

Metabolic and Microbiome Profiling in Healthy Adults: Correlating Agni Bala Variations with Gut Microbial Diversity and Fecal Short-Chain Fatty Acid Dynamics for Targeted Delivery Systems

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Abstract

Ayurveda conceptualizes digestive and metabolic competence through the construct of Agni, whose functional strength (Agni Bala) is classically described as manifesting in four states — Sama (balanced), Tikshna (intense), Manda (weak), and Vishama (irregular). Contemporary microbiome science has independently established that the gut microbial community and its fermentation end-products, particularly short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, are central determinants of host digestive and metabolic physiology. This review synthesizes evidence from validated Agni assessment instruments, Prakriti-microbiome association studies, gut microbial diversity research in healthy adults, and the biochemistry of fecal SCFA production, in order to construct an integrative framework linking Agni Bala variation to gut microbial diversity and SCFA dynamics. We further examine how such biological correlates could inform the rational design of microbiome- and metabolism-responsive targeted drug delivery systems, particularly colon-targeted platforms that exploit microbial enzymatic activity for site-specific drug release. Evidence indicates that Ayurvedic Prakriti phenotypes are associated with reproducible, if modest, differences in gut bacterial taxa, and that SCFA output is tightly coupled to microbial diversity, fiber intake, and host receptor signaling through GPR41/GPR43. However, direct empirical studies correlating validated Agni Bala scores with 16S rRNA-based diversity indices and fecal SCFA quantification remain absent from the literature, representing a substantial research gap. We propose a structured methodological framework for future correlative studies in healthy adults and outline how resulting biomarker signatures could be translated into individualized colon-targeted delivery strategies. Bridging traditional digestive physiology constructs with quantitative microbiome and metabolomic science may offer a biologically grounded route toward personalized nutraceutical and pharmaceutical delivery.

Keywords: Agni Bala; Ayurveda; gut microbiome; short-chain fatty acids; Prakriti; colon-targeted drug delivery; personalized medicine; 16S rRNA sequencing.

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Introduction

Traditional systems of medicine are increasingly being subjected to evidence-based scrutiny as they gain wider global acceptance, and Ayurveda — the classical Indian system of medicine — is no exception (Roy et al., 2026). Central to Ayurvedic physiology is the concept of Agni, broadly translated as “digestive fire,” which is held to govern digestion, absorption,

and the transformation of ingested substances into forms usable by body tissues. The functional strength of this digestive and metabolic capacity, termed Agni Bala, is not viewed as a fixed trait but as a dynamic state that varies between individuals and, within an individual, over time. Classical Ayurvedic texts describe four principal functional states of Agni: Sama

Agni, in which digestion proceeds in a balanced and regular manner; Tikshna Agni, characterized by unusually rapid or intense digestive activity; Manda Agni, marked by sluggish or weak digestive capacity; and Vishama Agni, defined by irregular or unpredictable digestive function (Singh et al., 2017). These states are further elaborated through a tiered model comprising Jatharagni (the principal alimentary digestive fire operating in the gastrointestinal lumen), Bhutagni (elemental or substrate-level metabolic transformation), and Dhatvagni (tissue-level metabolic processes that nourish the seven Dhatus, or bodily tissues) (Patwardhan, 2008).

Independently of this classical framework, biomedical science has, over the past two decades, established the human gut microbiome as a metabolically active organ system in its own right, exerting profound influence over host energy balance, immune regulation, and systemic metabolism. The gut is host to a dense and individually distinctive community of bacteria, archaea, and fungi that ferment otherwise indigestible dietary components — principally complex carbohydrates and dietary fiber — into short-chain fatty acids (SCFAs), chiefly acetate, propionate, and butyrate. These molecules serve simultaneously as energy substrates for colonocytes and as signaling ligands for host G-protein-coupled receptors, linking microbial fermentation directly to host metabolic and inflammatory physiology. The human microbiome has, in this sense, come to be described as the “latest discovered” human organ, and its characterization is now considered essential to a complete understanding of individualized digestive and metabolic physiology (Chaudhari et al., 2019).

A natural point of convergence between these two bodies of knowledge is the observation that both frameworks describe inter-individual variation in digestive-metabolic capacity using constructs that are, at least conceptually, homologous: Agni Bala in Ayurveda, and gut microbial diversity together with SCFA output in contemporary microbiome science. If Agni Bala reflects an integrated phenotype of digestive and metabolic competence, it is biologically plausible that this phenotype has measurable correlates in the composition, diversity, and fermentative output of the gut microbial community. Establishing such correlates would carry not only theoretical interest for integrative physiology but also practical significance: a validated biological signature of Agni Bala could inform the rational, individualized design of drug and nutraceutical delivery systems that are triggered by, or calibrated to, colonic microbial activity.

Agni and Agni Bala: Conceptual and Physiological Basis

In Ayurvedic physiology, Agni is not a single anatomical entity but a distributed functional principle

operating at multiple levels of biological organization. At the most proximate level, Jatharagni operates within the gastrointestinal tract, converting ingested food into a form suitable for absorption; this tier corresponds most closely to the biomedical processes of gastric, pancreatic, and intestinal digestion. At an intermediate level, five Bhutagnis are described, each acting upon one of the five classical elemental constituents (Earth, Water, Fire, Air, and Space) that are held to compose absorbed nutrients, transforming them into forms assimilable at the tissue level. At the most granular level, seven Dhatvagnis govern the metabolic transformation and nourishment of the seven Dhatus, or structural tissues of the body, extending from plasma (Rasa) through to reproductive tissue (Shukra) (Patwardhan, 2008). This tiered architecture bears a loose conceptual resemblance to the biomedical distinction between luminal digestion, intermediary metabolism, and tissue-specific anabolism, although the correspondence remains descriptive rather than mechanistically validated (Agrawal et al., 2010).

The strength or functional adequacy of this system — Agni Bala — is held to determine an individual’s capacity to digest, absorb, and metabolize food without residual toxic byproduct (Ama) accumulation. Ayurvedic nosology recognizes four functional states. Sama Agni represents an optimally functioning digestive fire, in which food of an appropriate quantity and quality is digested at an expected rate, without discomfort, and is regarded as the physiological ideal associated with overall health. Tikshna Agni describes an excessively intense digestive capacity, in which even large quantities of food are rapidly digested; while superficially advantageous, this state is classically associated with a propensity toward excessive hunger and, in some descriptions, downstream metabolic strain. Manda Agni denotes a weak or sluggish digestive capacity, in which even modest meals are poorly tolerated or slowly processed, and which is classically linked to symptoms resembling dyspepsia, bloating, and lethargy. Vishama Agni describes an irregular or unpredictable digestive pattern, alternating between phases of adequate and inadequate function, and is associated with variable bowel habit (Singh et al., 2017).

From a physiological standpoint, several contemporary authors have proposed that these four states might correspond, at least in a general sense, to observable variation in gastric emptying rate, gut transit time, digestive enzyme secretion, and basal metabolic rate, with mitochondrial oxidative metabolism suggested as a candidate cellular substrate for Agni activity (Agrawal et al., 2010). These correspondences remain hypothetical and have not

been subjected to rigorous mechanistic testing; nonetheless, the existence of measurable inter-individual variation in digestive and metabolic parameters provides an empirically tractable substrate against which the Agni Bala construct can, in principle, be validated. Figure 1 summarizes a proposed conceptual framework linking the tiered Agni system, Agni Bala states, gut microbial diversity, fecal SCFA output, and downstream applications in targeted drug delivery, situating the remainder of this review within a coherent explanatory structure.

An important corollary of this tiered model is that Agni Bala is not conceived as a single scalar quantity but as an emergent property of the coordinated function of multiple sequential and parallel metabolic processes. A deficit at any one tier — for instance, inadequate Jatharagni activity resulting in incomplete luminal digestion — is held to propagate downstream, producing incompletely processed substrates (Ama) that in turn compromise Bhutagni and Dhatvagni function at the tissue level. This systems-level framing bears a notable structural resemblance to the way in which modern gastroenterology and microbiome science now conceptualize the gut as an integrated axis linking luminal digestion, microbial fermentation, metabolite absorption, and downstream tissue metabolism, in which a perturbation at any one level (for example, reduced pancreatic enzyme output, altered microbial fermentative capacity, or impaired short-chain fatty acid receptor signaling) can produce cascading effects on overall metabolic health. This structural parallel, while not itself constituting evidence of a specific biological mechanism, strengthens the conceptual rationale for treating Agni Bala as a plausible candidate for correlation with an equally systems-level microbiome and metabolomic readout, rather than attempting to map it onto any single, isolated biomarker.

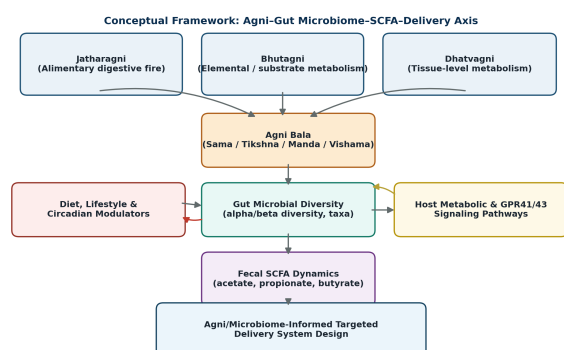


Figure 1. Conceptual framework linking the tiered Agni system (Jatharagni, Bhutagni, Dhatvagni), the four functional Agni Bala states, gut microbial diversity, fecal short-chain fatty acid (SCFA) dynamics, host metabolic signaling, and

Agni/microbiome-informed targeted drug delivery system design.

Assessment Tools for Agni Bala

Translating a classically descriptive construct such as Agni Bala into a quantifiable, reproducible measure has been the focus of several independent instrument-development efforts over the past two decades, each adopting a somewhat different methodological approach. One of the earliest formal attempts was a clinician-administered scale developed by Patil and colleagues, which operationalized digestive strength through the controlled administration of a defined quantity of ghee (clarified butter) and observation of the resulting digestive response, alongside assessment of bowel movement pattern (Koshtha) (Patil et al., 2008). While innovative in anchoring assessment to an observable physiological challenge, this approach requires direct clinical supervision and a controlled test meal, limiting its feasibility for large-scale epidemiological or microbiome-correlation studies.

A second major instrument, developed by Eswaran and colleagues, took the form of an extensive 64-item questionnaire derived from classical Ayurvedic textual descriptions of Vishmagagni, Tikshnagagni, Mandagni, and Samagni, employing a five-point Likert scale for each item and validated in a cohort of 250 healthy individuals (Eswaran et al., 2015). Although comprehensive, this tool has been criticized for its length and for the absence of a clearly specified scoring algorithm mapping individual item responses onto a definitive Agni state classification, limiting its practical usability in subsequent research.

A more concise and psychometrically validated alternative was subsequently developed by Singh and colleagues: a self-administered tool aligned explicitly with the four functional states of Jatharagni, validated in 300 healthy volunteers aged 18–40 years and further tested for external biological validity by demonstrating that classified Agni states corresponded to statistically significant differences in serum lipid parameters (Singh et al., 2017). This external validation against an objective biochemical outcome represents an important methodological advance, as it demonstrates that self-reported Agni Bala classification is not merely an internally consistent psychological construct but carries measurable physiological correlates — a finding of direct relevance to the hypothesis that Agni Bala may similarly correlate with gut microbial and metabolomic parameters.

More recently, a 26-item Agni Assessment Scale (AAS) was developed and psychometrically evaluated in a two-phase study involving 467 participants, incorporating exploratory factor analysis and reliability testing, and demonstrating promising factorial structure and conceptual coherence with

Ayurvedic theory (Ashwathykutty et al., 2026). Complementing these instrument-development efforts, a broader methodological literature has emerged addressing how diagnostic and assessment tools in Ayurveda should be developed and evaluated in accordance with contemporary psychometric standards, including content validity, construct validity, and test-retest reliability (Edavalath & Bharathan, 2021). A registered systematic review protocol has further been developed specifically to critically appraise and synthesize the psychometric properties of existing Agni assessment instruments using the COSMIN (Consensus-based Standards for the Selection of Health Measurement Instruments) methodology, highlighting persistent heterogeneity in administration mode, instrument length, and scoring transparency across the field (Roy et al., 2026). Collectively, this body of instrument-development work provides a methodologically credible, if still maturing, basis for the standardized ascertainment of Agni Bala required for any rigorous correlative study with gut microbiome or metabolomic parameters.

For the purposes of a microbiome-correlation study specifically, instrument selection carries practical as well as psychometric considerations. Clinician-administered, test-meal-based approaches such as that of Patil and colleagues offer strong ecological validity but are resource-intensive and difficult to standardize across multiple recruitment sites, whereas self-administered questionnaire-based tools such as the Agnibala self-assessment tool or the Agni Assessment Scale offer superior scalability, lower participant burden, and greater feasibility for the sample sizes typically required for adequately powered microbiome association analyses, which must account for the substantial inter-individual variability inherent in 16S rRNA sequencing data. A pragmatic study design might therefore favor a validated self-administered instrument as the primary Agni Bala classification tool, with a subset of participants additionally undergoing clinician-administered assessment to formally establish concordance between the two approaches and thereby strengthen confidence in the resulting classification before it is correlated with microbiome and metabolomic outcomes.

Gut Microbiome Diversity in Healthy Adults

The human gastrointestinal tract harbors a dense and taxonomically diverse microbial ecosystem, with the colon alone estimated to contain several hundred bacterial species occupying distinct ecological niches along the length of the gut (Sinha & Kumria, 2003). In healthy adults, this community is typically dominated by two bacterial phyla, Firmicutes and Bacteroidetes, which together account for the substantial majority of identifiable taxa, alongside smaller but functionally important contributions from Actinobacteria,

Proteobacteria, and Verrucomicrobia. Within these phyla, genera such as *Bacteroides*, *Prevotella*, *Faecalibacterium*, and *Ruminococcus* recur consistently across population-level surveys as core constituents of the “healthy” gut microbiome, alongside mucin-degrading specialists such as *Akkermansia muciniphila* (Wexler, 2007).

Two complementary metrics are conventionally used to characterize this community quantitatively. Alpha diversity indices, most commonly the Shannon entropy index and Chao1 richness estimator, capture the diversity and evenness of taxa present within a single individual’s microbiome, and are typically computed from 16S rRNA gene amplicon sequencing data processed through operational taxonomic unit (OTU) or amplicon sequence variant (ASV) clustering pipelines. Beta diversity metrics, such as the Bray-Curtis dissimilarity index and weighted or unweighted UniFrac distances, instead quantify the degree of compositional divergence between individuals or groups, and are typically visualized through ordination methods and tested statistically using permutational multivariate analysis of variance (PERMANOVA). Both classes of metric have become standard tools for characterizing inter-individual and inter-group variation in gut microbial ecology, and both are directly applicable to any prospective study seeking to correlate microbiome parameters with Agni Bala classification.

A substantial body of evidence indicates that gut microbial diversity is not a static trait but is shaped continuously by diet, lifestyle, and host physiological state. Short-term controlled feeding studies have demonstrated that gut microbial community structure can shift measurably within as little as 24 hours of a dramatic macronutrient change, with plant-based diets favoring fiber-fermenting Firmicutes such as *Roseburia* and *Eubacterium rectale*, and animal-based diets favoring bile-tolerant taxa such as *Bacteroides* and *Alistipes* (David et al., 2014). At the same time, cross-sectional comparisons between lean and obese individuals, and complementary murine transplantation experiments, have shown that a reduced Firmicutes-to-Bacteroidetes ratio and diminished overall microbial richness are frequently observed in association with obesity and altered energy-harvesting capacity from the diet, with this altered metabolic potential shown to be transmissible upon fecal microbiota transplantation into germ-free recipients (Turnbaugh et al., 2006; Ley et al., 2006). These findings collectively establish gut microbial diversity as a dynamic, diet- and phenotype-responsive parameter, providing strong methodological precedent for the hypothesis that it may likewise vary systematically with Agni Bala classification, a construct that is itself understood in

Ayurveda to fluctuate with dietary habit, meal timing, and lifestyle.

Beyond these summary statistics, it is increasingly recognized that no single diversity metric fully captures the functional state of the gut ecosystem, and that alpha and beta diversity should be interpreted alongside taxon-level differential abundance testing and, where possible, functional pathway prediction from 16S rRNA marker gene data. This methodological nuance is particularly relevant to any future Agni Bala correlation study, since two individuals with numerically similar Shannon diversity scores could nonetheless harbor markedly different functional fermentative capacity if the specific taxa present differ in their SCFA-producing potential. A robust study design linking Agni Bala to microbiome parameters should therefore report alpha- and beta-diversity indices alongside genus- and species-level differential abundance analysis, rather than relying on diversity indices in isolation.

It is also well established that gut microbial diversity reflects a cumulative history of early-life colonization, geography, host genetics, and long-term dietary pattern, layered upon shorter-term fluctuations driven by recent food intake, physical activity, sleep, and stress. This layered temporal structure maps intuitively onto the Ayurvedic distinction between an individual's underlying constitutional Prakriti, which is relatively stable across the lifespan, and their current Agni Bala state, which is explicitly understood to fluctuate with diet, season, and lifestyle. A rigorous correlative study design should therefore anticipate that Prakriti may correlate more strongly with relatively stable, trait-like features of the gut microbiome such as core taxon presence, whereas Agni Bala, particularly transient classifications such as Vishama Agni, may correlate more closely with short-term, state-like fluctuations best captured through repeated longitudinal sampling rather than a single cross-sectional snapshot.

Prakriti, Agni, and the Gut Microbiome: Emerging Correlations

The most direct empirical precedent for an Agni-microbiome correlation comes not from studies of Agni Bala itself, but from a small yet growing body of research examining Ayurvedic Prakriti — the constitutional typology of Vata, Pitta, and Kapha that is held to determine baseline physiological and psychological tendencies, including digestive capacity, and which has been proposed to possess a partially genomic basis with associations to metabolic phenotype and chronic disease susceptibility (Dey & Pahwa, 2014). Because Agni Bala is understood in classical Ayurveda to be substantially influenced by an individual's underlying Prakriti — Pitta-predominant individuals, for instance, being classically associated

with Tikshna Agni, and Kapha-predominant individuals with Manda Agni — Prakriti-microbiome studies represent the closest existing analogue to a direct test of the Agni-microbiome hypothesis.

In one of the earliest such investigations, comprehensive 16S rRNA gene-based metagenomic profiling of gut, oral, and skin microbiome samples from 18 healthy individuals of defined Prakriti type revealed differential abundance of several bacterial genera, including *Bacteroides*, *Desulfovibrio*, *Parabacteroides*, *Slackia*, and *Succinivibrio*, across the three Prakriti groups, alongside Prakriti-specific enrichment of distinct taxa in each constitutional type (Chaudhari et al., 2019). Notably, this study also reported that bacteria known to protect against gut inflammation by fermenting resistant starch were disproportionately abundant in Pitta Prakriti individuals — a population classically considered more susceptible to inflammatory digestive conditions — offering a striking, if preliminary, point of convergence between Ayurvedic constitutional theory and microbial functional capacity.

In a larger and geographically distinct cohort, gut microbial diversity was examined specifically in individuals representing extreme Prakriti endo-phenotypes drawn from a rural Western Indian population, again using 16S rRNA sequencing; this study identified signature microbial taxa associated with each extreme constitutional type and proposed that such microbial signatures might eventually serve as objective biomarkers capable of stratifying otherwise phenotypically heterogeneous healthy populations for precision-medicine applications (Chauhan et al., 2018). A subsequent narrative synthesis of this literature concluded that, across the available studies, gut microbiome profiles are consistently dominated by Firmicutes and Bacteroidetes, with Pitta Prakriti individuals showing relative enrichment of taxa associated with nutrient metabolism, reactive oxygen species generation, and inflammatory signaling, and Kapha Prakriti individuals showing increased abundance of *Bifidobacterium adolescentis*, *Faecalibacterium prausnitzii*, and *Akkermansia muciniphila* — species associated with anti-inflammatory and lipid-metabolic functions that align conceptually with the classically described Kapha tendency toward efficient nutrient assimilation and adiposity.

A separate line of investigation has emphasized the broader concept of a shared “core gut microbiome” — comprising *Bacteroides*, *Faecalibacterium*, *Prevotella*, and *Ruminococcus* — that persists across Prakriti types even as specific taxa vary in relative abundance, suggesting that Prakriti-associated (and, by extension, potential Agni Bala-associated) microbial signatures are likely to manifest as graded shifts in relative

abundance against a broadly conserved compositional backbone, rather than as categorically distinct microbial communities (Jnana et al., 2020). This distinction carries an important methodological implication for future Agni Bala correlation studies: analytical approaches should prioritize differential abundance testing and functional pathway inference over simple presence/absence taxonomic comparison. Synthesizing these findings, Figure 2 proposes a hypothetical, literature-derived framework mapping the four Agni Bala states onto anticipated patterns of microbial diversity, representative taxa shifts, and fecal SCFA profile — a framework intended explicitly as a set of testable hypotheses for future empirical validation rather than as an established diagnostic model.

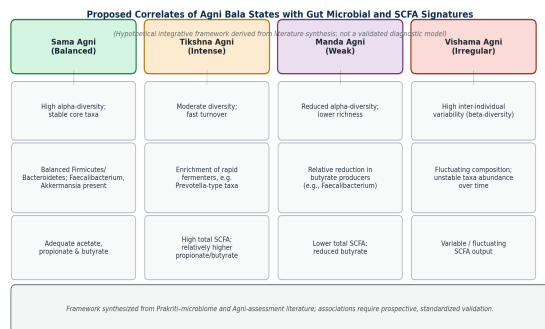


Figure 2. Proposed, literature-derived correlates of the four Agni Bala states (Sama, Tikshna, Manda, and Vishama) with anticipated gut microbial diversity patterns, representative taxa shifts, and fecal short-chain fatty acid (SCFA) profiles. This is a hypothetical integrative framework synthesized from Prakriti-microbiome and Agni-assessment literature and requires prospective, standardized empirical validation.

It must be emphasized that no published study to date has directly measured validated Agni Bala scores alongside 16S rRNA-based diversity indices and fecal SCFA concentrations in the same cohort. The correlations summarized above are drawn from the conceptually related, but distinct, construct of Prakriti, and the mapping proposed in Figure 2 should therefore be regarded as a set of biologically plausible, hypothesis-generating associations rather than an established empirical finding. This gap constitutes the central motivation for the integrated methodological framework proposed later in this review (Section 9).

Short-Chain Fatty Acids: Biosynthesis, Receptors, and Host Metabolism

Short-chain fatty acids are organic acids of fewer than six carbon atoms, produced predominantly through anaerobic bacterial fermentation of dietary fiber and other undigested carbohydrates, as well as, to a lesser extent, dietary and endogenous proteins, within the colonic lumen (Du et al., 2024). The three

principal SCFAs — acetate (C2), propionate (C3), and butyrate (C4) — together account for more than 95% of total colonic SCFA content, typically occurring at an approximate molar ratio in the region of 3:1:1, although this ratio varies considerably with dietary substrate composition and the specific bacterial taxa involved in fermentation. Members of the Bacteroidetes phylum are characteristically associated with high acetate and propionate production, whereas members of the Firmicutes phylum (particularly the Clostridial clusters IV and XIVa, including Faecalibacterium prausnitzii and Roseburia species) are the principal producers of butyrate (Du et al., 2024).

Once produced, SCFAs exert their physiological effects through two principal mechanisms. First, they act as direct energy substrates: butyrate is preferentially oxidized by colonocytes as their primary energy source, while acetate and propionate are absorbed into the portal circulation and metabolized systemically, contributing an estimated meaningful proportion of daily host caloric intake in individuals consuming fiber-rich diets. Second, and of particular mechanistic significance, SCFAs act as signaling ligands for a family of G-protein-coupled receptors, principally GPR43 (also termed free fatty acid receptor 2, FFAR2) and GPR41 (FFAR3). GPR43 is preferentially activated by acetate and propionate and is expressed prominently in adipose tissue, the gastrointestinal epithelium, and immune cells, where its activation has been shown to suppress fat accumulation, enhance systemic energy expenditure, and modulate gut hormone secretion; GPR41, in contrast, is activated with comparable potency by propionate and butyrate and is expressed in adipose tissue, gut enteroendocrine cells, and the peripheral nervous system, contributing to SCFA-dependent regulation of energy homeostasis via sympathetic nervous system activation and gut hormone release (Kimura et al., 2014).

The physiological importance of this SCFA-receptor signaling axis is underscored by its consistent implication in the pathophysiology of metabolic disease. The gut microbiota has been shown to interact with host genetic and environmental factors to influence nutrient acquisition and energy regulation, with disrupted microbial fermentation capacity implicated in the development of obesity, insulin resistance, and related metabolic disorders (Cani & Delzenne, 2009). Large-scale metagenomic cohort studies have further demonstrated that reduced overall gut microbial gene richness — and, by extension, reduced fermentative capacity — correlates with an adverse metabolic marker profile, including dyslipidemia and low-grade inflammation, independent of body mass index (Le Chatelier et al.,

2013). Beyond systemic metabolism, SCFAs, and butyrate in particular, exert well-documented anti-inflammatory effects at the level of the intestinal mucosa: reduced mucosal activity of butyrate-producing taxa such as *Butyrivibrio* has been shown to correlate with impaired epithelial tight-junction protein expression in patients with ulcerative colitis, illustrating the direct mechanistic link between SCFA-producing microbial function and gut barrier integrity (Devriese et al., 2017). Consistent with this mechanistic picture, systematic reviews of the inflammatory bowel disease literature have documented significant, reproducible reductions in fecal butyrate, acetate, and propionate concentrations in both Crohn's disease and ulcerative colitis relative to healthy controls, reinforcing the interpretation of fecal SCFA concentration as a biologically meaningful marker of gut microbial fermentative health (Ventura et al., 2024; Chulenbayeva et al., 2025).

The physiological reach of SCFAs extends further still, into immune regulation and gut-brain signaling. Butyrate and propionate are established histone deacetylase (HDAC) inhibitors, capable of modulating gene expression in colonic epithelial and immune cells and thereby promoting the differentiation of regulatory T cells that help maintain mucosal immune tolerance; SCFA depletion has consequently been linked, in mechanistic and observational studies, to a pro-inflammatory mucosal immune profile. In parallel, SCFAs generated in the colon can act on vagal afferent nerve terminals and enteroendocrine cells to influence appetite-regulating hormone release, and can cross into the systemic circulation to exert effects on hepatic and even central nervous system metabolism, forming part of the increasingly well-characterized gut-brain axis. This expanded physiological scope reinforces the interpretation of SCFA output as a systems-level integrator of digestive, immune, and neuroendocrine function — a scope that resonates closely with the holistic, whole-person conceptualization of Agni in Ayurvedic physiology, in which digestive strength is understood to influence not merely gastrointestinal comfort but overall vitality, immunity (Vyadikhshamatva), and mental clarity.

Taken together, this evidence base supports the biological plausibility of the central hypothesis motivating this review: because fecal SCFA output integrates both microbial community fermentative capacity and substrate (dietary) availability, and because these same factors are held in Ayurvedic physiology to determine Agni Bala, fecal SCFA profile represents a biochemically tractable candidate readout for digestive-metabolic strength that merits direct empirical correlation with validated Agni Bala classification.

Fecal SCFA Dynamics: Analytical Approaches and Correlation with Digestive Strength

Accurate quantification of fecal SCFA concentration is a nontrivial analytical undertaking, owing to the volatility, high polarity, and relatively low molecular weight of these compounds, and considerable methodological effort has been directed toward developing robust, reproducible quantification protocols. Gas chromatography coupled with mass spectrometry (GC-MS) has emerged as the principal analytical platform for this purpose, owing to its combination of high sensitivity, specificity, and quantitative accuracy. One widely cited derivatization-free GC-MS protocol employs ethanol extraction followed by alkaline vacuum centrifugation and acidification with succinic acid to enable volatilization, with SCFAs detected in selected ion monitoring mode and quantified against deuterated internal standards; this method has demonstrated excellent linearity ($R^2 > 0.99$ for most analytes), strong recovery rates of 95–117%, and good reproducibility (relative standard deviation of 1–4.5%) across plasma, fecal, cecal, hepatic, and adipose tissue matrices (Rohde et al., 2022). A complementary approach uses acidification with hydrochloric acid and extraction with methyl tert-butyl ether, employing distilled water as a surrogate matrix for calibration, and has likewise demonstrated robust analytical performance across human plasma, serum, and fecal samples as well as murine cecal tissue (Kim et al., 2022).

Beyond instrumentation, methodological rigor in sample handling is critical to obtaining biologically meaningful SCFA measurements: fecal samples must be collected under standardized conditions and stabilized rapidly (typically through immediate freezing at -80°C or the use of stabilizing buffer) to prevent *ex vivo* microbial fermentation from continuing after collection, which would otherwise introduce substantial measurement artifact and obscure any true biological signal linking SCFA concentration to host digestive-metabolic phenotype.

Sampling frequency and timing relative to meals represent additional methodological variables of direct relevance to the Agni Bala hypothesis. Because fecal SCFA concentration reflects a time-integrated product of recent dietary intake and microbial fermentative activity, a single fecal sample provides only a snapshot of an inherently dynamic process; this is of particular concern when attempting to characterize Vishama Agni, whose defining clinical feature is precisely its temporal irregularity. Repeated sampling protocols — for example, collection at multiple time points across one to two weeks, potentially paired with a brief structured dietary log — would allow investigators to distinguish genuine trait-level SCFA differences associated with Agni Bala classification from

transient, meal-driven fluctuation, and would additionally allow within-individual SCFA variability itself to be tested as a candidate quantitative marker of Vishama Agni.

When considered alongside the Agni Bala framework, fecal SCFA quantification offers a distinct advantage over microbiome sequencing alone: whereas 16S rRNA diversity metrics characterize microbial community structure, SCFA concentration captures functional fermentative output, integrating the combined influence of microbial composition, enzymatic activity, and substrate availability into a single, quantifiable biochemical readout. It is plausible, following the pattern established in the Prakriti-microbiome literature and the proposed framework in Figure 2, that Sama Agni individuals would exhibit a balanced fecal SCFA profile with adequate concentrations of all three principal SCFAs; that Tikshna Agni individuals, characterized by rapid digestive throughput, might show elevated total SCFA output; that Manda Agni individuals, associated with sluggish digestion and reduced abundance of key butyrate-producing taxa, might exhibit reduced total SCFA and particularly reduced butyrate; and that Vishama Agni individuals might exhibit high intra-individual variability in SCFA output over repeated sampling, mirroring their classically described unpredictable digestive pattern. As emphasized in Section 5, however, these remain literature-derived hypotheses rather than established findings, and their confirmation requires the direct, prospective correlative study design outlined in Section 9.

Microbiome- and Metabolism-Informed Targeted Drug Delivery Systems

Independent of its relevance to Agni Bala physiology, the density and enzymatic diversity of the colonic microbiota has, for over three decades, been actively exploited as an engineering resource in pharmaceutical formulation science, giving rise to the field of microbiota-triggered colon-targeted drug delivery. The clinical rationale for colon-specific delivery is substantial: local, high-concentration drug delivery to the colon enables topical treatment of conditions such as ulcerative colitis and Crohn's disease while minimizing systemic exposure and associated side effects, and the colon additionally offers an absorptive site for protein and peptide therapeutics that would otherwise be degraded by upper gastrointestinal enzymatic activity (Sinha & Kumria, 2003).

Three broad mechanistic strategies have been developed to achieve colon-specific release, and these are often used in combination within a single formulation. pH-dependent systems exploit the characteristic pH gradient of the gastrointestinal tract, employing polymer coatings (most commonly

methacrylate-based Eudragit formulations) that remain intact in the acidic stomach and near-neutral small intestine but dissolve upon reaching the relatively higher pH environment of the colon. Time-dependent systems instead employ a lag-time coating designed to release the drug payload following a fixed transit interval calibrated to typical small intestinal transit time, independent of local pH. Microbiota- or enzyme-triggered systems, the strategy of greatest direct relevance to this review, exploit the fact that colonic bacterial density and associated enzymatic activity — including azoreductases, glycosidases, and polysaccharidases — are dramatically higher than in the upper gastrointestinal tract; drug carriers formulated from natural polysaccharides such as pectin, guar gum, chitosan, and inulin remain largely intact until they reach the colon, where they are selectively degraded by resident bacterial enzymes, releasing the entrapped drug specifically at the target site (Bakshi et al., 2021). Figure 3 illustrates this tripartite mechanistic framework schematically, situating the microbiota-triggered mechanism within the broader gastrointestinal transit sequence.

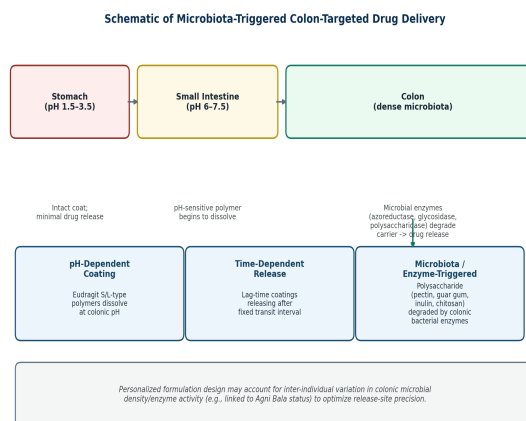


Figure 3. Schematic overview of microbiota-triggered colon-targeted drug delivery, illustrating the gastrointestinal pH gradient across the stomach, small intestine, and colon, alongside the three principal mechanistic strategies used to achieve colon-specific drug release: pH-dependent coatings, time-dependent (lag-time) release systems, and microbiota/enzyme-triggered polysaccharide carriers.

A substantial and continually expanding literature has documented refinements to this basic approach, including multilayer coating systems that combine pH-triggered and buffer-accelerated dissolution mechanisms, enzyme-triggered nanoparticle systems incorporating curcumin-cyclodextrin inclusion complexes within chitosan and alginate shells for the treatment of experimental colitis, and, most recently, hot-melt extrusion and three-dimensional printing technologies that enable

precise, individualized control over dosage form geometry, polymer composition, and consequently drug release kinetics (Alshammari et al., 2024). This latter development is of particular significance for the integrative framework proposed in this review, as additive manufacturing technologies are inherently well suited to producing individualized dosage forms calibrated to a specific patient's measured physiological parameters — including, potentially, their microbiome-derived enzymatic activity profile or Agni Bala classification.

A recognized and clinically significant limitation of purely microbiota-triggered delivery systems, however, is their dependence on an assumption of relatively uniform, “normal” colonic microbial density and enzymatic activity across the patient population — an assumption that is frequently violated in practice. Gut microbiota composition is known to vary substantially between healthy individuals, and is further altered in states of dysbiosis associated with gastrointestinal disease, aging, or concurrent pharmacotherapy (particularly antibiotic use), any of which may compromise the reliability, consistency, or timing of microbiota-triggered drug release. This inter-individual variability, rather than representing merely a limitation to be minimized, can instead be reframed as an opportunity for rational personalization: if an individual's baseline colonic microbial density and fermentative enzymatic capacity can be predicted, even approximately, from a rapid, non-invasive physiological assessment such as validated Agni Bala classification, this information could in principle be used to select or calibrate the specific release-triggering mechanism, polymer composition, or coating thickness most likely to achieve reliable, site-specific drug release in that individual.

Beyond conventional colon-targeted formulations, the same underlying microbiome-metabolism logic is increasingly being extended to nutraceutical and probiotic/prebiotic delivery, where formulation timing and carrier design can be adjusted to match an individual's baseline fermentative capacity: individuals with lower predicted colonic enzymatic activity or SCFA output, for example, might benefit from prebiotic fiber formulations paired with a co-encapsulated, slow-release probiotic consortium selected to include efficient butyrate-producing strains, effectively supplementing rather than merely relying upon endogenous fermentative capacity. Such an approach would extend the microbiome-informed personalization principle beyond pharmaceutical drug delivery into the broader domain of digestive-health nutraceuticals, an application area of particular relevance given that Agni Bala is itself most classically and directly

modified in Ayurvedic practice through dietary and nutraceutical intervention (Agni-deepana and Amapachana therapies) rather than through pharmaceutical treatment.

Toward an Integrated Framework: Agni Bala–Microbiome–SCFA-Guided Personalized Delivery

Synthesizing the evidence reviewed above, we propose that a structured, prospective correlative study — conducted in healthy adults to establish baseline associations before any extension to disease populations — represents the necessary next step in testing the Agni Bala–microbiome–SCFA hypothesis and translating it toward practical application in targeted delivery system design. Figure 4 outlines a proposed seven-stage methodological framework for such a study. The framework begins with careful recruitment and screening of healthy adult participants, with explicit exclusion of individuals with diagnosed gastrointestinal disease, recent antibiotic or probiotic use, or other dietary and pharmacological confounders likely to obscure the microbiome–Agni relationship of interest. Agni Bala is then formally assessed using one of the validated instruments discussed in Section 3, ideally the psychometrically evaluated Agnibala self-assessment tool or the more recently developed Agni Assessment Scale, selected on the basis of their demonstrated reliability and, in the case of the former, established external validity against serum biochemical parameters (Singh et al., 2017; Ashwathykutty et al., 2026).

Proposed Methodological Framework for Correlative Study Design

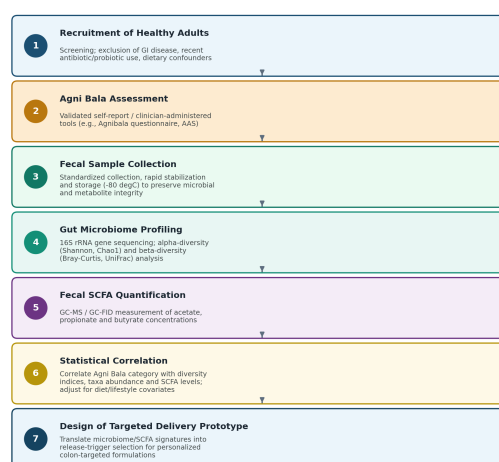


Figure 4. Proposed seven-stage methodological framework for a correlative study design linking validated Agni Bala assessment, gut microbiome diversity profiling, and fecal short-chain fatty acid (SCFA) quantification in healthy adults, culminating

in the design of a personalized, microbiome-informed targeted delivery prototype.

Following Agni Bala classification, standardized fecal sample collection would be undertaken with rapid stabilization and low-temperature storage to preserve both microbial DNA integrity and metabolite composition, enabling downstream 16S rRNA gene sequencing for alpha- and beta-diversity characterization (Section 4) and GC-MS-based fecal SCFA quantification using an established, validated protocol (Section 7). Statistical analysis would then examine the association between Agni Bala category and both microbial diversity indices/taxa abundance and fecal SCFA concentrations, with appropriate adjustment for dietary intake, physical activity, and other lifestyle covariates known to independently influence gut microbial ecology (David et al., 2014). Should this analysis identify reproducible, statistically robust associations — for instance, a consistent reduction in butyrate-producing taxa and fecal butyrate concentration among individuals classified as Manda Agni — such findings would provide the first direct empirical validation of the hypothesized Agni-microbiome-SCFA axis and would simultaneously generate the biomarker signatures required to inform personalized delivery system design.

In the final stage of the proposed framework, resulting biomarker signatures would be translated into concrete formulation parameters for microbiome-informed targeted delivery prototypes. For example, individuals whose Agni Bala classification and corresponding microbiome/SCFA profile predict lower colonic bacterial enzymatic activity might be matched to formulations employing a combined pH- and time-dependent release trigger with a secondary, less activity-dependent microbiota-triggered component, reducing reliance on potentially attenuated microbial enzymatic capacity; conversely, individuals with robust, high-diversity microbiomes and correspondingly high fermentative SCFA output might be well suited to more purely microbiota-triggered polysaccharide-based formulations, potentially permitting simpler, more cost-effective dosage form design. Advances in hot-melt extrusion and three-dimensional printing technology (Section 8) make such individualized calibration of polymer composition, coating thickness, and dosage form geometry increasingly feasible at a practical manufacturing level (Alshammari et al., 2024), suggesting that the translational endpoint of this proposed framework is not merely theoretical but is compatible with existing and emerging pharmaceutical manufacturing capability.

Challenges and Future Directions

Several substantive challenges must be addressed before the integrated framework proposed in this review can be considered scientifically mature. First and most fundamentally, standardization of Agni Bala assessment remains incomplete: as highlighted by a recently registered systematic review protocol, existing instruments vary considerably in administration mode, item length, and scoring transparency, and few have been cross-validated against one another in the same population (Roy et al., 2026). Without a single, widely accepted, and psychometrically robust instrument, cross-study comparison and meta-analytic synthesis of any future Agni-microbiome correlative findings will remain difficult.

Second, the existing Prakriti-microbiome literature, while suggestive, is characterized by small sample sizes, predominantly cross-sectional designs, and considerable heterogeneity in sequencing methodology, Prakriti/Agni assessment criteria, and statistical reporting conventions, limiting the strength of causal inference that can currently be drawn (Chulenbayeva et al., 2025). Larger, prospective, and ideally longitudinal cohort studies — incorporating repeated Agni Bala assessment and repeated fecal sampling to capture within-individual variability, particularly relevant to the classically described instability of Vishama Agni — will be required to establish robust, reproducible associations.

Third, translation of any validated biomarker signature into personalized delivery system design will require close interdisciplinary collaboration between Ayurvedic physiology researchers, microbiome scientists, analytical chemists, and pharmaceutical formulation scientists, together with careful attention to regulatory pathways governing individualized or stratified drug delivery products. The additive manufacturing technologies discussed in Section 8, while promising, remain at a relatively early stage of clinical translation for colon-targeted applications specifically, and reproducibility of microbiota-triggered release across the substantial inter-individual variability in colonic microbial ecology documented throughout this review will need to be rigorously characterized in vivo before any personalized formulation strategy could be considered for clinical development (Bakshi et al., 2021).

A further, more conceptual challenge concerns causal directionality. Even a robust statistical association between Agni Bala classification and gut microbial or SCFA parameters would not, by itself, establish whether variation in digestive-metabolic function drives corresponding microbial adaptation, whether the gut microbiome and its fermentative output are themselves a principal determinant of the subjectively and clinically assessed Agni Bala

phenotype, or whether both are shaped in parallel by shared upstream factors such as habitual diet, meal timing, and circadian rhythm. Disentangling these possibilities will likely require interventional study designs — for example, examining whether a standardized dietary or probiotic intervention capable of shifting gut microbial diversity and SCFA output produces a corresponding, measurable shift in Agni Bala classification over time — rather than cross-sectional correlation alone.

Finally, future research should extend beyond 16S rRNA amplicon sequencing and targeted SCFA quantification toward integrated multi-omics approaches — incorporating shotgun metagenomics for finer taxonomic and functional resolution, untargeted metabolomics to capture the fuller repertoire of microbial metabolites beyond the three principal SCFAs, and host transcriptomic or proteomic profiling of intestinal GPR41/GPR43 signaling activity — in order to build a more mechanistically complete picture of how Agni Bala, as an integrated physiological construct, relates to the multilayered biology of the gut microbiome and host metabolism.

Conclusion

The Ayurvedic construct of Agni Bala and the contemporary biomedical characterization of gut microbial diversity and fecal short-chain fatty acid dynamics represent two independently developed, yet conceptually convergent, frameworks for describing inter-individual variation in digestive and metabolic capacity. Validated instruments now exist for the reproducible assessment of Agni Bala, and a growing body of Prakriti-microbiome research provides preliminary, biologically plausible evidence that Ayurvedic constitutional and functional typologies are associated with measurable differences in gut microbial composition. Parallel advances in gas chromatography-mass spectrometry-based SCFA quantification and in microbiota-responsive colon-targeted drug delivery technology provide both the analytical tools required to test this hypothesis directly and a compelling translational rationale for doing so. We have proposed an integrated, seven-stage methodological framework by which validated Agni Bala assessment, 16S rRNA-based gut microbiome profiling, and fecal SCFA quantification could be jointly characterized in a cohort of healthy adults, with the explicit long-term aim of informing the rational, individualized design of microbiome- and metabolism-responsive targeted delivery systems. Realizing this aim will require sustained interdisciplinary collaboration and rigorous, adequately powered empirical study; nonetheless, the convergence of traditional digestive physiology with quantitative microbiome and metabolomic science offers a scientifically grounded and translationally

promising direction for future research in personalized nutraceutical and pharmaceutical delivery.

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