activates the mechanistic target of rapamycin complex 1 (mTORC1) pathway, stimulating muscle protein synthesis and repair (Fig. 2A). Dysbiosis can diminish microbial contributions to BCAA pools and alter intestinal transport and hepatic clearance, collectively blunting mTOR-driven anabolism and, over time, compromising muscle maintenance and functional capacity [56, 57].

The third barrier–inflammation axis links microbial imbalance to low-grade chronic inflammation through a process driven by intestinal barrier dysfunction. Increased gut permeability facilitates translocation of lipopolysaccharide (LPS) into systemic circulation, triggering the subsequent activation of Toll-like receptor 4 (TLR4) and NF-κB signaling (Fig. 2A). The resulting cytokine milieu (e.g., TNF-α, IL-6) upregulates ubiquitin–proteasome E3 ligases MuRF-1 (TRIM63) and Atrogin-1 (FBXO32), accelerating muscle protein degradation and fiber atrophy [58–61]. These axes are interdependent—metabolic insufficiency sensitizes inflammatory pathways, while inflammation suppresses anabolic signaling—forming a coherent framework that links gut dysbiosis to functional decline in sarcopenia and highlights tractable targets for intervention.

Metabolic roles of the tongue-coating microbiome and links to muscle function

Interest is growing in the metabolic regulation exerted by the tongue-coating microbiome, particularly its relevance to sarcopenia. Metabolites produced on the tongue—such as SCFAs, ammonia, and hydrogen sulfide—can enter the gastrointestinal tract via saliva, contributing to systemic metabolic control [62]. Taxonomic shifts on the tongue have functional implications: reduced *Streptococcus* has been associated with diminished SCFA biosynthetic potential [63], whereas reduced *Veillonella* often co-occurs with heightened inflammatory tone [64] (Fig. 2B). Moreover, tongue-community structure and function exhibit circadian fluctuation tightly coupled to host energy rhythms, suggesting a potential role in timing of interventions [51, 65].

Recent reviews and meta-analyses report modest, overall positive pooled effects of microbiome-directed interventions on strength and physical performance in older adults or individuals with sarcopenia, but with substantial heterogeneity across endpoints [6–8]. Some studies suggest that fitness-related measures (e.g., gait speed, composite functional tests) may be more consistently responsive than handgrip, yet higher-quality, stratified randomized controlled trials are needed. Emerging

Table 1 Comparison of sampling characteristics between tongue coating and fecal samples

Dimension of Comparison	Tongue Coating Samples	Fecal
Sampling method	Non-invasive, easy to perform	Mildly invasive, less convenient to collect
Compliance in older adults compliance	High, allows frequent sampling	Low, limited
Dynamic responsiveness state	Reflects short-term metabolic rhythm changes	Primarily reflects terminal metabolic
Data reproducibility	High stability, suitable for longitudinal analysis	More susceptible to sampling conditions, greater variability
Cost and operational feasibility	Convenient on-site collection, low-cost	Requires cold-chain transport and laboratory infrastructure

Sampling features of tongue-coating vs. fecal matrices for microbiome monitoring in sarcopenia—method, adherence in older adults, dynamic responsiveness, reproducibility, and operational feasibility. Tongue coating is non-invasive, supports frequent, short-term rhythm tracking with stable data and low on-site cost; fecal sampling is more burdensome, reflects terminal metabolic state, shows greater variability, and typically requires cold-chain logistics

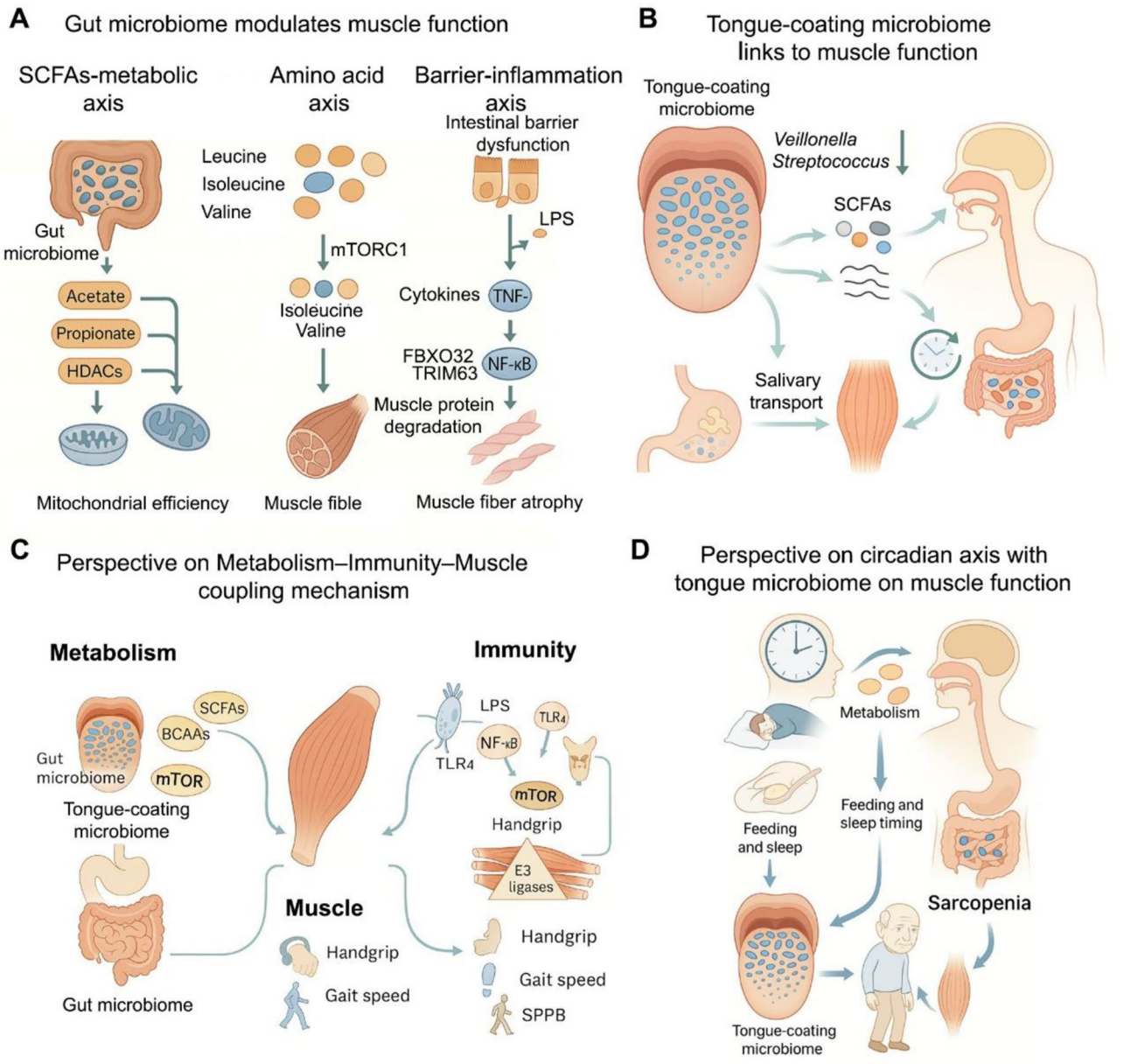


Fig. 2 Microbial mechanisms linking the tongue–gut axis to sarcopenia through metabolic, inflammatory, and circadian pathways. (**A**) The tongue-coating microbiome forms a metabolically active niche generating local metabolites (e.g., short-chain fatty acids, amino acid derivatives) and immune signals that may influence systemic muscle homeostasis. (**B**) Salivary microbiota and metabolites can be swallowed, modulating gut microbial composition and metabolite availability, thereby impacting skeletal muscle function and physical performance. (**C**) Coordinated metabolic–immune–muscle regulation is mediated through pathways such as SCFA–AMPK–mTOR for anabolic support and LPS–TLR4–NF-κB for inflammatory degradation, forming a bidirectional regulatory triad across the oral–gut–muscle continuum. (**D**) The circadian axis couples feeding and sleep timing with diurnal oscillations in the oral microbiome, which in turn interact with inflammatory and metabolic processes to affect muscle regulation. This time-domain window enables potential stratification and optimization of microbiome-targeted interventions for sarcopenia

reports (e.g., niacin from Bifidobacterium adolescents enhancing the NAD^+–SIRT1–PGC-1α axis; *Lacticaseibacillus rhamnosus* and *Faecalibacterium prausnitzii* promoting energy pathways and muscle phenotypes in models) (Fig. 2B) provide cross-level support for a microbe–metabolite–muscle bioenergetics pathway [64, 66, 67].

As a proximal, low-burden readout, salivary SCFAs have been proposed as early indicators of systemic change. However, their quantitative linkage to whole-body flux and muscle outcomes remains to be established and will likely require stable-isotope tracing and harmonized longitudinal designs [53].

The metabolism–immunity–muscle triadic coupling mechanism

The gut and tongue-coating microbiomes exhibit shared and coupled influences on metabolism, immunity, and muscle homeostasis. Metabolically, SCFAs—notably butyrate and propionate—enhance mitochondrial efficiency and insulin sensitivity, supporting muscle energetics; branched-chain amino acids (BCAAs) activate the mechanistic target of mTORC1 to promote protein synthesis and repair. Reports of reduced SCFA supply and impaired BCAA handling in sarcopenia suggest that metabolic pathway deficits align with diminished muscle function [54, 55].

On the immune side, microbial dysbiosis and barrier dysfunction permit translocation of LPS into the circulation, triggering TLR4 and NF-κB signaling. The resultant cytokine milieu (e.g., IL-1β, IL-18) upregulates the ubiquitin–proteasome E3 ligases MuRF-1 (TRIM63) and Atrogin-1 (FBXO32), accelerating muscle protein breakdown and atrophy (Fig. 2C). Animal and cellular studies underscore the centrality of the LPS–TLR4–NF-κB axis in inflammatory myopathy; inhibiting NF-κB or augmenting sirtuin 1 (SIRT1) can mitigate LPS-induced damage, and select nutrients (e.g., vitamin K1) show protective effects in models [68–72]. Clinically, recurrent observations of periodontal inflammation and elevated salivary IL-1β/IL-18 support the use of salivary inflammatory markers plus tongue imaging/omics as proximal readouts of the inflammatory pathway [57, 61, 73–76].

At the signaling-integration level, TLR4/NF-κB and mTOR act as cross-organ control nodes: inflammatory activation suppresses mTOR-driven anabolism, whereas SCFAs and SIRT1 place countervailing constraints on NF-κB, yielding bidirectional regulation of the metabolism–immunity–muscle triad [34,35]. This logic motivates a stratified research and intervention framework: upstream measurable indicators (tongue/salivary SCFAs, IL-1β/IL-18, tongue-image phenotypes), midstream checkpoints (LPS–TLR4–NF-κB, mTOR), and downstream functional endpoints (handgrip strength, gait speed, Short Physical Performance Battery [SPPB]). Overall, human evidence is directionally consistent, but causal strength and external validation require further reinforcement.

Circadian axis: microbiome coupling with feeding and sleep timing and its impact on muscle function

High–temporal-resolution oral studies and tongue–gut linkage work indicate that salivary and tongue-coating communities display diurnal rhythms aligned with feeding and sleep, with concurrent interactions with inflammatory and metabolic markers (Fig. 2D) [24, 29, 77, 78].

Regarding time-restricted eating (TRE), recent meta-analyses suggest benefits for adiposity and body composition, whereas gains in muscle strength or muscle mass are inconsistent; when TRE is combined with resistance training, fat mass can be reduced without compromising strength, but consistent improvements in handgrip or other functional endpoints have not been established [31, 79, 80]. Mechanistically, diurnal oscillations of gut microbe–derived SCFAs can entrain host metabolic rhythms, and emerging evidence highlights three-way interactions among microbiota, circadian control, and stress, pointing to behavioral/nutritional timing as a candidate variable for individualized stratification [81–83].

Operationally, the circadian axis provides a tractable time-domain handle: fix sampling windows (e.g., morning/evening), log wearable-derived rhythm metrics, and analyze AM–PM deltas alongside changes in functional endpoints to test the sequence "rhythm recalibration → functional improvement."

Building a tongue–gut–muscle three-tier regulatory model

Drawing on shared metabolic, immune, and signaling mechanisms between the gut and tongue-coating microbiomes, we propose a three-tier regulatory model that positions the tongue coating as an upstream niche influencing muscle function through multiple routes. First, microbes shed into saliva and swallowed routinely can shape intestinal colonization and community structure. Second, tongue-derived microbial metabolites entering the circulation may directly modulate myocellular energetics and substrate use. Third, shifts in oral ecology can elicit local and systemic inflammatory responses, indirectly altering muscle protein turnover via pathways such as LPS–Toll-like receptor 4–NF-κB, with downstream effects on ubiquitin–proteasome ligases.

While direct interventional confirmation in sarcopenia is still lacking, cross-condition evidence (e.g., in diabetes) links oral–gut dysbiosis with functional decline, supporting the model's practical plausibility. This framework yields testable hypotheses and stratification targets for future studies, guiding the design of upstream measurements and mechanistically informed interventions.

Upstream measurable window: the potential role of the tongue-coating microbiome in sarcopenia

Ecological niche and measurability

Situated on the dorsal tongue, the tongue coating forms a stable ecological niche composed of bacteria, biofilm matrix, and host components (mucins, desquamated epithelium, immune molecules), enabling standardized tongue imaging for objective quantification and saliva-based assays that provide proximal metabolic and immune readouts (Fig. 3A) [26, 84, 85]. Compared with stool, tongue/saliva measures are more sensitive to short-term perturbations in diet, inflammation, and circadian

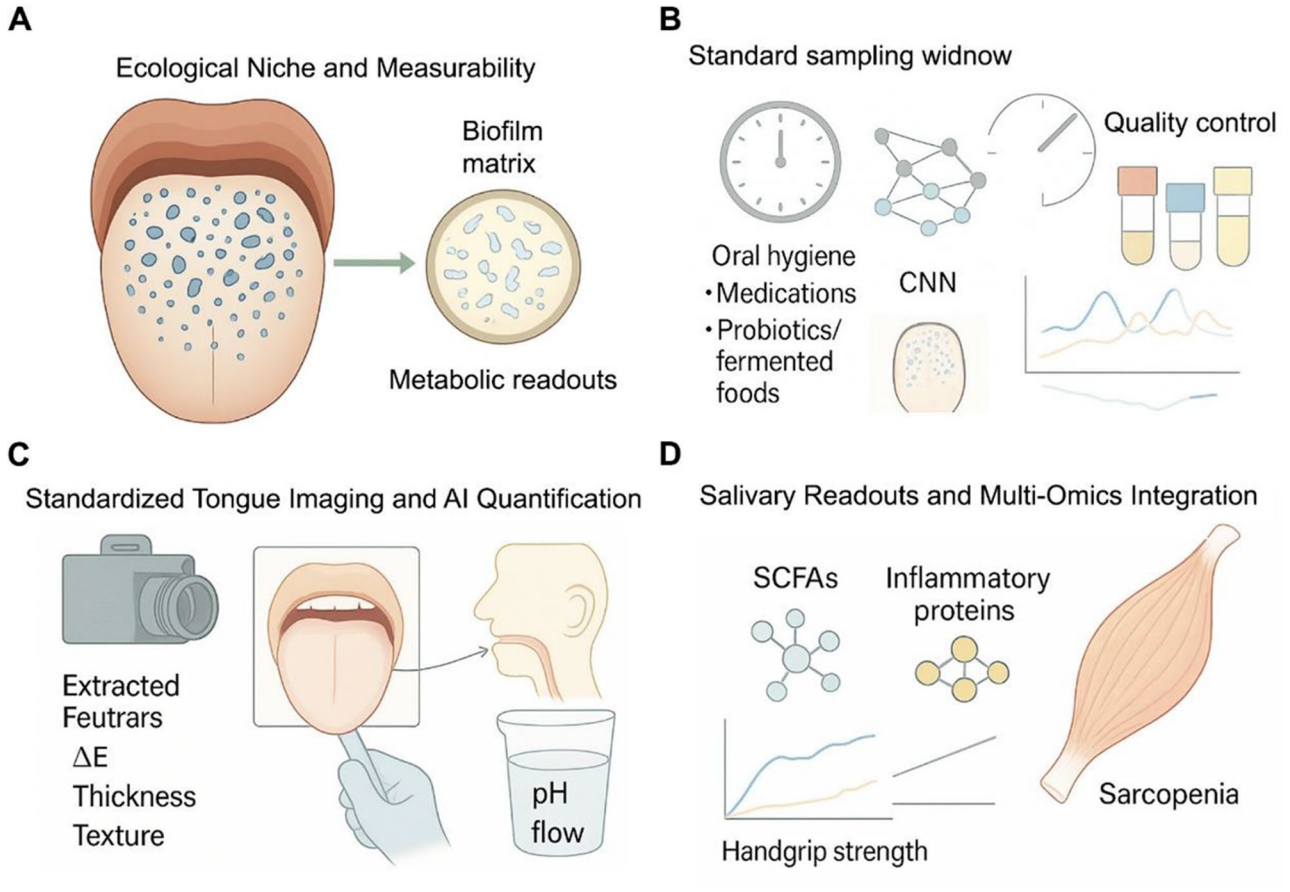


Fig. 3 A minimal methods framework for standardized tongue-coating microbiome assessment in sarcopenia research. (**A**) The tongue coating forms a stable ecological niche composed of biomatrix, host-derived components (e.g., mucins, desquamated epithelium), and immune molecules. This structure allows for consistent spatial sampling and supports its role as a measurable microbial niche. (**B**) High-fidelity tongue-coating sampling requires a fixed pre-breakfast window, adherence to low-biomass quality control standards (e.g., negative/positive controls), and appropriate oral hygiene recording. (**C**) Standardized tongue imaging protocols—with fixed illumination, position, and timing—can be coupled with artificial intelligence (AI) for quantitative phenotype extraction (e.g., coating thickness, color, texture), enabling real-time, portable biomarker development. (**D**) Saliva collected in parallel enables measurement of functional biochemical markers such as SCFAs, inflammatory proteins, and epithelial barrier indicators, integrated with multi-omics (metagenomics, metabolomics) to link tongue-image features with molecular pathways

rhythms, and their low sampling burden makes them well suited to high-frequency longitudinal follow-up.

At the same time, they are more susceptible to oral-hygiene behaviors and exogenous contamination; therefore, robust inference requires strict standard operating procedures (SOPs) and low-biomass quality control, including fixed pre-breakfast sampling windows, adequate negative/positive controls with decontamination workflows, and explicit batch-effect correction to ensure cross-study comparability [23, 44].

Sampling window and low-biomass quality control

To enable time-series comparability and circadian analyses, a fixed pre-breakfast window (30–60 min), fasting, and without tooth-brushing or mouthwash is advisable. Record potential confounders—oral-hygiene habits, recent medications (e.g., proton-pump inhibitors, metformin), and intake of probiotics/fermented foods (Fig. 3B) [45]. For tongue coating, use sterile swabs along the midline and both lateral dorsum three passes with standardized pressure and trajectory, then stabilize/freeze promptly. Collect saliva by passive drool, and log flow rate and pH for downstream model adjustment [22].

Because tongue and saliva are low-biomass matrices, include adequate negative controls (≥ 10% of sample count) and positive standards/mock communities to assess process bias; apply frequency/abundance- or regression-based decontamination to remove features co-occurring with negatives; and correct batch effects explicitly. Reporting should follow STORMS and Minimum Information about any (x) Sequence (MIxS) guidelines, including sampling context, DNA quantitation, PCR cycle number, batch structure, and decontamination strategy [23, 36, 86]. This pragmatic "minimal methods set" establishes the reproducible foundation for

subsequent imaging–omics integration and cross-center comparability.

Standardized tongue imaging and AI quantification

After establishing sample SOPs, the imaging goal is to move from visible to comparable. Standardize the capture pipeline by fixing camera–tongue position–distance–angle–illumination, using a gray card for white balance and exposure calibration, and—where possible—maintaining the same device across longitudinal visits to minimize hardware drift [87]. Feature extraction should combine color and morpho-texture cues: compute ΔE in the CIE L*a*b* space for color shifts; quantify coating thickness/coverage; and derive texture descriptors such as gray-level co-occurrence matrix (GLCM), local binary patterns (LBP), and frequency-domain features to build a multidimensional phenotype profile (Fig. 3C) [88].

For modeling, apply convolutional neural networks (CNNs) for segmentation and scoring, paired with cross-center external validation and occlusion/sensitivity analyses to improve interpretability and robustness. Anchor evaluation to clinical function by testing correlation and regression with handgrip strength, gait speed, and the Short Physical Performance Battery (SPPB), and quantify added value using decision-curve analysis (DCA) for net clinical benefit [89]. Report test–retest reliability with targets of intraclass correlation coefficient (ICC) ≥ 0.75 and coefficient of variation (CV) ≤ 20%, and visualize agreement via Bland–Altman plots [90]. The overarching aim is to convert subjective tongue impressions into portable, quantitative, and generalizable optical biomarkers.

Salivary readouts and multi-omics integration

In parallel with imaging, the biochemical layer should create a two-way bridge from proximal functional readouts to pathway interpretation. SCFAs—acetate, propionate, and butyrate—can be quantified in saliva by gas or liquid chromatography–mass spectrometry (GC–MS/LC–MS) with isotopic internal standards, then linked to tongue-image thickness/texture features and to metagenomic SCFA pathway scores (e.g., KEGG/MetaCyc) for mechanistic coherence. Inflammation/barrier proteins (e.g., IL-1β, IL-18, TNF-α) can be profiled via multiplex immunoassays, while oral health status (caries, gingivitis, periodontal pockets) is recorded to mitigate confounding [26, 91].

To capture time-domain effects, collect AM/PM paired samples to derive diurnal deltas/rhythm divergence indices, and couple these with wearable-derived sleep/activity metrics (e.g., M10/L5, interdaily stability [IS], intradaily variability [IV]) to support circadian-type stratification [92]. At the multi-omics layer, 16S rRNA gene sequencing provides community structure and diversity, shotgun metagenomics resolves species, gene families, and pathway annotation, and metabolomics with optional proteomics runs in parallel (Fig. 3D). LC/GC platforms should use pooled quality-control (QC) samples and interleaved injections to monitor drift, with LOESS or ComBat applied for batch correction [93–95].

For data governance and reproducibility, preregister protocols, lock primary/secondary endpoints, maintain a versioned data dictionary, and run containerized pipelines. Report methods under STORMS/MIxS (microbiome) and, when building prediction models, TRIPOD. Ensure cross-center comparability via simple calibration (e.g., image color-card harmonization; omics QC drift checks) and validate with site-stratified or leave-one-site-out testing. Emphasize discrimination and calibration in model evaluation; where relevant, add decision-curve analyses and anchor thresholds to minimal clinically important difference (MCID) to enable actionability. Finally, plan external validation across devices and populations and track feasibility metrics (adherence, per-test cost) to support real-world deployment [35].

Metric framework and clinical alignment

To be actionable, imaging and omics readouts must align with clinical functional endpoints. We propose a three-tier metric stack: upstream indicators—standardized tongue-image features (thickness, ΔE in CIE L*a*b*, texture) plus SCFAs and inflammatory proteins; midstream pathway scores—SCFA synthesis/transport, LPS biosynthesis–TLR4–NF-κB, and circadian-related pathways; and downstream function—handgrip strength, gait speed, and the Short Physical Performance Battery (SPPB) with their minimal clinically important differences (MCIDs) [96].

In longitudinal cohorts, use mixed-effects models to estimate time lags linking upstream changes to functional change, and anchor action thresholds to MCIDs (e.g., a ≥ x% reduction in coating thickness or a ≥ y mmol/L rise in salivary butyrate corresponding to a ≥ p% probability of achieving ≥ MCID in SPPB). Assess clinical utility with decision-curve analysis (DCA) and net reclassification improvement (NRI) to quantify the added value of stratification or prediction models [97]. Here, x and y are illustrative placeholders; action thresholds should be empirically derived from prospective cohorts and explicitly linked to MCID-defined changes in muscle strength, performance, or mass.

For real-world deployment, establish monthly drift panels in geriatric/community and rehab clinics with inter-site comparisons to track system error, refine SOPs, and improve sampling training and operability, supporting robust generalization and scale-up [98].

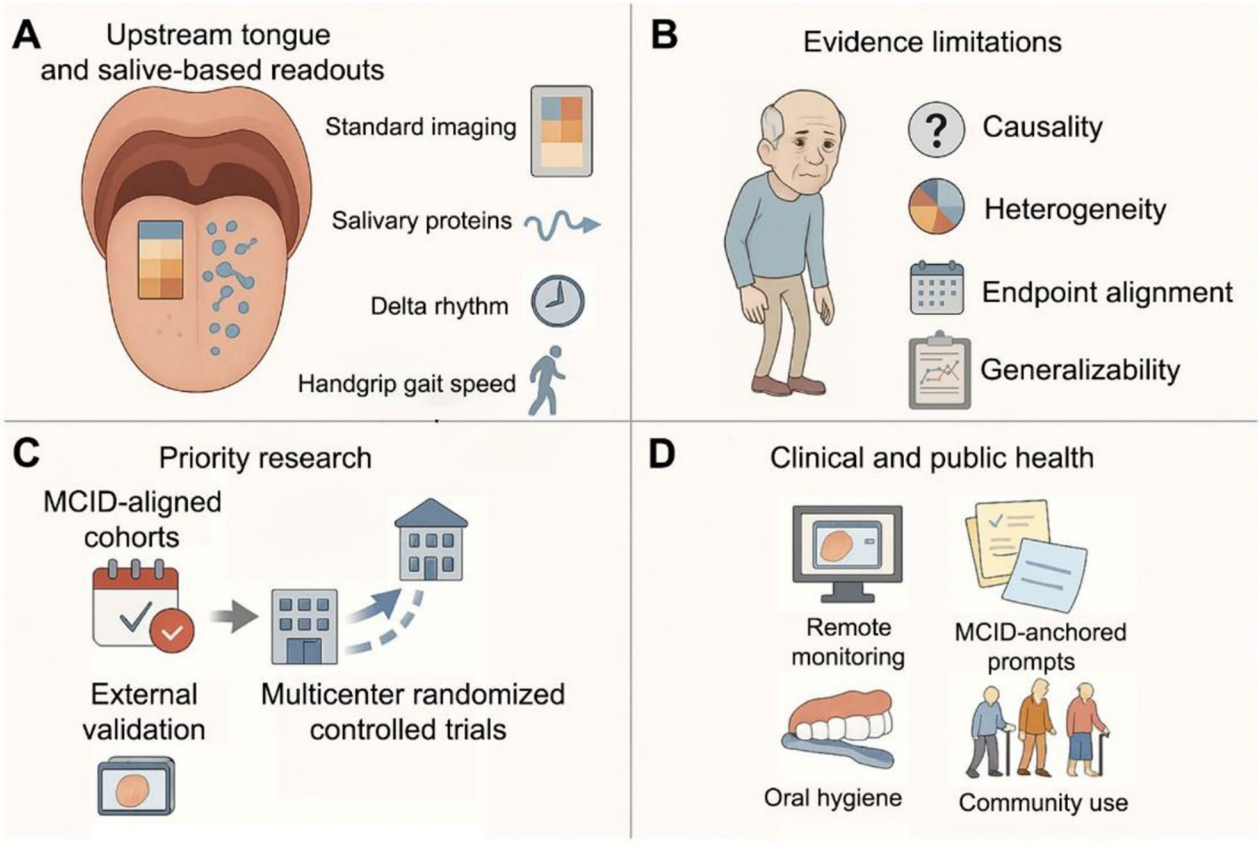


Fig. 4 Current limitations and future directions for tongue-coating microbiome research in sarcopenia. (**A**) Tongue and saliva-based readouts represent an upstream, low-burden phenotyping window aligned with metabolic, inflammatory, and circadian axes. These signals may co-vary with functional outcomes (e.g., handgrip, gait speed), but are best used as adjunct tools rather than replacements for stool or clinical gold standards. (**B**) Major evidence gaps remain in causality, generalizability, and cross-study comparability. Heterogeneity in populations, oral hygiene, medications, and study design impairs inference and functional alignment, especially without minimal clinically important difference (MCID) anchoring. (**C**) Research priorities include MCID-aligned prospective cohorts and harmonized multicenter randomized controlled trials (RCTs) with fixed sampling windows, decontamination protocols, and integrated omics and functional outcomes. (**D**) In clinical and public health settings, tongue–saliva signals can support longitudinal follow-up and stratified intervention. Practical integration into workflows requires baseline capture, regular re-assessment, oral health confounder control, and user-friendly tools for remote monitoring, with the aim of bridging upstream signals to actionable care pathways

Limitations and future directions

Key consensus (plain-language summary)

Current evidence supports tongue coating and saliva as an upstream, low-burden phenotyping window. Their proximal readouts—standardized tongue-image features, salivary short-chain fatty acids (SCFAs) and inflammatory proteins, and AM/PM rhythm deltas—track the metabolic, inflammatory, and circadian axes and, in certain contexts, co-vary with small-to-moderate improvements in handgrip strength, gait speed, or the SPPB (Fig. 4A) [6, 32, 33].

These measures do not replace stool testing or functional gold standards; they are best viewed as complementary tools that enable high-frequency, community/home follow-up. Data quality hinges on rigorous low-biomass quality control and transparent reporting (e.g., STORMS): a fixed pre-breakfast sampling window, adequate negative/positive controls with decontamination, explicit batch correction, and device/lighting calibration for imaging [23].

For clinical use, interpretation should be anchored to the minimal clinically important difference (MCID): a proximal change (e.g., ΔE, coating thickness, salivary butyrate) is not a clinical outcome by itself. Thresholds and models should be aligned to MCID, with utility assessed via discrimination/calibration and, where relevant, decision-curve or reclassification analyses [97].

Practical implementation of this framework requires attention to key operational factors. To improve comparability and fairness, capture key confounders (oral hygiene and periodontal status, antibiotics/proton-pump inhibitors/metformin, probiotic intake), consider AM/PM pairing, and favor device-agnostic imaging with simple training materials to support real-world use (Fig. 4A). Stratifying by metabolic / inflammatory / circadian phenotype can clarify who benefits and when, while

reporting per-test burden and adherence supports uptake in routine care.

Evidence limits on upstream tongue/saliva signals and functional outcomes

There is a lack of direct longitudinal evidence to establish the causal pathway from oral sensory signals to mechanistic pathways and subsequently to functional improvements meeting the MCID. Several constraints temper current inferences. First (causality), longitudinal evidence that explicitly links tongue/salivary signals → mechanistic pathways → functional endpoints reaching the minimal clinically important difference (MCID) is limited; existing studies are small and short in follow-up (Fig. 4B) [99]. Second (heterogeneity/confounding), results vary with demographics and comorbidities, medications (e.g., proton-pump inhibitors, metformin), oral health and hygiene behaviors, nutrition and training background, as well as sampling and batch effects [100]. Third (endpoint alignment), reporting for handgrip strength, gait speed, and the SPPB lacks uniform thresholds and time windows; diurnal control and sampling timing are often unmanaged, complicating comparisons across studies [24]. Fourth (generalizability), cross-center portability and external validation remain scarce, limiting methodological uptake and real-world translation [95]. Consequently, throughout this Perspective we treat the proposed tongue-coating–microbiome–muscle links as testable hypotheses, and we recommend explicitly reporting evidence level (direct human longitudinal, indirect human, animal/in vitro) when interpreting mechanisms.

Sex and gender differences may further shape tongue and gut microbiota composition, immune tone, and muscle trajectories. Recent oral microbiome studies report sex-stratified differences in community structure and host–microbe relationships, and oral microbial signatures of age/frailty show sex-dependent patterns, supporting the need for sex-stratified modeling and interaction testing in tongue–microbiota–muscle analyses [52, 101]. In addition, gut microbiome–muscle associations may differ by sex, implying hormonal and behavioral modifiers [102]. Future studies should pre-specify sex-stratified sampling and evaluate whether effect sizes and intervention responses differ between women and men.

Several confounders can distort tongue-coating signals and their apparent links to sarcopenia, including oral health status (periodontitis, dental caries), denture use, xerostomia/saliva flow, oral hygiene behaviors (brushing, mouthwash), diet and fasting status, smoking and alcohol use, recent antibiotics/probiotics, and systemic medications and comorbidities that also influence muscle outcomes. We therefore recommend harmonized metadata capture, repeated measures, negative controls, and sensitivity analyses for unmeasured confounding, alongside low-biomass contamination controls [23, 36].

Priority research: MCID-aligned cohorts, multicenter RCTs, and external validation/interpretable modeling

First, pursue minimal clinically important difference (MCID)–aligned prospective cohorts that are stratified by metabolic, inflammatory, and circadian phenotypes. Use a fixed pre-breakfast sampling window (with optional AM/PM pairing), reassess at 4–8 weeks, and model time-dependent links from upstream changes (tongue imaging, salivary short-chain fatty acids and inflammatory proteins) to functional outcomes (handgrip, gait speed, Short Physical Performance Battery). Pre-register protocols, handle confounding with mixed-effects or distributed-lag models, and quantify added clinical value using decision-curve analysis (DCA) and net reclassification improvement (NRI) (Fig. 4C). Define MCID-anchored action thresholds to translate signals into decisions [103].

To quantify "how much" of an intervention or exposure effect propagates through the oral–gut–muscle continuum, future studies should pre-specify a causal graph (DAG) and implement causal/mediation analyses that separate (i) direct effects of tongue-coating features from (ii) indirect effects mediated by gut taxa, metabolites, and inflammatory markers. Because microbiome mediators are compositional and high-dimensional, methods tailored to compositional mediation and multimodal mediation (microbiome + metabolome) are preferable, and should be paired with sensitivity analyses for unmeasured confounding [104–106]. In longitudinal settings, time-lagged mixed models and dynamic Bayesian/SEM frameworks can further test directionality (tongue → gut → muscle) while accounting for circadian timing and short-term diet/hygiene perturbations.

Second, run multicenter randomized trials under a harmonized minimal methods set (fixed sampling window; negative/positive controls with decontamination; unified optical imaging; pooled QC samples and batch correction). Compare sequential strategies—for example, short-course oral de-inflammation or rhythm alignment followed by nutrition/resistance training—against simultaneous approaches. Predefine primary endpoints (handgrip, gait speed, Short Physical Performance Battery), track adherence and feasibility, and include safety and implementation readouts for real-world use [107].

Third, prioritize external validation and interpretable modeling. Build compact, device-agnostic models from standardizable features (tongue ΔE, thickness/texture; salivary SCFAs; IL-1β/IL-18; AM/PM deltas), report both discrimination and calibration, and provide simple, MCID-anchored decision tools (e.g., threshold cards, individual trajectories). Validate across sites and devices, release containerized code with a versioned data

dictionary, and assess cost and adherence to support community deployment [35].

Clinical and public health implications

In geriatric clinics, prehabilitation, and rehabilitation follow-up, tongue–saliva readouts are best used as adjuncts to standard care. A practical workflow is to establish a baseline (standardized tongue image plus a small saliva panel—short-chain fatty acids, IL-1β/IL-18), then repeat in a fixed pre-breakfast window at weekly or biweekly intervals. Signals are translated into minimal clinically important difference (MCID)–anchored prompts: for example, sustained rises in coating thickness/ΔE or inflammatory markers may trigger a short course of oral de-inflammation (hygiene optimization, targeted oral care) or rhythm alignment (sleep/eating timing) before escalating nutrition or resistance training. Embed capture in the EHR flowsheet, note key confounders (periodontal status, antibiotics, proton-pump inhibitors, metformin, probiotics), and flag conditions that complicate interpretation (xerostomia, acute infections) to close the loop from upstream change → decision → functional reassessment [108].

To improve real-world feasibility and reduce cost, we suggest a tiered implementation package: Tier 1 (community/primary care) uses standardized smartphone tongue imaging with minimal metadata (diet, oral hygiene, key medications) and a small set of targeted saliva markers; Tier 2 (sentinel centers) adds 16 S/shotgun profiling and targeted metabolomics; Tier 3 (research hubs) performs full multi-omics integration and causal modeling. This staged approach allows broad coverage while reserving high-cost assays for mechanistic validation, and it aligns with low-biomass quality-control requirements and transparent reporting/model validation [23, 35, 36].

For community and home use, smartphone tongue imaging paired with at-home saliva kits lowers barriers to participation (Fig. 4D). Provide a simple SOP card (lighting/angle, on-screen color reference, no brushing/mouthwash beforehand), reminder nudges, and brief coaching for older adults or caregivers. Remote QC can check image exposure and sample adequacy; kits can ship with prepaid returns. Display results as trend plots with traffic-light thresholds tied to functional goals, while making clear that upstream improvements are not clinical benefit unless aligned with functional endpoints and MCID. Favor device-agnostic capture and multilingual instructions, and track feasibility metrics such as adherence, turnaround time, and per-test cost to guide real-world adoption [109].

At the population level, integrating oral-health education and hygiene adherence into community programs reduces oral confounding and improves the signal-to-noise of tongue-based metrics (Fig. 4D). Health systems can pilot sentinel cohorts in senior centers that combine standardized imaging, brief saliva panels, and periodic functional testing to monitor trends and target outreach. Reporting should follow common method standards (e.g., STORMS/MIxS), with de-identified, policy-compliant data flows and simple cross-site calibration. Evaluate implementation with public-health metrics—uptake, completeness, cost per person-year, avoidable referrals—and explore reimbursement pathways and caregiver training to sustain community-scale deployment [110].

Conclusion

The tongue-coating microbiome and the associated lingual mucosal niche provide a stable, accessible window into the oral–gut–muscle continuum, enabling standardized tongue imaging plus saliva/tongue-swab multiomics to capture upstream metabolic, inflammatory, and circadian signals relevant to sarcopenia risk. By synthesizing oral–gut coupling routes and organizing candidate mechanisms across three axes, we propose a testable framework for earlier risk signaling and high-frequency monitoring that complements stool microbiome profiling and conventional functional assessments. However, current evidence directly linking tongue-derived signals to MCID-defined changes in muscle outcomes remains limited; therefore, rigorous multicenter longitudinal cohorts and interventional trials—with harmonized SOPs, low-biomass quality control, and sex-stratified, confounder-aware causal modeling—are required before routine clinical adoption.

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Author contributions

X. Zhao and Y.M. Hu wrote the manuscript text and made substantial contributions to the conception. Y.R. Weng and R. Huang made suggestions to the design of the work and construction of figures.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not required.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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