The Sweetener Problem: What "Natural" Doesn't Mean — Mechanisms of Microbiome-Mediated Metabolic and Neurophysiological Risk

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1. Introduction: Reassessing Biological Inertness in the Colonic Niche

The modern food industry has positioned plant-derived high-intensity sweeteners, steviol glycosides (stevia), mogrosides (monk fruit), and thaumatin, as metabolic alternatives that bypass small-intestinal absorption and glycemic response. Regulatory assessments have historically focused on acute toxicity and glycemic neutrality, concluding that these compounds are safe within established acceptable daily intakes (ADIs) (Conz et al., 2023; Magnuson et al., 2016). However, this framework rests on a critical, increasingly tenuous assumption: that non-absorption equates to biological inertness. For many non-nutritive sweeteners (NNS), the physicochemical properties that prevent proximal absorption, high molecular weight, polarity, or glycosidic bonds, ensure transit to the colon, where they encounter a dense microbial ecosystem of approximately 10^{14} cells that mediates systemic immunity, neuroendocrine signaling, and metabolic homeostasis (Conz et al., 2023; Camilleri, 2021). The notion that these compounds are "just sweeteners" ignores their potential role as bioactive xenobiotics capable of remodeling microbial communities, altering fermentation profiles, and modulating barrier integrity, particularly in metabolically or immunologically vulnerable subpopulations (Panyod et al., 2024; Arnold et al., 2022).

This review synthesizes mechanistic, preclinical, and clinical evidence on the microbiome-mediated effects of high-intensity sweeteners, distinguishing between synthetic compounds (e.g., sucralose, saccharin, acesulfame-K) and plant-derived glycosides, while also evaluating sugar alcohols (polyols) as a separate nutritive sweetener class. The central argument is that the current ADI-based safety paradigm inadequately accounts for person-specific, microbiome-dependent responses that may

manifest as metabolic endotoxemia, neuroinflammatory priming, and appetitive dysregulation at doses relevant to chronic consumption. Critically, however, the magnitude and clinical significance of these effects vary substantially by chemical class, host baseline microbiome composition, and exposure context, necessitating a more nuanced risk framework.

2. Non-Nutritive Sweeteners: Definitions and Mechanisms

2.1 Definition and Core Properties

Non-nutritive sweeteners (NNS), also referred to as *non-caloric artificial sweeteners* (NCAS) or *high-intensity sweeteners* (HIS), provide intense sweetness, ranging from 30 to more than 20,000 times that of sucrose, while contributing negligible caloric value at customary use levels (Ahmad et al., 2020; Ruiz-Ojeda et al., 2019). Their defining characteristics include (a) high sweetness intensity requiring only microgram-to-milligram quantities in food matrices; (b) negligible caloric contribution at practical doses; and (c) distinctive metabolic fates in which the parent compound and/or its metabolites often bypass traditional energy pathways while interacting with gut microbes and host tissues (Conz et al., 2023; Magnuson et al., 2016).

NNS can be broadly divided into *synthetic (artificial)* sweeteners and *natural high-intensity* sweeteners of botanical origin. Both categories have received regulatory acceptance, yet their mechanisms of biological interaction and potential harm differ substantially.

2.2 Microbiome-Dependent Metabolic Risks of Non-Nutritive Sweeteners

The principal risk of NNS demonstrated in controlled human studies is the induction of personalized, microbiome-dependent impairments in glucose tolerance (Suez et al., 2022). This finding directly challenges the long-held assumption that NNS are metabolically inert. Importantly, the glycemic impairment occurred only in a subset of *responders*, indicating that baseline microbiome composition predicts individual susceptibility (Suez et al., 2022). Even though aspartame and stevia did

not produce cohort-wide glycemic effects, all four NNS, saccharin, sucralose, aspartame, and stevia. elicited distinct alterations in microbiome function, suggesting subclinical or context-dependent effects that may emerge with prolonged exposure or in metabolically vulnerable hosts (Suez et al., 2022; Burke & Small, 2015). Collectively, these findings support Suez et al.'s (2022) conceptualization of the gut microbiome as a *responsiveness hub* in which microbial configurations determine whether NNS consumption results in metabolic neutrality or dysregulation.

2.3 Impaired Glycemic Responses to Saccharin and Sucralose

In healthy adults with no prior NNS exposure, short-term supplementation with saccharin or sucralose significantly impaired glycemic control. Both sweeteners elevated the incremental area under the glucose curve (iAUC) during oral glucose-tolerance testing relative to both glucose-vehicle and no-supplement controls (Suez et al., 2022). The glycemic impairment persisted through the first and second weeks of exposure and returned toward baseline after discontinuation. In contrast, aspartame and stevia produced no cohort-wide change in glucose tolerance, though both altered microbial composition and metabolic signaling in subtle, compound-specific ways (Suez et al., 2022).

2.4 Microbiome Alterations and Causal Mediation

All four NNS, saccharin, sucralose, aspartame, and stevia, produced distinct functional alterations in the oral and intestinal microbiomes, whereas no such modulation occurred in the control groups (Suez et al., 2022). Causality was demonstrated through microbiota-transfer experiments: germ-free mice conventionalized with fecal samples from human *top responders* (individuals showing the greatest glycemic disruption) developed significantly higher glycemic responses than mice receiving baseline microbiota from the same donors. The transplanted metabolic phenotype mirrored that of the

human donors, confirming that the adverse effect was personalized and microbiome-mediated (Suez et al., 2022).

2.5 Molecular and Functional Pathways of Risk

Each NNS generated unique microbial and host-metabolic signatures (Suez et al., 2022):

- **Sucralose** reduced microbial purine biosynthesis while increasing mixed-acid fermentation and tricarboxylic-acid-cycle pathways. Correspondingly, plasma levels of TCA intermediates such as isocitrate and trans-aconitate rose—metabolites linked to impaired glycemic control.
- **Saccharin** increased *Prevotella copri* abundance and appeared to promote degradation of the cyclic amide caprolactam. Circulating indoxyl sulfate, a uremic toxin associated with vascular disease, also increased.
- **Aspartame** altered microbial polyamine metabolism and raised plasma kynurenine, a biomarker associated with diabetes risk.
- **Stevia** elevated microbial fatty-acid-biosynthesis pathways and increased plasma amino acids serine and lysine as well as arginine-derived metabolites ornithine and citrulline.

These convergent metabolomic shifts highlight the potential for NNS to influence host metabolism indirectly through microbiome-driven mechanisms rather than through direct caloric contribution.

2.6 Study Context and Limitations

However, interpretation requires consideration of the study's design parameters. Participants were healthy, lean, normoglycemic adults without prior NNS exposure, meaning that metabolic or microbiome perturbations could differ in individuals with pre-existing insulin resistance or obesity (Suez et al., 2022). The sweeteners were delivered as commercially available sachets containing

glucose as a bulking agent, and glycemic impairment occurred only when saccharin or sucralose were combined with this glucose vehicle, not with glucose alone, suggesting synergistic interactions between NNS and carbohydrates. Although all participants ingested comparable glucose loads, insulin responses rose only in the stevia and glucose-vehicle groups, implying that saccharin and sucralose may blunt glucose-stimulated insulin secretion (Suez et al., 2022).

Collectively, these data demonstrate that consumption of saccharin and sucralose, particularly in real-world formulations, can elicit individualized, microbiome-driven impairments in glycemic regulation. The results underscore the need for long-term, mechanistic clinical studies across diverse populations to clarify the metabolic and microbiome consequences of habitual NNS exposure, an issue further illuminated when contrasted with polyols and other "natural" sweeteners in subsequent sections.

3. Artificial Sweeteners Are Not Inert: Insights from Human Trials

3.1 Evidence from Human and Translational Studies

Evidence from human and translational studies demonstrates that artificial sweeteners are far from biologically inert. Rather than acting as passive sugar substitutes, they exert individualized, microbiome-dependent effects that can alter glucose regulation and cardiovascular risk.

In a landmark randomized controlled trial, Suez et al. (2022) showed that the metabolic consequences of non-nutritive sweeteners (NNS) depend on the composition and function of the gut microbiome. One hundred twenty adults with no habitual NNS exposure were randomized to consume saccharin, sucralose, aspartame, or stevia for two weeks, with matched glucose-vehicle and water control groups. Continuous glucose monitoring revealed that saccharin and sucralose produced the most consistent impairments in glycemic control, whereas aspartame and stevia elicited more variable

responses. Participants who exhibited post-prandial glucose elevations shared distinct baseline microbial profiles, suggesting that the microbiome could predict susceptibility to metabolic disruption.

To confirm causality, fecal samples from “top responders” (those developing glucose intolerance) and “non-responders” were transplanted into germ-free mice. Mice colonized with responder microbiota reproduced the same impaired-glucose-tolerance phenotype as their human donors, whereas mice colonized with non-responder microbiota remained normoglycemic. These results provided direct proof that sweetener-induced metabolic effects are mediated by the human microbiome (Suez et al., 2022). What distinguished responders from non-responders was not the sweetener dose but their baseline gut-microbiome configuration.

3.2 Foundational Evidence: The 2014 Translational Model

This discovery built upon earlier work by Suez et al. (2014), which first identified the causal pathway from artificial-sweetener exposure to dysglycemia. In that *Nature* study, healthy volunteers naïve to saccharin were administered doses at the U.S. FDA’s acceptable daily intake for one week. Roughly half developed significant glucose intolerance accompanied by compositional and functional alterations in their gut microbiota, including enrichment of *Bacteroides* and *Clostridiales* taxa. When fecal samples from these “responders” were transplanted into germ-free mice, the recipient animals—despite never being directly exposed to saccharin—developed the same glucose-intolerant phenotype, whereas mice receiving microbiota from human non-responders did not. This human-to-mouse transmission established that the adverse metabolic effects of artificial sweeteners are microbiome-driven rather than a result of direct absorption or host metabolism (Suez et al., 2014).

Together, the 2014 and 2022 studies form a coherent mechanistic narrative: NNS exposure alters microbial ecology, which in turn modulates host glucose regulation through shifts in microbial

carbohydrate metabolism, purine biosynthesis, and short-chain-fatty-acid pathways. These pathways can amplify systemic inflammation and insulin resistance even in the absence of caloric intake. The findings dismantle the traditional toxicological notion that “non-nutritive” implies “non-biological.” Artificial sweeteners are best described as **microbiome-active xenobiotics** whose metabolic impact is determined by the ecological context of the host gut.

3.3 Expanding the Scope: Cardiometabolic Implications

Beyond glucose regulation, emerging data implicate NNS in broader metabolic and cardiovascular disturbances. Witkowski et al. (2023) reported that circulating erythritol, a commonly used sugar alcohol—was strongly associated with major adverse cardiovascular events in humans. Mechanistic experiments demonstrated that erythritol enhances platelet activation and thrombosis potential, identifying a plausible causal link between chronic polyol exposure and vascular risk. These results extend concern from glycemic regulation to systemic cardiometabolic pathways, illustrating that low-calorie sweeteners can act well beyond the gastrointestinal tract.

3.4 Global Policy and Scientific Debate

At the public-health level, the World Health Organization (2023) released its first formal guideline on non-sugar sweeteners, concluding that such compounds *“should not be used as a means of achieving weight control or reducing the risk of noncommunicable diseases”* (p. 3). The WHO’s synthesis of more than 280 studies found that short-term randomized trials show trivial or inconsistent effects on body weight, while long-term cohort studies associate habitual NNS consumption with increased incidence of obesity, type 2 diabetes, and cardiovascular disease.

This recommendation has provoked significant scholarly debate. Khan et al. (2023) contend that the WHO’s evidence base combined heterogeneous study designs and overemphasized low-certainty

findings. When randomized controlled trials are analyzed separately, they argue, non-sugar sweeteners generally yield neutral or modestly beneficial outcomes for body-weight management compared with caloric sugars. Khan and colleagues propose that substituting sugars with NNS may constitute a harm-reduction strategy for individuals with obesity or diabetes, provided such substitutions occur within an overall healthful diet.

Despite their disagreement, both perspectives converge on a key principle: artificial sweeteners are not metabolically inert. Their effects vary with individual microbiome composition, baseline metabolic health, and the broader nutritional environment. Consequently, the safety or efficacy of any sweetener cannot be defined universally but must be interpreted through a personalized, microbiome-aware lens.

3.5 Conceptual Integration: The Microbiome as a Responsiveness Hub

These findings reposition the gut microbiome as a *responsiveness hub* that dictates host reaction to environmental compounds. A formulation that is harmless in one person may provoke dysglycemia in another whose microbial community has been shaped by prior diet, antibiotic exposure, or inflammation. This inter-individual variability challenges the adequacy of population-level dietary guidelines and underscores the need for precision-nutrition approaches.

3.6 Synthesis

Translational evidence from human and animal models demonstrates that saccharin and sucralose pose the greatest risk for microbiome-mediated glucose dysregulation, whereas aspartame and stevia produce subtler or context-dependent effects. The identification of a microbiome-to-host causal mechanism redefines how artificial sweeteners should be evaluated for safety. Moving forward, nutritional policy and clinical practice must shift from a calorie-centric to a systems-biology

perspective, one that accounts for microbial ecology, metabolic individuality, and long-term cardiometabolic outcomes.

Non-Sugar Sweeteners as a Dual-Class System

Non-sugar sweeteners (NSS) encompass a chemically and functionally diverse group of compounds unified by their ability to provide sweetness with minimal or no caloric contribution (World Health Organization [WHO], 2023). Within this regulatory category, two principal subtypes emerge: synthetic (artificial) non-nutritive sweeteners (NNS), such as aspartame, sucralose, saccharin, acesulfame K, neotame, and advantame, and "natural" high-intensity sweeteners derived from botanical or fermentative sources, including stevia glycosides and mogrosides from monk fruit. Both groups are legally sanctioned as sugar substitutes, yet their biochemical fates and physiological interactions differ markedly. Synthetic NNS are largely xenobiotic, chemically stable molecules that resist human digestion and reach the colon intact, where they interact directly with gut microbes. In contrast, natural high-intensity sweeteners often comprise glycosidic structures that undergo partial microbial hydrolysis, producing bioactive metabolites with potential prebiotic or dysbiotic effects depending on the host context. Understanding these divergent mechanisms is critical, as both classes, despite their contrasting origins, exert non-trivial influences on the gut microbiome and host metabolism, challenging the notion that either can be considered biologically inert.

4. Synthetic (Artificial) Non-Nutritive Sweeteners (NNS)

4.1 Aspartame: Metabolite-Mediated Dysbiosis and Neurochemical Change

Aspartame is an outlier among non-nutritive sweeteners (NNS) because it is rapidly and almost completely hydrolyzed in the small intestine into *L-aspartic acid*, *L-phenylalanine*, and *methanol*; thus, little of the intact compound reaches the colon (Conz et al., 2023; Magnuson et al., 2016).

Nevertheless, its metabolic fragments exert biologically significant effects. In rat models, low-dose aspartame increased total bacteria, *Enterobacteriaceae*, and *Clostridium leptum*, indicating that the downstream metabolic environment favors compositional shifts in the microbiota (Palmnäs et al., 2014, as cited in Conz et al., 2023). These changes correlated with increased fasting glucose and impaired insulin-stimulated glucose disposal independent of body composition (Palmnäs et al., 2014, as cited in Conz et al., 2023).

Human and animal data further indicate that aspartame alters polyamine-related metabolic pathways and may induce long-lasting neurochemical changes in mesolimbic reward circuitry when exposure occurs during critical developmental windows (Nettleton et al., 2020; Suez et al., 2022). Thus, aspartame's risk profile is not limited to its component amino acids; its metabolite-driven alterations in microbial composition and host metabolism make it a potent modulator of the gut–brain–metabolic axis.

4.2 Sucralose: Colonic Persistence, Dysbiosis, and Inflammatory Signaling

Sucralose is a chlorinated sucrose derivative that is highly stable and poorly absorbed; more than 85 % of ingested sucralose reaches the colon unchanged, providing sustained exposure to gut microbes (Conz et al., 2023; Ruiz-Ojeda et al., 2019). In a randomized controlled trial, two weeks of sucralose consumption impaired glycemic responses in previously healthy individuals. Transplantation of microbiota from human *responders* into germ-free mice reproduced glucose intolerance, establishing a causal role for sucralose-induced dysbiosis in metabolic dysfunction (Suez et al., 2022).

Animal studies consistently show that sucralose reduces beneficial taxa such as *Lactobacilli*, total anaerobes, and *Clostridium* cluster XIVa while enriching *Proteobacteria*, a phylum strongly associated with intestinal inflammation and Crohn-like ileitis (Abou-Donia et al., 2008; Rodriguez-

Palacios et al., 2018, as cited in Conz et al., 2023). At the functional level, sucralose increases expression of microbial genes involved in lipopolysaccharide (LPS) and flagellar-protein synthesis, thereby enhancing pro-inflammatory potential (Bian et al., 2017, as cited in Conz et al., 2023). *In vitro* findings that sucralose promotes the spread of antibiotic-resistance genes by altering bacterial-membrane properties further underscore its ecological impact (Conz et al., 2023).

Sucralose exposure has also been linked to ΔFosB accumulation in reward-related brain regions, suggesting maladaptive plasticity and potential reinforcement of compulsive intake patterns (Salaya-Velazquez et al., 2020). Overall, sucralose is best conceptualized as a colonic bacteriostatic and pro-inflammatory agent that induces dysbiosis, metabolic endotoxemia, and neurobehavioral alterations.

4.3 Saccharin: Driver of Endotoxemia and Hepatic Inflammation

Saccharin is only partially absorbed, leaving a substantial fraction to interact with colonic microbes (Conz et al., 2023; Ruiz-Ojeda et al., 2019). Both mouse and human studies demonstrate that saccharin impairs glucose tolerance through microbiome-mediated mechanisms; microbiota transfer from saccharin-exposed donors into germ-free mice is sufficient to induce glucose intolerance (Suez et al., 2014; Suez et al., 2022, as cited in Conz et al., 2023).

Microbiologically, saccharin consumption increases *Bacteroides* and multiple *Clostridiales* taxa. Functionally, it enriches pathways related to LPS biosynthesis, bacterial chemotaxis, and flagella assembly (Bian et al., 2017, as cited in Conz et al., 2023). These microbial changes are accompanied by hepatic inflammation and up-regulation of inducible nitric-oxide synthase (iNOS) and tumor-necrosis-factor α (TNF-α), implicating saccharin in the progression to metabolic endotoxemia and hepatocellular stress (Bian et al., 2017, as cited in Conz et al., 2023).

4.4 Acesulfame Potassium (ACE-K): Neuro-Metabolic Disruption and Sex-Specific Dysbiosis

Acesulfame potassium (ACE-K) is highly absorbed and largely excreted unchanged in urine, leading early researchers to assume minimal colonic interaction (Conz et al., 2023; Ruiz-Ojeda et al., 2019). However, chronic-exposure studies in mice reveal a distinct neuro-metabolic toxicity profile. Long-term ACE-K intake (40 weeks) impairs cognitive memory, inhibits glycolysis, and depletes ATP in hippocampal neurons, partly via dysregulation of TrkB-mediated brain-derived neurotrophic-factor (BDNF) signaling (Cong et al., 2013). Critically, ACE-K crosses the blood–brain barrier and accumulates in cortical tissue, indicating direct central-nervous-system exposure (Cong et al., 2013).

Microbiome analyses demonstrate sex-specific dysbiosis: ACE-K increases *Bacteroides* and other potentially pathogenic genera in males, while decreasing *Lactobacillus* and *Clostridium* in females, with parallel up-regulation of LPS-synthesis genes in both sexes (Bian et al., 2017, as cited in Conz et al., 2023). Thus, ACE-K functions as a systemic neuro-metabolic disruptor with both central and microbiome-mediated effects.

4.5 Neotame and Advantame: Epithelial Toxicity at Trace Concentrations

Neotame and advantame are N-substituted aspartame derivatives with extremely high sweetness potency, historically assumed to have negligible biological effects due to their low absolute doses (Ruiz-Ojeda et al., 2019). However, recent data challenge this presumption. Neotame damages intestinal epithelial cells and disrupts monolayer integrity at concentrations as low as 1 μM, increasing apoptosis and epithelial permeability (Shil et al., 2020, as cited in Conz et al., 2023). These effects are mediated via the T1R3 sweet-taste receptor expressed in gut epithelium, confirming that sweet-taste receptors serve as functional signaling hubs beyond the tongue (Shil et al., 2020, as cited in Conz et al., 2023).

Neotame also enhances the pathogenicity of model gut bacteria, increasing biofilm formation, adhesion, and invasion by *Escherichia coli* and *Enterococcus faecalis* (Shil et al., 2020, as cited in Conz et al., 2023). For advantame, empirical microbiome data remain sparse, but its structural similarity to neotame and shared receptor affinity suggest a potential for comparable T1R3-mediated epithelial effects (Ruiz-Ojeda et al., 2019).

Table 1

Synthetic Non-Nutritive Sweeteners: Mechanisms and Metabolic Consequences

Sweetener	Chemical Classification	Primary Mechanism	Key Outcomes in Literature
Aspartame (ASP)	Dipeptide methyl ester (L-aspartyl-L-phenylalanine)	Rapidly hydrolyzed in the small intestine; limited direct colonic contact. Indirect metabolic effects via metabolites (phenylalanine, aspartate, methanol) that can influence neurotransmission and gut microbial composition (Magnuson et al., 2016; Butchko et al., 2002; Stegink, 2020).	Increased fasting glucose and altered gut microbiota composition in rats (↑*Enterobacteriaceae*, ↓*Clostridium leptum*) (Palmnäs et al., 2014); elevated kynurenine pathway activity in human plasma (Suez et al., 2022); minimal microbiome disruption at realistic intake levels (Ahmad et al., 2020).
Sucralose (SUC)	Chlorinated disaccharide (1,6-dichloro-1,6-dideoxyfructose + 4-chloro-4-deoxygalactose)	Poorly absorbed; >85 % reaches the colon unmetabolized (Roberts et al., 2000; Magnuson et al., 2016). Direct bacteriostatic and pro-inflammatory actions; alters purine metabolism and nucleotide biosynthesis (Suez et al., 2022).	Induces glucose intolerance in humans and mice, transmissible via fecal microbiota transplant (Suez et al., 2022); ↑*Proteobacteria* (Rodriguez-Palacios et al., 2018); up-regulation of LPS synthesis and antibiotic-resistance genes (Bian et al., 2017; Yu et al., 2021); disrupts *Clostridium cluster XIVa* (Uebanso et al., 2017).
Saccharin	Benzoisothiazol-3(2H)-one	5–15 % of ingested dose	Causes glucose intolerance

Sweetener	Chemical Classification	Primary Mechanism	Key Outcomes in Literature
(SAC)	1,1-dioxide derivative	reaches the colon (Renwick, 1985). Inhibits microbial glucose fermentation (Pfeffer et al., 1985) and enriches glycan-degradation and endotoxin pathways (Suez et al., 2014).	in humans; phenotype transferrable to germ-free mice (Suez et al., 2014); promotes hepatic inflammation via TNF-α/iNOS (Bian et al., 2017); ↑*Bacteroides* and LPS-biosynthesis genes (Suez et al., 2014; Bian et al., 2017); contributes to antibiotic resistance (Yu et al., 2021).
Acesulfame K (ACE-K)	Potassium salt of 6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide	Crosses the blood–brain barrier and accumulates in brain tissue, altering energy metabolism (Cong et al., 2013). Sex-specific gut microbial modulation with pro-inflammatory effects (Bian et al., 2017).	Impaired hippocampal glycolysis and memory in mice (Cong et al., 2013); ↑*Bacteroides* (males), ↓*Lactobacillus* (females) (Bian et al., 2017); up-regulation of LPS and antibiotic-resistance genes (Yu et al., 2021; Li et al., 2022).
Neotame (NEO)	N-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine 1-methyl ester	Damages intestinal epithelial cells via T1R3 sweet-taste receptor signaling; promotes pathogenic biofilm formation (Shil et al., 2024).	Induces Caco-2 cell apoptosis and barrier loss ≥100 μM (Shil et al., 2024); enhances *E. coli* and *E. faecalis* invasion and biofilm activity (Shil et al., 2024).
Advantame (ADV)	N-substituted aspartame derivative	Ultra-potent (~20 000× sucrose); consumed in trace amounts. Direct microbial effects negligible, though T1R3 signaling potential remains unexamined (Ruiz-Ojeda et al., 2019).	No significant microbiome or metabolic alterations (Ruiz-Ojeda et al., 2019); EFSA (2013) ADI = 5 mg/kg, well below microbiologically active range.

Note.

This table summarizes current mechanistic evidence linking synthetic non-nutritive sweeteners to microbiome and host-metabolic effects. Data integrate human, animal, and in vitro findings emphasizing microbial mediation, dose dependence, and translational uncertainty.

5. “Natural” High-Intensity Sweeteners: The Natural Fallacy

Regulators and the food industry frequently classify stevia, monk fruit, thaumatin, and neohesperidin dihydrochalcone (NDC) as *natural* and implicitly safer alternatives to synthetic non-nutritive sweeteners (NNS). This classification, however, is scientifically misleading. These compounds are highly processed extracts whose biological activity depends heavily on colonic microbial metabolism, generating aglycones and other metabolites with poorly characterized, or in some cases explicitly harmful—profiles (Conz et al., 2023; European Food Safety Authority [EFSA] Panel, 2019; Wang, 2021).

5.1 Steviol Glycosides (Stevia): Genotoxic Aglycone and Functional Dysbiosis

Steviol glycosides (e.g., stevioside, rebaudioside A) are not absorbed intact in the small intestine. Instead, they reach the colon, where *Bacteroides* species hydrolyze glycosidic bonds, releasing steviol that is subsequently absorbed systemically (Ruiz-Ojeda et al., 2019; Wang, 2021). Steviol has tested positive in Ames mutagenicity assays, is cytotoxic to human colon epithelial cells at physiologically relevant concentrations, and exhibits weak androgenic and anti-estrogenic activity in rodents (Conz et al., 2023; EFSA Panel, 2019; Magnuson et al., 2016).

Metaproteomic analyses show that stevia induces profound functional shifts despite modest taxonomic changes. Steviol glycosides suppress butyryl-CoA:acetate CoA-transferase, a key enzyme in butyrate synthesis, by over 40 %, thereby reducing the availability of this barrier-protective short-chain fatty acid (Camilleri, 2021; Wang, 2021). Concurrently, stevia enriches LPS-producing *Bacteroides fragilis* and *B. vulgatus* while depleting *Faecalibacterium prausnitzii* and *Roseburia*, keystone butyrate producers whose loss is associated with inflammation and insulin resistance (Panyod et al., 2024; Wang, 2021).

At the host level, steviol activates TLR4 signaling in hepatic Kupffer cells and brain microglia, promoting LPS translocation, hepatic steatosis, hippocampal neuroinflammation, and suppression of BDNF (Arnold et al., 2022; Chassaing et al., 2022). Behavioral studies link stevia exposure to reward-system desensitization, ΔFosB accumulation in the nucleus accumbens, and altered sugar-motivated behaviors (Nettleton et al., 2020; Salaya-Velazquez et al., 2020; Tsan et al., 2022). Collectively, these findings challenge the notion that stevia is safe for chronic consumption.

5.2 Mogrosides (Monk Fruit Extract): Unknown Metabolite Risk and Hepatic Injury

Mogrosides, particularly mogroside V, are the principal sweetening constituents of monk fruit extract (MFE). EFSA's (2019) safety assessment concluded that genotoxicity data were insufficient to evaluate microbial-metabolite safety, including the aglycone form. A 90-day rat study reported 15–20 % reductions in testis weight at doses near the proposed ADI, alongside systemic bioavailability of mogrosides and their metabolites (EFSA Panel, 2019).

Ex vivo RapidAIM analyses demonstrate that MFE profoundly reshapes the human-microbiome metaproteome, suppressing butyrate-producing pathways in *Faecalibacterium* and *Roseburia*, increasing LPS-biosynthesis gene expression in *Bacteroides*, and up-regulating bacterial-motility proteins such as flagellin and chemotaxis factors (Chassaing et al., 2022; Naimi et al., 2021; Wang, 2021). In vivo, MFE elevates serum LPS, increases hepatic transaminases, promotes SREBP-1c-driven steatosis, and reduces GLP-1 secretion from L cells, thereby impairing post-prandial insulin responses (Camilleri, 2021; Chassaing et al., 2022). Short-term human trials showing reduced post-prandial glucose and insulin relative to sucrose (Tey et al., 2017) highlight a dangerous dichotomy: acute glycemic benefit versus chronic microbiome- and liver-related risk.

5.3 Thaumatin: Regulatory Assumptions Amid Microbiome Data Gaps

Thaumatin, a sweet-tasting protein roughly 100 000 times sweeter than sucrose, is presumed safe because it is digested in the small intestine into amino acids and small peptides (Ruiz-Ojeda et al., 2019). This presumption is tenuous given that: (a) no human or animal studies have examined its microbiome effects; (b) no long-term toxicity data exist; and (c) other sweet proteins, such as monellin, can activate TLR4 on immune cells (Chassaing et al., 2022; Conz et al., 2023). In the absence of microbiome data, thaumatin's safety remains assumed rather than demonstrated.

5.4 Neohesperidin Dihydrochalcone (NDC): Context-Dependent Dysbiosis and LPS Amplification

NDC, a flavonoid-derived sweetener from immature citrus fruits, is often portrayed as beneficial because it increases *Lactobacillus* and is metabolized to apparently innocuous products (Ruiz-Ojeda et al., 2019). However, in dysbiotic or inflamed contexts, *Lactobacillus* overgrowth can be maladaptive. Expansion within the inner mucus layer displaces mucin-degrading specialists such as *Akkermansia muciniphila*, promoting barrier disruption and bacterial translocation (Naimi et al., 2021; Panyod et al., 2024).

NDC-driven lactic-acid production acidifies the colonic lumen and solubilizes LPS from Gram-negative bacteria, facilitating its translocation across an already compromised barrier (Camilleri, 2021). This creates a feed-forward cycle of LPS release, inflammation, and further dysbiosis.

5.5 Summary: The "Natural" Sweetener Problem

Across stevia, monk fruit, thaumatin, and NDC, a consistent theme emerges: microbial biotransformation into bioactive metabolites that suppress protective SCFA pathways, enrich LPS-producing taxa, and potentiate inflammatory signaling. "Natural" origin therefore confers no guarantee of microbiome safety.

Table 2

Natural Non-Nutritive Sweeteners: Mechanisms and Context-Dependent Effects

Sweetener	Botanical / Chemical Classification	Primary Mechanism of Concern	Key Outcomes in Literature
Steviol glycosides (Stevia)	Diterpene glycosides from *Stevia rebaudiana* (e.g., stevioside, rebaudioside A)	Not absorbed in small intestine; *Bacteroides* hydrolyze glycosides to steviol (Ruiz-Ojeda et al., 2019). Steviol is mutagenic and alters butyrate metabolism.	Mutagenic in Ames assays; cytotoxic to colon cells (EFSA Panel, 2019). Suppression > 40 % of butyryl-CoA:acetate CoA-transferase (Wang, 2021); enrichment of *B. fragilis/vulgatus* and depletion of *F. prausnitzii* and *Roseburia*. Human RCTs report no cohort-wide changes (Ahmad et al., 2020).
Mogrosides (Monk fruit extract)	Cucurbitane-type triterpene glycosides from *Siraitia grosvenorii* (e.g., mogroside V)	Poorly characterized microbial metabolism; conversion to aglycones with uncertain toxicology (EFSA Panel, 2019).	EFSA found insufficient genotoxicity data; 15–20 % testis-weight reduction near ADI (EFSA Panel, 2019). RapidAIM data: suppression of butyrate pathways, ↑ LPS genes, ↑ flagellin (Wang, 2021). Short-term human data show lower glucose but possible chronic liver risk (Tey et al., 2017).
Thaumatin	Sweet protein (≈ 2 000–3 000 × sucrose) from *Thaumatococcus daniellii*	Presumed complete digestion to amino acids; microbiome impact unstudied (Ruiz-Ojeda et al., 2019).	No long-term microbiome or toxicity data (Kaim & Labus, 2025). EFSA notes lack of data and parallels to monellin that can activate TLR4 (EFSA Panel, 2019).
Neohesperidin dihydrochalcone (NDC)	Hydrogenated flavonoid derivative from citrus neohesperidin	Fermented by colonic microbes; lactic-acid production increases LPS solubility (Ruiz-Ojeda et al., 2019).	↑ *Lactobacillus* and lactic acid (Ruiz-Ojeda et al., 2019); acidification promotes LPS translocation (Shil et al., 2020). Human trials lacking; effects context-dependent.

Note. "Natural" high-intensity sweeteners are highly processed extracts whose biological effects depend on microbial metabolism. Current evidence suggests that stevia and monk-fruit extracts can impair butyrate pathways and promote LPS-related signaling in ex vivo and animal models, whereas thaumatin and NDC remain under-characterized, particularly in long-term human studies.

Integrative Perspective

Although Tables 1 and 2 distinguish between synthetic and natural non-nutritive sweeteners (NNS), the mechanistic evidence reveals a common flaw—the assumption of inertness. Synthetic compounds such as sucralose and saccharin directly alter microbial composition and host metabolism through colonic persistence and pro-inflammatory signaling, whereas "natural" counterparts like stevia and monk-fruit extracts exert comparable downstream effects via microbial biotransformation into bioactive or cytotoxic metabolites (Conz et al., 2023; EFSA Panel, 2019; Suez et al., 2022). The "natural" label thus functions as a marketing construct rather than a biological safeguard. Across both groups, the intestinal microbiome emerges as the principal mediator of risk, transforming these sweeteners from presumed inert sugar substitutes into modulators of metabolic, immune, and neuroendocrine homeostasis.

6. Nutritive Sweeteners: Sugar Alcohols (Polyols) and the Polyol Paradox

6.1 Polyols as Nutritive Sweeteners With Non-Trivial Microbiome Effects

Sugar alcohols (polyols) occupy an ambiguous regulatory category. They are classified as *nutritive* sweeteners because they provide some caloric value, yet they are frequently marketed as "gut-friendly" or even "prebiotic" due to partial fermentation by purportedly beneficial taxa (Ruiz-Ojeda et al., 2019). However, the mechanistic literature reveals a *dose-dependent toxicity profile* in which modest, sub-therapeutic doses may yield limited benefits, whereas realistic consumption levels induce osmotic stress, functional dysbiosis, and pathobiont expansion (Conz et al., 2023; Panyod et al., 2024; Wang, 2021).

6.2 Osmotic Overload, Mucus Disruption, and Barrier Compromise

Polyols are poorly absorbed osmotic agents that draw water into the intestinal lumen. Sorbitol, for instance, is malabsorbed in 71 % of healthy adults at doses as low as 10 g, producing flatulence, abdominal discomfort, and mild laxative effects (Ruiz-Ojeda et al., 2019). At 20 g/day, sorbitol induces

pronounced abdominal pain and diarrhea (Conz et al., 2023). This osmotic overload physically disrupts the mucus layer, increases shear stress on epithelial surfaces, and facilitates lipopolysaccharide (LPS) translocation—mechanistically analogous to synthetic emulsifiers that erode mucosal integrity (Camilleri, 2021; Panyod et al., 2024).

6.3 Functional Dysbiosis Despite "Beneficial" Taxa

Compositional analyses often suggest that polyols enrich genera such as *Bifidobacterium* and *Lactobacillus*. Yet metaproteomic data demonstrate that polyols cluster with highly fermentable controls (e.g., glucose, fructo-oligosaccharides) in provoking strong *functional perturbations*, including suppression of butyrogenic enzyme expression (Wang, 2021). Specifically, activity of butyryl-CoA:acetate CoA-transferase is markedly reduced, indicating that short-chain fatty acid (SCFA) output does not match the apparent taxonomic enrichment. This disconnect exemplifies functional dysbiosis—a state where the microbiome appears compositionally favorable but is metabolically impaired and less capable of maintaining barrier integrity and immune tolerance.

6.4 Pathobiont Amplification and Endotoxin Burden

Polyols selectively enrich pathobionts. Xylitol and sorbitol increase *Bacteroides* and *Anaerostipes* species, including LPS-rich *B. fragilis* and *B. vulgatus* (Ruiz-Ojeda et al., 2019; Panyod et al., 2024). Maltitol enriches *Eubacterium rectale* but simultaneously depletes *Faecalibacterium prausnitzii*, a keystone butyrate producer whose loss correlates with inflammation and metabolic disease (Wang, 2021). These microbial shifts correspond with elevated fecal endotoxin levels and low-grade systemic inflammation in animal models (Arnold et al., 2022; Panyod et al., 2024). Even erythritol—often regarded as microbiome-neutral due to limited fermentation, remains osmotically

active, contributes no SCFAs, and may synergize with other additives to increase LPS translocation in complex food matrices (Wang, 2021).

6.5 Polyols in Real-World Ultra-Processed Diets

Acceptable daily intakes (ADIs) for polyols are derived from acute osmotic endpoints rather than long-term metabolic data. Yet ex vivo metaproteomic analyses show that sugar alcohols cluster with fermentable carbohydrates in producing some of the most pronounced functional disruptions among tested compounds (Wang, 2021). In modern ultra-processed foods, polyols rarely occur in isolation—they coexist with emulsifiers and both synthetic and "natural" NNS, creating additive or synergistic dysbiotic effects (Chassaing et al., 2022; Panyod et al., 2024).

Thus, the widely promoted "prebiotic" narrative surrounding polyols is misleading. At doses typical of "sugar-free" confectionery, baked goods, and nutritional bars, polyols act more like osmotic laxatives and dysbiosis amplifiers than benign microbial substrates.

Table 3

The Polyol Problem: Reported Benefits Versus Mechanistic Risks

Sweetener (Polyol)	Reported "Beneficial" Effects	Pathogenic Mechanism (Literature-Based)
Erythritol	Non-fermentable; minimal microbiome alteration (Ruiz-Ojeda et al., 2019).	Largely non-fermentable but osmotically active; provides no SCFAs; may increase LPS when co-consumed with other additives (Wang, 2021).
Xylitol	Increases butyrate and *Anaerostipes* abundance (Ruiz-Ojeda et al., 2019).	Acts as osmotic laxative at >10 g/day; enriches *Bacteroides* (LPS producers); suppresses *F. prausnitzii* in murine models (Wang, 2021; Panyod et al., 2024).
Maltitol	Elevates *Bifidobacterium* and *Eubacterium rectale* levels (Ruiz-Ojeda et al., 2019).	Provokes strong metaproteomic dysregulation; suppresses butyrogenic enzymes; enriches *Bacteroides* at expense of *F. prausnitzii* (Wang, 2021; Panyod et al., 2024).
Lactitol	Increases *Bifidobacterium* and	Reduces *Enterobacteriaceae* but also depletes

Sweetener (Polyol)	Reported "Beneficial" Effects	Pathogenic Mechanism (Literature-Based)
	Lactobacillus (Ruiz-Ojeda et al., 2019).	commensal *Clostridium* clusters; induces flatulence and bloating at therapeutic doses (Conz et al., 2023).
Sorbitol	Enhances butyrate and *Lactobacillus* production (Ruiz-Ojeda et al., 2019).	71 % malabsorption at 10 g; osmotic overload and diarrhea ≥20 g/day; associated with LPS translocation (Conz et al., 2023; Panyod et al., 2024).

Note. Polyols are conditionally beneficial at sub-therapeutic doses but functionally disruptive and pro-inflammatory at realistic intake levels—particularly within ultra-processed food matrices. Their "prebiotic" label obscures their osmotic, inflammatory, and dysbiotic liabilities.

6.6 Reframing Polyols: From Prebiotics to Conditional Stressors

Polyols epitomize how regulatory classifications lag behind emerging mechanistic evidence. Once regarded as "safe bulking agents" or "low-glycemic prebiotics," these compounds are now understood as conditional stressors—capable of exerting beneficial or deleterious effects depending on dose, co-ingredients, and host context (Wang, 2021; Panyod et al., 2024). Their osmotic activity and fermentative potential create a narrow therapeutic window: modest exposure may transiently favor beneficial taxa, yet typical consumption in ultra-processed formulations triggers barrier disruption, endotoxemia, and chronic low-grade inflammation (Camilleri, 2021; Conz et al., 2023).

Just as carboxymethylcellulose and carrageenan erode the mucus layer and facilitate LPS translocation in both murine and human trials (Camilleri, 2021; Chassaing et al., 2022), polyols at realistic doses generate comparable osmotic shear stress that compromises epithelial integrity. While direct human randomized controlled trials linking polyols to chronic endotoxemia are limited, the microbiome-mediated glycemic impairments observed with saccharin and sucralose—transmissible via fecal transplants to germ-free mice (Suez et al., 2022)—reinforce the principle that chronic dysbiosis drives systemic metabolic dysfunction.

In this light, the polyol paradox underscores a broader regulatory blind spot: compounds deemed "safe" on the basis of acute gastrointestinal tolerance may, under real-world dietary conditions,

undermine mucosal and metabolic integrity. A microbiome-informed risk framework—integrating dose, food matrix, and host susceptibility—is therefore essential to replace the obsolete "safe versus unsafe" binary with a precision-based nutritional toxicology paradigm.

7. Molecular Mechanisms of Microbial–Metabolic Disruption and Its Systemic Consequences

7.1. Direct Microbial Interactions and Growth Inhibition

Non-nutritive sweeteners (NNS) reach the colon largely unabsorbed, ensuring direct contact with the gut microbiota (Conz et al., 2023). Sucralose, for instance, is poorly absorbed (< 15%) and transits intact to the large intestine (Burke & Small, 2015; Conz et al., 2023), while a measurable fraction of saccharin similarly persists in the colon despite partial absorption (Burke & Small, 2015). This luminal availability allows NNS to act as selective antimicrobial or bacteriostatic agents. *In vitro* evidence shows that sucralose and saccharin inhibit bacterial growth, with saccharin reducing total anaerobic populations and sucralose displaying bacteriostatic effects on *Escherichia coli*, *Lactobacillus*, and *Bacteroides* species (Suez et al., 2022; Burke & Small, 2015).

Neotame—a newer synthetic sweetener—amplifies biofilm formation in *E. coli* and *Enterococcus faecalis*, increasing adhesion to and invasion of intestinal epithelial cells (Shil et al., 2023). These pathogenic alterations occur at physiologically relevant concentrations (0.1–1,000 μM) and are mediated by the T1R3 sweet-taste receptor, as co-treatment with zinc sulfate attenuates the effect (Shil et al., 2023; Burke & Small, 2015).

This antimicrobial selectivity drives dysbiosis, a shift away from commensal, anti-inflammatory taxa toward pro-inflammatory species. Saccharin consumption enriches *Bacteroides fragilis* and *Clostridiales* while depleting *Akkermansia muciniphila*, a keystone mucin-degrader linked to metabolic health and barrier stability (Suez et al., 2022). Sucralose exposure increases *Turicibacter*, *Roseburia*,

and *Akkermansia* but reduces *Ruminococcus* and *Streptococcus* (Wang, 2021). Even aspartame—rapidly hydrolyzed in the small intestine, modulates microbial composition in vivo, increasing *Enterobacteriaceae* and *Clostridium leptum* in diet-induced obese rats (Ruiz-Ojeda et al., 2019; Suez et al., 2022). Collectively, saccharin and sucralose exhibit the strongest dysbiotic signatures among tested NNS (Suez et al., 2022).

7.2. Functional Pathway Alterations: From SCFAs to Bile Acids

Beyond taxonomic disruption, NNS reprogram microbial metabolic function. Butyrate-producing pathways are especially vulnerable. Butyrate supports colonocyte energy metabolism, maintains tight-junction integrity, and suppresses inflammation (Suez et al., 2022; Wang, 2021).

Metaproteomic profiling demonstrates that sweeteners, particularly sugar alcohols and glycosides, induce broader functional perturbations than classical artificial NNS (Wang, 2021). Enzymes involved in butyrate synthesis (e.g., butyryl-CoA dehydrogenase, phosphate butyryltransferase) are differentially regulated depending on the sweetener (Wang, 2021). Xylitol, for example, enhances *Anaerostipes caccae*-mediated butyrate production, whereas sucralose and saccharin suppress butyrogenic enzyme expression in *Faecalibacterium*, *Roseburia*, and *Eubacterium* (Wang, 2021). This functional erosion of short-chain-fatty-acid (SCFA) output reduces epithelial energy supply and increases intestinal permeability.

NNS also alter bile-acid metabolism, a key regulator of lipid and glucose homeostasis via FXR and TGR5 signaling. Saccharin and sucralose increase fecal bile-acid concentrations and modulate bile-salt-hydrolase (BSH) activity (Conz et al., 2023). Elevated luminal bile acids disrupt tight-junction proteins (claudin-3, ZO-1) and facilitate LPS translocation into circulation (Burke & Small, 2015; Shil et al., 2023). Binding of LPS to TLR4 on immune cells triggers systemic inflammation—a recognized driver of insulin resistance and neuroinflammation. In murine models, sucralose-induced dysbiosis

elevates circulating LPS and hepatic pro-inflammatory markers (TNF-α, iNOS), establishing a mechanistic link between microbial perturbation and metabolic dysfunction (Suez et al., 2022; Thomson et al., 2019).

Just as carboxymethylcellulose and carrageenan erode the mucus layer and potentiate LPS translocation in both murine and human studies (Camilleri, 2021; Chassaing et al., 2022), polyols and certain NNS create comparable osmotic and biochemical stressors that compromise barrier integrity. While direct human RCTs linking polyols or NNS to chronic endotoxemia remain sparse, the causal glucose-intolerance phenotypes observed with saccharin and sucralose—reproducible through microbiome transplants to germ-free mice (Suez et al., 2022)—affirm that microbiome-mediated inflammation can drive systemic metabolic dysfunction.

7.3. Purine Biosynthesis and Energy Harvest

An additional mechanism involves microbial purine biosynthesis. In sucralose "responders," upregulation of purine-pathway genes and increased plasma purine metabolites correlated with impaired glycemic control (Suez et al., 2022). Simultaneous activation of mixed-acid fermentation and tricarboxylic-acid (TCA)-cycle genes enhances microbial energy yield, a phenotype previously linked to obesity (Suez et al., 2014). Increased expression of ATP-binding-cassette (ABC) transporters and phosphotransferase-system (PTS) genes allows microbes to more efficiently scavenge host carbohydrates, thereby increasing caloric extraction and promoting adiposity (Suez et al., 2022). This mechanism explains how ostensibly "non-caloric" sweeteners can paradoxically augment energy harvest and weight gain in susceptible individuals.

8. Neurophysiological Risk Pathways: The Gut–Brain Axis

8.1 Sweet-Taste Receptor Signaling Beyond the Tongue

The discovery of extra-oral sweet-taste receptors (T1R2/T1R3 heterodimers) in the gastrointestinal tract and brain fundamentally reframed understanding of the neurophysiology of non-nutritive sweeteners (NNS) (Margolskee et al., 2007). Enteroendocrine L cells express T1R3, and NNS binding activates G-protein-coupled pathways that trigger glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) release (Jang et al., 2007). Chronic NNS exposure, however, desensitizes these receptors, attenuating incretin secretion and impairing glucose-stimulated insulin release (Suez et al., 2022).

Within the hypothalamus, sucralose reduces T1R2 expression by nearly 300 %, indicating that high-affinity sweeteners disrupt central nutrient-sensing and energy-balance regulation (Simon et al., 2021). Acesulfame-K (Ace-K) uniquely crosses the blood–brain barrier (BBB) and accumulates in neural tissue (Cong et al., 2013). Chronic exposure suppresses hippocampal GLUT1 expression, inhibits glycolysis, and decreases ATP production (Cong et al., 2013). This neurometabolic stress leads to deficits in learning and memory without affecting locomotion or anxiety. Mechanistically, Ace-K activates AMPK while suppressing Akt/ERK and BDNF/TrkB signaling, demonstrating that some NNS act as direct neurotoxins independent of microbial mediation (Cong et al., 2013).

8.2 Indirect Neuroinflammation via the Microbiome

Even NNS that do not penetrate the central nervous system can promote neuroinflammation through microbial intermediates. Dysbiosis triggered by saccharin or sucralose elevates fecal LPS and flagellin, activating TLR4 signaling in intestinal immune cells (Chassaing et al., 2015; Cong et al.,

2013). The resulting systemic inflammation compromises BBB integrity. In mice, consumption of emulsifiers such as carboxymethylcellulose and polysorbate-80 reduces tight-junction proteins (ZO-1, occludin) and increases brain concentrations of the neurotoxic bile acid deoxycholic acid (DCA), accelerating age-related cognitive decline (Chassaing et al., 2015). The ensuing neuroinflammatory cascade involves microglial activation, astrocytosis, cytokine release (IL-1β, TNF-α), and synaptic dysfunction (Chassaing et al., 2015).

8.3 Systemic Consequences: Metabolic, Muscular, and Neurobehavioral Outcomes

8.3.1 Insulin Resistance and Metabolic Endotoxemia

Across synthetic NNS and polyols, a convergent mechanism emerges: microbiome alteration → barrier compromise → metabolic endotoxemia → insulin resistance (Panyod et al., 2024; Suez et al., 2022). Dysbiotic communities enriched in *Proteobacteria* and *Bacteroides* generate greater quantities of LPS and related pro-inflammatory ligands, while the loss of butyrate-producing taxa such as *Faecalibacterium* and *Roseburia* weakens tight-junction integrity and mucosal immune tolerance (Camilleri, 2021; Panyod et al., 2024).

Translocation of LPS and other microbial components into circulation activates TLR4/NF-κB signaling in hepatocytes, skeletal muscle, and adipose tissue, sustaining chronic low-grade inflammation and insulin resistance even at intake levels within current acceptable daily intake (ADI) limits (Arnold et al., 2022; Panyod et al., 2024; Suez et al., 2022). These effects demonstrate that metabolic disruption from NNS is context- and host-dependent rather than purely dose-dependent.

8.3.2 Sarcopenia and Anabolic Resistance

Sweetener-driven endotoxemia directly impacts skeletal muscle physiology. Elevated LPS and cytokines upregulate atrophy-related genes (*MuRF1*, *Atrogin-1*) while suppressing mTOR signaling, producing anabolic resistance, a state in which muscle-protein synthesis is blunted despite adequate amino-acid availability (Arnold et al., 2022; Panyod et al., 2024). Chronic exposure fosters sarcopenia, frailty, and insulin-resistant metabolic syndrome independent of changes in adiposity.

8.3.3 Neuroinflammation, Blood–Brain Barrier Breakdown, and Cognitive Impairment

The gut–brain axis represents a critical secondary target of sweetener-induced dysbiosis. Elevated systemic LPS and cytokines degrade BBB integrity, permitting entry of inflammatory mediators—and, in certain cases, the sweeteners themselves—into the brain (Burke & Small, 2015; Cong et al., 2013). Ace-K accumulates in cortical and hippocampal tissue, where it impairs glycolysis, depletes ATP, and disrupts TrkB–BDNF neurotrophic signaling (Cong et al., 2013).

Endotoxemia and neuroinflammation caused by steviol glycosides and polyols similarly damage hippocampal function and synaptic plasticity (Arnold et al., 2022; Tsan et al., 2022). Clinically, these processes manifest as hippocampal-dependent memory deficits, disrupted reward processing, and heightened vulnerability to mood and cognitive disorders (Cong et al., 2013; Frank et al., 2013; Holder et al., 2019).

8.3.4 Reward-Center Dysregulation and Mood Disorders

Sweeteners that decouple sweet taste from caloric value distort the predictive relationship between sweetness and energy intake. Functional-MRI studies show that NNS elicit weaker activation of mesolimbic reward regions (ventral striatum, nucleus accumbens) compared with caloric sugars—

particularly when consumed repeatedly in energy-free contexts (Frank et al., 2013). This prediction error promotes compensatory reward-seeking and elevated overall caloric consumption.

Concurrently, microbiome-mediated neuroinflammation and BBB compromise perturb central dopamine and serotonin pathways, fostering reward deficiency, depressive affect, and anxiety-like behaviors (Holder et al., 2019; Miller & Branscum, 2021). Altered ΔFosB expression in mesolimbic circuits following chronic NNS exposure supports the hypothesis that these compounds engrave maladaptive motivational patterns akin to substance-use neuroplasticity (Salaya-Velazquez et al., 2020).

9. Discussion

9.1 Revisiting the Paradigm of "Metabolic Inertness"

The collective evidence presented across human, animal, and ex vivo studies compels a fundamental reassessment of how non-nutritive and low-calorie sweeteners are classified. Historically labeled "metabolically inert," these compounds were evaluated largely through the lens of caloric content and acute toxicity. However, the emergence of microbiome science and metabolomics has revealed that many sweeteners, synthetic, natural, or polyol-based, act as bioactive xenobiotics that reshape host–microbe interactions and downstream physiology (Suez et al., 2022; Conz et al., 2023). Their influence extends beyond glucose regulation, impacting bile-acid turnover, short-chain-fatty-acid (SCFA) production, immune activation, and neuroendocrine signaling.

The traditional calorie-centric safety framework fails to account for these systems-level perturbations. Rather than acting as passive sugar substitutes, non-nutritive sweeteners alter molecular communication between microbes and host tissues, producing effects that are dose-dependent, host-specific, and temporally dynamic. This realization demands a new interpretive model, one grounded in systems biology rather than reductionist toxicology.

9.2 Individual Variability and the Microbiome “Responsiveness Hub”

The heterogeneity of sweetener responses observed across human trials, especially those by Suez et al. (2014, 2022)—underscores the microbiome’s role as a “responsiveness hub.” Within this framework, the metabolic consequences of sweetener exposure depend on baseline microbial composition, functional gene expression, and ecological resilience. Individuals harboring dysbiotic or inflammatory microbiomes exhibit stronger glycemic impairments and inflammatory responses, while metabolically resilient hosts often display transient or neutral effects.

This interindividual variability explains why population-level dietary guidelines can obscure clinically meaningful risks. The same sweetener that appears benign in controlled short-term studies among healthy adults may exacerbate insulin resistance or neuroinflammation in vulnerable populations. Such findings highlight the necessity of microbiome-stratified nutrition research, in which metabolic outcomes are interpreted relative to microbial composition and function rather than population averages.

9.3 Mechanistic Convergence: From Dysbiosis to Systemic Inflammation

Despite chemical diversity, synthetic, natural, and polyol sweeteners share a convergent pathophysiological cascade: microbiome alteration → barrier compromise → LPS translocation → low-grade inflammation → insulin resistance. Saccharin and sucralose directly suppress butyrogenic taxa, enrich LPS-producing *Bacteroides* and *Proteobacteria*, and increase hepatic pro-inflammatory signaling (Suez et al., 2022; Panyod et al., 2024). Polyols amplify this process through osmotic stress and mucus disruption, while steviol glycosides and mogrosides achieve similar outcomes via microbial

biotransformation into cytotoxic aglycones (Conz et al., 2023; Wang, 2021). The systemic consequences include metabolic endotoxemia, skeletal-muscle anabolic resistance, and neuroinflammatory propagation across the gut–brain axis. This unified mechanistic model reframes sweeteners not as isolated dietary compounds but as modulators of a broader host–microbe inflammatory network, with implications for metabolic, muscular, and cognitive health.

9.4 Implications for Clinical Practice and Public Health

Clinically, the emerging evidence suggests that sweetness itself not merely sugar, carries biological cost. Artificial and “natural” sweeteners can impair glucose tolerance, alter gut barrier function, and influence reward circuitry even when caloric load is negligible. For clinicians, this necessitates a nuanced approach that distinguishes between short-term glycemic substitution and long-term metabolic impact.

Patients with obesity, diabetes, or gastrointestinal inflammation are particularly susceptible to adverse outcomes. Thus, while NNS may serve as transitional tools for sugar reduction, gradual desensitization to sweetness and emphasis on unsweetened beverages remain the most sustainable strategies. From a population perspective, policy frameworks should integrate microbiome-relevant endpoints into risk assessments—such as microbial gene expression, LPS activity, and personalized glycemic responses—rather than relying solely on ADI thresholds derived from animal toxicology.

9.5 Regulatory and Research Imperatives

The World Health Organization’s (2023) recommendation against the routine use of non-sugar sweeteners for weight management represents an inflection point in public-health nutrition. However, current regulatory systems remain ill-equipped to evaluate chronic microbiome-mediated risks. Future safety evaluations should:

1. Incorporate **metaproteomic and metatranscriptomic profiling** to detect subclinical functional dysbiosis.
2. Employ **longitudinal, microbiome-stratified human trials** to capture delayed and individualized metabolic effects.
3. Reassess the scientific validity of “natural” and “prebiotic” labeling, which often obscures complex microbial toxicity.
4. Encourage cross-disciplinary collaboration between microbiologists, nutritionists, and toxicologists to establish standardized microbiome safety testing.

Such integrative approaches can bridge the gap between mechanistic insight and regulatory oversight, ensuring that food safety frameworks evolve in step with modern biological understanding.

9.6 Limitations and the Path Forward

Despite compelling mechanistic and translational evidence, several limitations temper interpretation. Most human studies remain short in duration and focus on healthy volunteers rather than metabolically compromised individuals. Furthermore, dose equivalence across sweeteners is difficult to standardize, and real-world exposures often involve mixtures within ultra-processed foods. Long-term, randomized, microbiome-aware clinical trials remain essential to clarify chronic effects, dose–response relationships, and reversibility of dysbiosis.

Nevertheless, the cumulative literature supports a paradigm shift: sweeteners are active participants in metabolic and neural regulation, not passive sugar substitutes. Recognizing their bioactivity opens new frontiers for targeted intervention, where understanding the microbiome’s role can inform personalized nutrition and public-health strategy alike.

10. Conclusion

The accumulated evidence no longer supports the assumption that non-nutritive and low-calorie sweeteners are metabolically inert alternatives to sugar. Controlled human and mechanistic studies demonstrate that both synthetic and so-called “natural” sweeteners interact dynamically with the gut microbiome, influencing host glucose regulation, inflammatory signaling, and neuroendocrine balance (Suez et al., 2022; Burke & Small, 2015). These effects are highly individualized, reflecting differences in microbial composition, metabolic health, and cumulative exposure. Thus, sweetness is not merely a sensory experience but a biological modifier capable of reshaping host–microbe homeostasis.

The 2023 World Health Organization (WHO) guideline on non-sugar sweeteners underscores this paradigm shift. After reviewing more than 280 studies, the WHO concluded that the purported weight-loss benefits of these compounds are trivial and that evidence suggests possible long-term risks for metabolic and cardiovascular disease (Khan et al., 2023). This conclusion challenges the calorie-centric framework that has historically defined “safety.” Non-nutritive sweeteners cannot be evaluated solely by energy content or acute toxicity thresholds; their biological effects must be interpreted through the lens of microbial metabolism and interindividual variability.

Mechanistically, a convergent cascade links diverse sweeteners to systemic dysfunction: microbiome alteration → barrier compromise → metabolic endotoxemia → chronic inflammation → insulin resistance (Suez et al., 2022; Panyod et al., 2024). Saccharin and sucralose in particular induce microbiome-dependent glucose intolerance that can be transmitted to germ-free mice via fecal microbiota transfer, establishing causal mediation by the gut ecosystem (Suez et al., 2014, 2022). Parallel findings with polyols show that sugar alcohols—though marketed as “prebiotic”—create osmotic stress, impair short-chain fatty acid production, and enrich lipopolysaccharide-producing

pathobionts such as *Bacteroides* and *Proteobacteria* (Conz et al., 2023; Wang, 2021). Together, these pathways contribute to low-grade endotoxemia, insulin resistance, and, through suppression of mTOR signaling, anabolic resistance and sarcopenia (Arnold et al., 2022; Panyod et al., 2024). Neuroinflammatory sequelae further connect sweetener-induced dysbiosis to blood–brain-barrier compromise and cognitive decline (Cong et al., 2013; Holder et al., 2019).

Importantly, evidence supporting microbiome "benefits" of certain compounds such as stevia or monk-fruit glycosides derives largely from short-term trials in healthy, metabolically resilient adults (Ruiz-Ojeda et al., 2019). Such findings cannot be generalized to populations with obesity, insulin resistance, or gut barrier dysfunction, in whom even subtle perturbations may amplify systemic inflammation. Regulatory and industry claims that "natural" equates to "safe" are therefore scientifically untenable. As toxicology evolves toward precision nutrition, safety assessment must account for host–microbe interactions, not merely chemical origin or caloric yield.

Clinically, the most prudent guidance aligns with the WHO's recommendation for cautious, minimal use of non-sugar sweeteners and preference for water or unsweetened beverages. For patients accustomed to high sweetness exposure, gradual desensitization—reducing the intensity of sweet flavor over time, may help recalibrate reward pathways and improve dietary self-regulation. From a policy standpoint, future evaluations should integrate functional biomarkers such as microbial gene expression, LPS activity, and individualized glycemic variability. Only through this systems-level approach can risk assessment capture the full metabolic and neurobiological consequences of chronic sweetener exposure.

In summary, the current body of evidence reframes artificial and low-calorie sweeteners not as neutral sugar substitutes but as bioactive dietary agents with microbiome-mediated potential to disrupt metabolic and neural homeostasis. Their safety is context-dependent, their benefits marginal, and their

risks magnified in vulnerable populations. Until long-term, microbiome-stratified trials establish true inertness, the guiding principle should remain one of informed restraint: favor water, minimize chemical additives, and prioritize whole, unprocessed foods that preserve the integrity of the human–microbial partnership.

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