Mechanistic Links Between Non-Nutritive Sweeteners, Intestinal Permeability, and Metabolic Endotoxemia

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1. Executive Summary

Non-nutritive sweeteners (NNS), including plant-derived sweeteners such as steviol glycosides (stevia) and mogrosides (monk fruit), are widely consumed as sugar substitutes. While regulatory bodies deem them toxicologically safe at established intake levels, their biological inertness is increasingly being challenged. This reassessment is driven by the 2023 World Health Organization (WHO) guideline, which conditionally recommends against using non-sugar sweeteners for weight control or disease prevention due to a lack of evidence for long-term benefit and the possibility of undesirable metabolic effects (WHO, 2023).

This paper synthesizes emerging evidence demonstrating that natural NNS function as bioactive compounds capable of reshaping the gut ecosystem. Mechanistic studies indicate that these sweeteners are metabolized by colonic bacteria, leading to functional microbial shifts that include suppression of pathways involved in butyrate synthesis, a key short-chain fatty acid (SCFA) essential for maintaining gut barrier integrity, and upregulation of genes associated with lipopolysaccharide (LPS) biosynthesis (Wang, 2021).

Furthermore, human randomized trials have established that, for certain NNS such as saccharin and sucralose, NNS-induced microbiome alterations can be causally linked to personalized impairments in glucose tolerance (Suez et al., 2022).

While some recent short-term trials in healthy adults report neutral effects on overall microbiome composition, these studies often lack the functional endpoints required to detect subtle metabolic alterations. The discrepancy between mechanistic risk and some clinical findings highlights a methodological gap, particularly the absence of intestinal permeability and endotoxemia measurements in human NNS research.

Given the established role of metabolic endotoxemia in driving insulin resistance and chronic inflammation (Cani et al., 2007), the biological activity of these sweeteners within the gut warrants a more cautious public health perspective aligned with the WHO's position.

2. Introduction: Public Health Context and the WHO Position

Cardiometabolic diseases, driven largely by the rising global prevalence of obesity and insulin resistance, represent a major public health challenge. In response, NNS have been widely promoted as tools for reducing the caloric and glycemic burden of modern diets. However, their long-term efficacy and safety remain subjects of ongoing scientific and regulatory debate.

In May 2023, the World Health Organization issued a formal guideline advising that non-sugar sweeteners not be used as a means of achieving weight control or reducing the risk of non-communicable diseases (WHO, 2023). This conditional recommendation was based on a systematic review of more than 280 studies. The review concluded that although short-term randomized controlled trials sometimes demonstrate modest reductions in body weight, long-term observational data associate habitual NNS consumption with increased risks of type 2 diabetes, cardiovascular disease, and mortality (Rios-Leyvraz & Montez, 2022).

This shift signals an important change in perspective: rather than being viewed as metabolically inert sugar replacements, non-nutritive sweeteners are increasingly recognized as biologically active

dietary components whose long-term systemic effects remain insufficiently understood. Understanding the biological plausibility of these associations requires examining the mechanistic pathways through which NNS interact with the gut microbiome and host metabolic systems.

3. Mechanistic Pathways: How NNS Reshape the Gut Ecosystem

The biological activity of NNS, including stevia and monk fruit, originates from their interaction with the gut microbiota. Because these compounds are largely unabsorbed in the small intestine, they reach the colon where they become substrates for microbial metabolism, thereby altering the gut environment.

3.1 Colonic Metabolism and Functional Microbial Shifts

Steviol glycosides (stevia) and mogrosides (monk fruit) are too large to be absorbed intact. Instead, they require microbial enzymes, particularly β-glucosidases produced by species such as *Bacteroides,* to cleave their sugar moieties and release the core aglycone molecules (steviol or mogroside aglycones) for absorption (Ruiz-Ojeda et al., 2019). This metabolic dependence makes the gut microbiome an obligatory partner in the digestion of these compounds.

Recent microbiome research highlights a critical distinction between taxonomic composition (which organisms are present) and functional microbial activity (what those organisms are doing). While some human trials report minimal changes in the relative abundance of major bacterial groups following dietary interventions, compositional stability does not necessarily reflect functional stability within the microbial ecosystem. Microbial communities exhibit substantial functional redundancy and strain-level metabolic diversity, meaning that closely related taxa can perform markedly different biochemical roles within the same ecological niche. As a result, taxonomic analyses based on 16S

rRNA sequencing may overlook meaningful shifts in microbial metabolism, gene expression, and ecological interactions (Shade & Handelsman, 2012; Costello et al., 2012; Zhao, 2025).

From an ecological perspective, microbial communities can maintain similar taxonomic profiles while undergoing substantial functional remodeling at the level of metabolic pathways, enzymatic activity, and host–microbe signaling. Advances in multi-omics technologies, including metaproteomics, metagenomics, and metabolomics, have therefore revealed that dietary exposures can reprogram microbial metabolic networks even when broad compositional metrics remain relatively unchanged. In the context of non-nutritive sweeteners, functional analyses have demonstrated alterations in pathways related to short-chain fatty acid metabolism, carbohydrate utilization, and inflammatory signaling despite limited detectable taxonomic shifts in microbial community structure (Wang, 2021).

An ex vivo study using the RapidAIM human microbiome culturing platform demonstrated that both stevia and monk fruit extracts induced significant changes in microbial enzyme expression related to short-chain fatty acid metabolism and other central metabolic pathways, even when large compositional shifts were not observed (Wang, 2021). This finding illustrates that an apparently "stable" microbiome composition can mask substantial functional remodeling within the microbial ecosystem.

3.2 Butyrate Suppression and Barrier Dysfunction

Butyrate is the primary energy source for colonocytes and is essential for maintaining a healthy intestinal barrier. It enhances the expression of tight junction proteins, stimulates mucus production, and exerts potent anti-inflammatory effects (Bischoff et al., 2014).

The ex vivo metaproteomic study by Wang (2021) provided mechanistic evidence that steviol glycosides suppress butyryl-CoA:acetate CoA-transferase, a key enzyme in the final step of microbial

butyrate synthesis, by more than 40%. These functional changes were associated with depletion of keystone butyrate-producing taxa such as *Faecalibacterium prausnitzii* and *Roseburia*.

Monk fruit extract demonstrated a similar pattern in the RapidAIM system, with suppression of butyrate-associated pathways and enrichment of LPS-related microbial functions even when overall compositional shifts remained modest. Reduced butyrate production may compromise intestinal barrier integrity, increasing susceptibility to inflammatory signaling.

3.3 Lipopolysaccharide (LPS) Signaling and Metabolic Endotoxemia

Metabolic endotoxemia refers to a chronic, low-grade elevation of circulating lipopolysaccharide derived from Gram-negative bacteria in the gut. Even modest increases in circulating LPS can activate Toll-like receptor 4 (TLR4) signaling, initiating inflammatory pathways that contribute to insulin resistance and metabolic disease (Cani et al., 2007). Under normal physiological conditions, the intestinal epithelial barrier limits translocation of bacterial endotoxins into systemic circulation. Mechanistic evidence suggests NNS may influence this pathway through two complementary mechanisms.

Increased LPS Production:

Ex vivo studies demonstrate that exposure to NNS such as stevia and monk fruit can increase microbial gene pathways associated with LPS biosynthesis (Wang, 2021). Certain dietary emulsifiers commonly consumed alongside sweeteners have also been shown to promote the growth of LPS-rich Proteobacteria (Naimi et al., 2021).

Increased Permeability:

Suppression of butyrate synthesis may weaken epithelial barrier integrity, increasing permeability to endotoxins produced in the gut lumen. Additionally, experimental research indicates that the

artificial sweetener neotame can directly increase epithelial permeability and induce cell death in intestinal epithelial models (Shil et al., 2023). Although this direct permeability effect has not yet been demonstrated for stevia in human trials, the mechanistic pathway, of reduced butyrate production, weakened barrier function, and increased LPS translocation, remains biologically plausible one.

3.4 Enteroendocrine and Receptor-Mediated Effects

Beyond microbiome-mediated pathways, non-nutritive sweeteners may interact directly with host physiology. Sweet taste receptors (T1R2/T1R3) are expressed not only on the tongue, but also on enteroendocrine cells within the gastrointestinal tract. Activation of these receptors influences secretion of metabolic hormones including glucagon-like peptide-1 (GLP-1), which plays a central role in glucose regulation and appetite signaling.

Although findings remain mixed, chronic non-caloric stimulation of these receptors may disrupt the coordinated signaling between taste perception, nutrient sensing, and hormonal responses involved in metabolic regulation (Burke & Small, 2015).

4. Re-evaluating the Human Clinical Evidence

The apparent conflict between mechanistic concerns and neutral findings in some human trials can largely be explained by differences in study design, analytical depth, and the biological endpoints being measured. Several recent randomized trials in metabolically healthy adults report that daily consumption of stevia for 4–12 weeks does not significantly alter overall gut microbiota α- or β-diversity or fecal short-chain fatty acid concentrations (Kwok et al., 2024; Singh et al., 2024). Similarly, the large SWEET trial observed only modest microbiome compositional shifts in overweight individuals consuming non-nutritive sweeteners over a one-year period (Pang et al., 2025).

However, these findings must be interpreted within the methodological limitations of the analytical approaches employed. Most of these studies relied primarily on 16S rRNA gene sequencing, a technique designed to characterize microbial taxonomic composition rather than microbial functional activity. While useful for identifying broad shifts in bacterial populations, 16S profiling cannot capture strain-level metabolic differences, gene expression changes, or alterations in microbial biochemical pathways that may influence host physiology.

Consequently, the absence of major compositional shifts should not be interpreted as evidence of biological neutrality. As discussed earlier, microbial communities can maintain relatively stable taxonomic profiles while undergoing substantial functional remodeling in metabolic pathways, enzymatic activity, and host-microbe signaling. Detecting these changes requires higher-resolution approaches such as shotgun metagenomics, metaproteomics, and metabolomics, which remain underutilized in many clinical nutrition trials.

Importantly, most existing randomized trials examining stevia or other non-nutritive sweeteners have also not measured key physiological endpoints predicted by the mechanistic literature, including intestinal permeability, circulating lipopolysaccharide (LPS), or other markers of metabolic endotoxemia. Without these measurements, it remains difficult to determine whether subtle microbiome alterations translate into meaningful changes in gut barrier integrity or inflammatory signaling.

In contrast, the landmark randomized controlled trial by Suez et al. (2022) employed shotgun metagenomics and a personalized analytical approach. The study demonstrated that while stevia did not impair glycemic control at the cohort level, exposure to multiple sweeteners, including saccharin, sucralose, aspartame, and stevia, produced distinct alterations in microbial metabolic function.

Crucially, the researchers demonstrated causality by transplanting fecal microbiota from human NNS "responders" into germ-free mice. The recipient animals developed the same glycemic impairments observed in their human donors, confirming that microbiome alterations induced by NNS can directly influence host metabolic responses.

5. Funding Structures and Research Integrity

Interpretation of nutrition research is frequently complicated by funding-related bias. Systematic reviews have demonstrated that industry-sponsored studies are significantly more likely to report conclusions favorable to sponsor interests (Lesser et al., 2007; Lundh et al., 2017). Although many recent trials investigating stevia disclose industry funding or product provision, this context may influence methodological choices such as reliance on less-sensitive endpoints (e.g., taxonomic sequencing rather than barrier function markers). Publicly funded studies, including the SWEET trial and the research conducted by Suez et al. (2022), provide important counterpoints and highlight the importance of independently funded investigations capable of examining potentially undesirable effects without conflicts of interest.

6. Vulnerable Populations: A Magnified Risk

The metabolic impact of NNS may not be uniform across all populations. Individuals with metabolic syndrome, type 2 diabetes, or non-alcoholic fatty liver disease frequently exhibit pre-existing gut dysbiosis, reduced butyrate production, and compromised intestinal barrier integrity (Bischoff et al., 2014). In such individuals, a weakened intestinal barrier may provide less protection against LPS translocation, while a chronically activated immune system may amplify inflammatory responses.

Consequently, exposures that appear metabolically neutral in healthy populations could potentially exacerbate inflammation and insulin resistance in metabolically vulnerable individuals. To

date, few long-term NNS trials have specifically investigated these populations with endpoints focused on barrier function or endotoxemia.

7. Integrated Interpretation

Current evidence does not indicate that stevia or monk fruit are acutely toxic at commonly consumed doses. Rather, the available data suggest that these compounds function as bioactive microbial modulators capable of interacting with the gut ecosystem. Mechanistic and ex vivo studies consistently demonstrate that non-nutritive sweeteners can influence microbial metabolic pathways involved in short-chain fatty acid production, inflammatory signaling, and intestinal barrier maintenance.

The apparent discrepancy between neutral findings in some human trials and concerning signals from mechanistic research is most plausibly explained by differences in analytical depth and biological endpoints. This was discussed above, but briefly many clinical trials evaluate microbiome composition using 16S rRNA sequencing and broad diversity metrics, which provide limited insight into microbial metabolic activity. As microbiome ecology research has emphasized, taxonomic stability does not necessarily imply functional stability, and microbial communities can undergo significant metabolic remodeling even when community composition appears largely unchanged.

Taken together, the current evidence base suggests that non-nutritive sweeteners should be understood not as inert sugar substitutes, but as dietary compounds capable of modulating host–microbe interactions. The World Health Organization’s precautionary recommendation is therefore consistent with the present scientific landscape, which combines plausible biological mechanisms, emerging functional microbiome evidence, and a relative lack of long-term human data demonstrating sustained metabolic benefit.

8. Conclusion

The classification of stevia and monk fruit as biologically inert sweeteners is increasingly inconsistent with emerging microbiome research. Mechanistic and ex vivo studies demonstrate that these compounds interact with intestinal microbial ecosystems and can alter pathways involved in short-chain fatty acid production, intestinal barrier maintenance, and lipopolysaccharide signaling.

Human clinical trials further indicate that, for certain sweeteners such as saccharin and sucralose, microbiome-mediated changes can contribute to individualized impairments in glucose tolerance (Suez et al., 2022).

However, definitive human evidence demonstrating increased intestinal permeability or metabolic endotoxemia following consumption of plant-derived sweeteners remains limited.

Importantly, the absence of long-term in vivo evidence demonstrating harm should not be interpreted as confirmation of biological neutrality. Many existing trials are short in duration, conducted primarily in metabolically healthy individuals, and rely heavily on microbiome compositional metrics rather than functional analyses or barrier integrity measurements.

Given the WHO's recommendation, the strong mechanistic rationale, and the lack of long-term safety data regarding microbiome-mediated endpoints, a prudent public health strategy should prioritize reducing overall sweet taste preference rather than promoting widespread substitution with non-nutritive sweeteners.

Future research must move beyond compositional microbiome analyses and incorporate functional metagenomics, metabolomics, and direct measurements of intestinal permeability and circulating lipopolysaccharide, particularly in metabolically vulnerable populations.

From a clinical perspective, healthcare practitioners operate under the ethical principle of non-maleficence, the obligation to avoid causing harm. Establishing definitive long-term safety for microbiome-active compounds may require decades of observation, particularly for chronic diseases with long latency periods such as obesity, insulin resistance, and cardiovascular disease. In the presence of plausible biological mechanisms and unresolved scientific uncertainty, a precautionary formulation strategy is justified.

For this reason, TLC PureOrigin™ has elected not to include isolated non-nutritive sweeteners in its product formulations. This decision does not assert that these compounds are definitively harmful; rather, it reflects a conservative interpretation of the current evidence base. Many modern sweeteners represent highly purified extracts removed from the fiber-rich plant matrices in which they naturally occur, a context that likely influences how these compounds interact with the gut microbiome.

Until long-term human research clarifies these interactions, prioritizing minimally processed ingredients and avoiding isolated non-nutritive sweeteners represents a precautionary and clinically responsible design choice.

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