*https://doi.org/10.31925/farmacia.2024.6.2* **REVIEW**

# ADDITIVE LOW CALORIE SWEETENERS AND THEIR IMPACT ON HEALTH

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**Abstract**

Concerns about the health effects of sugary foods and drinks have led to an increased consumption of sugar-free alternatives. Artificial sweeteners, often hundreds of times sweeter than sugar, are widely used as substitutes. Despite being considered safe, their impact on the gut microbiota, glucose intolerance and sweet taste receptors remains controversial. Emerging evidence suggests that artificial sweeteners like sucralose, saccharin and aspartame may disrupt the gut microbiota, leading to reduced diversity and balance. This disruption, transmissible through faecal transplantation, has been linked to glucose intolerance, a risk factor for metabolic disorders such as insulin resistance and obesity. Given the growing evidence connecting gut microbiota to metabolic health, understanding the effects of sweeteners on the microbiome and overall body homeostasis is essential. This review highlights the need for more in-depth research on the health implications of sweetener consumption to guide informed dietary choices.

**Rezumat**

Intensificarea preocupărilor legate de efectele alimentelor și băuturilor zaharoase asupra sănătății a condus la o extindere a consumului de produse fără zahăr, în care îndulcitorii artificiali sunt utilizați ca substituenți. Acești compuși, de sute de ori mai dulci decât zahărul, sunt considerați siguri pentru consumul uman, însă impactul lor asupra microbiotei intestinale, toleranței la glucoză și a receptorilor gustativi pentru dulce rămâne un subiect de dezbatere. Studii recente indică faptul că îndulcitorii artificiali, precum sucraloza, zaharina și aspartamul, pot afecta diversitatea și echilibrul microbiotei intestinale, având potențialul de a provoca modificări transmisibile, conform experimentelor de transplant fecal. Aceste perturbări ale microbiotei au fost corelate cu apariția intoleranței la glucoză, un factor de risc cunoscut pentru dezvoltarea tulburărilor metabolice, inclusiv rezistența la insulină și obezitatea. Având în vedere legătura tot mai bine documentată dintre metabolism și microbiota intestinală, se impune o evaluare detaliată a efectelor îndulcitorilor asupra acesteia și, implicit, asupra homeostaziei organismului. Acest articol evidențiază necesitatea unor cercetări suplimentare, riguroase și multidisciplinare, pentru a clarifica efectele îndulcitorilor hipocalorici și pentru a fundamenta recomandări dietetice mai informate.

**Keywords**: additive sweeteners, gut microbiota, human health, dysbiosis, mechanism

## Introduction

Sweet-flavoured foods are generally appealing and are known to stimulate appetite, as highlighted by McCaughey's preloading research in 2008 [1]. There has been a significant increase in the global consumption of sugars like glucose and sucrose, raising concerns about their adverse health effects [2]. Consequently, the use of sweeteners as sugar substitutes has grown, offering a low-energy alternative that replicates sugar's sweet taste [3, 4]. Many individuals turn to sweeteners for weight management, believing they aid in weight loss. The market offers various sweeteners including sucralose, saccharin, aspartame, acesulfame potassium and stevia. Sucralose, a synthetic sweetener, is prevalent in numerous products and holds a significant position in the artificial sweetener market. Stevia, a natural sweetener derived from the *Stevia rebaudiana* plant's steviol glycosides, is 200 - 300 times sweeter than sucrose and has recently entered the market [5, 6]. However, emerging studies suggest potential unexpected health effects of sweeteners, linking their consumption with glucose intolerance and increased weight gain in both animal and human studies [7-10].

The gut microbiota, a diverse bacterial community, is essential for various physiological functions including metabolism, and its imbalance can lead to obesity and insulin resistance [11, 12]. Recent findings indicate that sweeteners significantly affect gut bacterial balance. Their health effects remain a topic of active debate,

even though they have been approved by regulatory bodies like United States Food and Drug Administration (USFDA) [3]. Research examining the link between sweeteners in beverages and obesity and diabetes risks has produced mixed results [13-20]. Additionally, there are indications that common sweeteners might promote glucose intolerance through changes in gut microbiota structure and function [21]. Livesey in 2003 reported that polyol sweeteners like the Sorbitol and the Xylitol could cause gastrointestinal issues, varying based on individual sensitivity and concurrent food intake [22]. Some sweeteners have been associated with lower blood glucose levels, mainly attributed to their low carbohydrate content rather than effects on sweet taste receptors [14]. Research has shown that saccharin can alter gut bacterial balance, increasing aerobic and decreasing anaerobic bacteria, while sucralose may affect gut microbiota in living organisms [23, 24]. Palmnäs *et al.* found that aspartame significantly alters intestinal bacteria in rats, increasing the abundance of Enterobacteriaceae and *Clostridium leptum* [25]. Suez *et al*. discovered that saccharin, aspartame and sucralose disrupt gut bacterial balance in mice, leading to dysbiosis [26].

This review endeavours to comprehensively summarize and evaluate the latest research on the interplay between various sweeteners and the gut microbiota, aiming to offer a detailed perspective on how these substances might affect gut homeostasis. It also critically examines the diverse range of sweeteners, including both artificial and natural, and their consumption patterns, assessing their regulatory status and perceived safety. The review delves deep into the physiological impacts these sweeteners have on the gut microbiota, exploring how they influence the development and composition of this vital microbial community and their broader implications for metabolic health. Additionally, it highlights the complex relationship between the gut microbiota and overall health, emphasizing the need for more research work in response to the wide use of these sweeteners. The ultimate review's goal is to bridge the gap in understanding the intricate connections between diet, gut health and metabolic disorders, providing a clearer picture of the role sweeteners play in modern nutrition and health.

## Methodology

A comprehensive literature search was conducted across PubMed and Embase (Elsevier) databases, as well as Google Scholar, to identify relevant articles on the impact of artificial sweeteners on human health. Specific search terms included “artificial sweeteners”, “gut microbiota”, “human health”, “physiology” and “GI microbiota.” To ensure the search was both comprehensive and focused, we included articles published from (starting year, *e.g.*, 2000) onwards, emphasizing recent findings. Articles were selected if they directly investigated the effects of artificial or natural sweeteners on the gut microbiota, provided mechanistic insights into their impact on metabolic health, and were peer-reviewed research or review articles. Studies were excluded if they did not focus on sweeteners or gut microbiota, were not peer-reviewed, or focused solely on non-human models without clear translational relevance to human health. Additionally, in Google Scholar, the search was limited to article titles to manage the high volume of results, while no further restrictions were applied to the PubMed and Embase searches.

Our initial search yielded approximately 500 articles, which were subsequently screened for relevance and quality, resulting in the inclusion of 150 articles for this narrative review comprising of original research and review articles. This review aims to synthesize current evidence on the interplay between sweeteners, gut microbiota and metabolic health outcomes. The search and data extraction processes were performed by three researchers, who used predefined inclusion criteria and manually removed duplicates. In cases of disagreement during article selection, decisions were made through discussion and consensus to ensure only the most relevant studies were included. Both *in vivo* and *in vitro* studies were considered to provide a well-rounded perspective on the topic.

## Sweeteners

As the global prevalence of obesity continues to rise, the world has seen an influx of both natural and synthetic alternatives to sucrose, receiving mixed reactions for their role in weight management. Research suggests that rare sugars (Allulose and Tagatose) and non-caloric sugar alcohols (Erythritol, Xylitol and Mannitol) can be fermented in the large intestine by the gut microbiota [27, 28]. This suggests a possible fermentation of sweeteners by the gut microbiota, yet this area remains under-researched. Artificial sweeteners, introduced over a century ago and first appearing in culinary use in the 18$^{th}$ century, were bent to provide sweetness without the high caloric content of sugar. Their popularity has surged since the turn of the century due to their low-calorie nature and perceived health benefits, such as aiding in weight loss and maintaining normal blood sugar levels [29]. They are commonly used by people with type 2 diabetes (T2D), glucose intolerance and those seeking weight loss, and are found in a variety of products like cereals, beverages and desserts [29]. Research shows that over 20% of overweight or obese individuals and 10% of those at a healthy weight consume diet drinks with low-calorie sweeteners daily [30]. Sylvetsky *et al*. in 2017 observed a notable escalation in the consumption of sweeteners within the United States. They reported that from 1999 to 2000, there was an increase of over 50% in sweetener intake among adults. Even more striking was the surge in consumption among children and adolescents, which nearly tripled during the same period [31]. The US

Food and Drug Administration has approved six artificial sweeteners, saccharin, acesulfame potassium (AceK), aspartame, sucralose, advantame and Neotame along with two natural options, stevia and monk fruit extract (Table I).

Discovered in 1879, saccharin, a calorie-free artificial sweetener, is often blended with other sweeteners like aspartame to mitigate its slightly acidic taste at higher concentrations [32]. Aspartame, identified in 1967 and known for its bitter aftertaste, is a methyl ester of a dipeptide made from aspartic acid and L-phenyl-alanine. It is frequently combined with sweeteners like sucralose, Ace-K and cyclamates for enhanced flavour [33, 34]. Acesulfame potassium, another sweetener discovered in the same year, is a potassium salt of asulfame, widely used in beverages, confectionaries and frozen desserts. Discovered in 1976, sucralose bears a chemical resemblance to sucrose and is known for its water solubility, heat stability and minimal impact on pH and viscosity [35, 36]. Neotame, related to aspartame chemically, was discovered in the 1980s and is known for its potent sweetness [33]. Advantame, the latest artificial sweetener approved for general use and flavour enhancement, was introduced in 2017 [37]. Natural sweeteners include extracts from the Luo Han Guo fruit and leaves of the stevia plant (Steviol glycosides), primarily found in Paraguay and Portugal. These steviol glycosides are made up of a steviol structure bound to various sugar molecules, contributing to their beneficial properties [37]. The sweetness of Luo Han Guo, known scientifically as *Siraitia grosvenorii* (Swingle) and native to Southern China, stems from its mogrosides, which are glycosylated cucurbitane-type triterpenoids [38, 39].

**Table I**

Some of the artificial sweeteners approved by FDA and their effects on the body

| Artificial sweetener | Natural/Artificial | Effects |
|---|---|---|
| Saccharin | Artificial | Changes glucose tolerance-related metabolic pathways and causes dysbiosis |
| Aspartame | Artificial | Regardless of body mass index, reduces caloric intake and weight growth; increases fasting glucose and impairs insulin-stimulated glucose clearance. |
| Acesulfame potassium (Ace-K) | Artificial | Restricts the ability of gut bacteria to ferment glucose and changes genes that are involved in energy metabolism in bacteria. |
| Sucralose | Artificial | Promotes intestinal dysbiosis, slows bacterial growth and may either selectively block or stimulate bacterial growth. |
| Neotame | Artificial | Avoiding or maintaining a low body weight is linked to using neotame for an extended period of time. |
| Stevia (Rebaudioside A) | Natural | The gut microbiome seems to be altered. |
| Erythritol | Natural/Artificial | Has no effect on the gut microbiome, insulin levels, or plasma glucose levels. |

Polyols represent another group of sweetening agents used in foods [40]. These substances, found naturally in certain fruits and vegetables, began to be synthetically produced in the last century to address health concerns associated with the overuse of other sweeteners. Eight polyols erythritol, sorbitol, xylitol, maltitol, lactitol, mannitol, isomalt and hydrogenated starch hydrolysates have been approved for human consumption by various food safety authorities, including *Codex Alimentarius*, the European Food Safety Authority, the US Food Administration and the FSA [41-43]. Erythritol, a sugar alcohol, is unique due to its low molecular weight, being comprised of only four carbon atoms and displaying distinct physical and chemical properties. It is calorie-free and naturally present in several foods like the mushrooms, grapes, pears, wine and beer [44]. While sweeteners share a common sweet taste, their chemical compositions differ, leading to varied effects on gut microbiota and gastrointestinal (GI) tract processes, including absorption, metabolism and excretion [36]. Saccharin, for example, is absorbed 80% to 90% in the small intestines, binds to plasma proteins and is distributed throughout the body. It is primarily excreted in urine, with a smaller portion eliminated through faeces [36, 45]. Its absorption is influenced by stomach pH lower pH levels increase absorption in humans, while higher pH levels decrease it in mice and rats [36].

Research involving *in vitro* studies indicates that saccharin alters the gut microbiome, increasing Bifido-bacteria while reducing Firmicutes in a dose-responsive manner [46]. In cases of inflammatory bowel disease, a shift from anaerobic to facultative anaerobic and aerobic organisms has been noted, aligning with saccharin's bacteriostatic and microbiome-altering attributes [24, 26, 47]. Moreover, saccharin-induced dysbiosis has been linked to changes in metabolic pathways related to glucose intolerance [26]. Once in the gut, saccharin is hydrolysed into amino acids, aspartate, phenylalanine and methanol, with only small amounts of the intact molecule entering the circulation. The duodenum and jejunum absorb and process it through the standard metabolic routes [36, 45]. According to a 2014 study, aspartame affects living things in a variety of ways. It was found to raise fasting glucose levels at the same time as reducing food intake and weight gain. In addition, the study observed a phenomenon that happens regardless of changes in body weight: a decrease in the effectiveness of insulin-stimulated glucose clearance [25]. Regarding acesulfame potassium (Ace-K), the small intestine almost entirely absorbs the intact molecule, subsequently distributing it to

different body tissues *via* the bloodstream [36]. It is primarily excreted in urine, with less than one percent ending up in faeces [36]. Although only a minimal amount of Ace-K reaches the colon bacteria, *in vivo* studies indicate it can alter gut microbiota composition [36, 48]. Research suggests that Ace-K inhibits glucose fermentation by gut bacteria and affects genes related to energy metabolism [49]. These alterations are associated with weight gain, and studies have shown reduced bacterial diversity in the guts of individuals consuming Ace-K and aspartate [50].

Sucralose is largely excreted unchanged in urine and faeces [36, 51]. Mass spectrometry analyses have shown that certain sucralose molecules undergo chemical transformations in the gastrointestinal tract of rats. This indicates possible metabolism and the formation of secondary metabolites. However, the small proportion of sucralose that is absorbed is predominantly excreted in urine without undergoing any change [51]. Studies demonstrate that sucralose can promote intestinal dysbiosis, either inhibiting bacterial growth or selectively affecting bacterial development [35, 48, 51].

Approximately half of absorbed neotame is eliminated through the kidney, with the remainder excreted in faeces. No toxicity has been observed with neotame, even at doses exceeding the ADI [37]. Conversely, most advantame is converted to de-esterified advantame in the intestines before absorption. Plasma conversion of the intact advantame to ANS9801 acid occurs proportionally to its absorption rate [52]. In the upper part of the gastrointestinal tract, enzymes are ineffective in breaking down steviol glycosides [53]. However, in the colon, Bacteroides species can convert steviol glycosides into steviol [53-55]. This steviol is then absorbed and undergoes a process in the liver where it's combined with glucuronic acid, forming steviol glucuronide, which is predominantly excreted through urine. Erythritol, a polyol, is largely passed in urine after its rapid uptake by diffusion in the small intestine, spreading broadly across tissues with minimal alteration [44]. As a result, erythritol doesn't impact glucose levels in plasma or alter gut microbiota [56].

Comprehensive tests have consistently shown that erythritol poses no toxicity, carcinogenic, or reproductive hazards [37]. Common fillers like silica, calcium silicate, or maltodextrin are used in commercial sweet products to increase their bulk. An innovative approach for safer sugar consumption involves using silica as a sugar framework, reducing the total caloric value of a product and offering a substitute to artificial sweeteners [57]. When examining branded artificial sweeteners, the researchers should be mindful of the complexity arising from the interaction of multiple components within the mixture, making it challenging to attribute effects to individual ingredients [58].

**Gut Microbiota**

The human intestinal tract is a bustling ecosystem (Figure 1), hosting a vast array of microorganisms which play crucial roles in maintaining the balance and health of their host [59]. This diverse community of gut flora, primarily comprising bacteria but also including other organisms like archaea, viruses and fungi, is essential for optimal health and disease prevention [60]. Particularly dense microbial populations are found in the large intestine, where they reach concentrations of about $10^{12}$ CFU *per* gram of intestinal content [59]. These microbes perform key functions, including water, vitamin and electrolyte absorption in the large intestine, highlighting the colon's critical role in overall health [60, 61]. Disruptions in the colon's functioning can lead to severe health issues and a decline in life quality, making the maintenance of a balanced gut microbiota essential for preventing gastrointestinal problems like bloating, constipation and infectious colitis [62]. The complexity and variety within an individual's gut flora, which can be analysed more accurately through advanced techniques like 16S ribosomal RNA gene sequencing, are indicators of nutritional status and overall health, especially in elderly populations [62-64].

**Physiology and Development of Gut Microbiota**

The microbiota, a complex system of microorganisms within the human body, exerts significant control over numerous physiological processes. The human stomach and intestines are teeming with bacterial populations that far exceed the body's total count of somatic cells [65]. Over a thousand bacterial species present in the human body, a mere hundred species make up almost 99 percent of this population [66]. In the small intestine, bacterial density starts at about $10^4$ CFU/g and escalates to $10^7$ CFU by the end, predominantly comprising Gram-negative aerobes [59]. These gut microorganisms, equipped with over 60,000 glycoside hydrolases and polysaccharide lyases, are skilful at adapting to various dietary, metabolic and health conditions, playing a pivotal role in breaking down complex compounds and aiding in dietary metabolism [67]. Fiber rich diets contribute significantly to health regulation by providing ample substrate for these microbes [68].

The Human Microbiome Project identified four primary bacterial phyla within the human microbiome: *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Proteobacteria*. However, the diversity and quantity of species in healthy individuals can vary widely [11]. Despite these variations, a common core microbiome with similar metabolic capabilities has been observed among healthy individuals [11, 65]. Notably, recent research has found microorganisms in womb tissues, casting doubt on the idea that microbiota formation begins at birth [69, 70]. The microbiota undergoes rapid colonization and changes post-birth, influenced by diet, illness and antibiotics [70, 71]. Initially, an infant's microbiota is

dominated by Actinobacteria and Proteobacteria and is relatively simple, but it evolves over the first year, eventually resembling an adult-like composition by around 2.5 years of age [70, 71]. While the microbiota remains mostly stable in adulthood, life events can cause disruptions around 70 years old were quite similar, centenarians exhibited significantly less microbial diversity noted that while the microbiota of young adults and seniors [70, 72]. Aging is associated with increased proteolytic activity in the microbiota and decreased metabolic functions, such as short-chain fatty acid production and amylolysis [73]. This reduction in short-chain fatty acids could accelerate intestinal aging, given their crucial role as metabolic and immunological mediators [74].

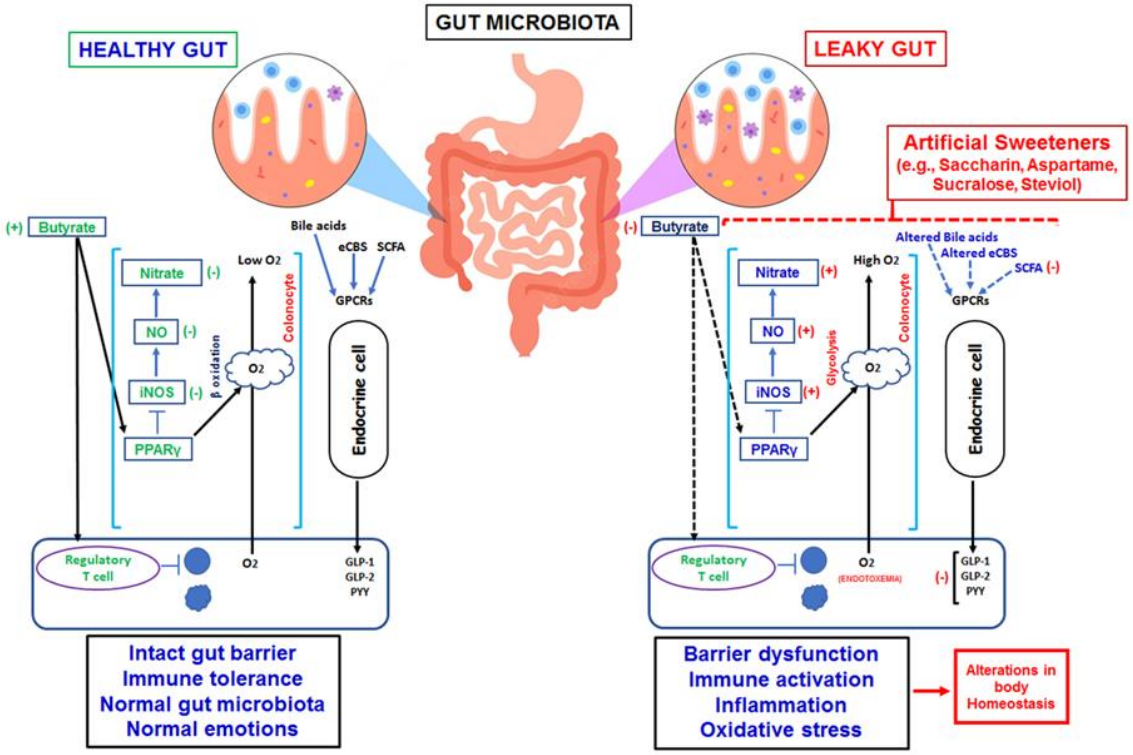


**Figure 1.**

Gut microbiota mechanism and in healthy and diseased situations. In a healthy colon, colonocytes use butyrate for energy by way of beta-oxidation in the mitochondria, which helps keep the lumen in good repair. The binding of butyrate to the peroxisome proliferator-activated receptor gamma (PPAR) further reduces NO and nitrate production by inhibiting the inducible nitric oxide synthase (iNOS). On the other hand, in pathological circumstances, PPAR activity decreases, glycolysis increases and oxygen consumption decreases when there is less butyrate in the lumen. The availability of nitrates for specific illnesses is boosted as a result of an increase in iNOS expression, which increases NO generation. Bile acids (BAs), endocannabinoids (eCBs) and short chain fatty acids (SCFAs) are the chemicals that activate G-protein coupled receptors (GPCRs). After these receptors are active, the secretion of important gastrointestinal peptides such as glucagon-like peptide (GLP)-1, GLP-2 and peptide YY (PYY) increases. The image source for the gut microbiota is from www.freepik.com

## Human Microbiota

Human gastrointestinal system's microbiota composition is closely aligned with the specific physiological attributes of each intestinal region [75, 76]. The gut's varying mineral, chemical and immunological gradients greatly influence both the density and makeup of these microbial communities. The small intestine, characterized by its highly acidic environment, abundant oxygen supply and the presence of antimicrobials, is primarily hospitable to fast-growing, facultative anaerobes that can adhere to epithelial surfaces [75]. In contrast, the colon's environment is conducive to a diverse array of microbial life, predominantly anaerobes that thrive on complex carbohydrates not digested in the small intestine [77]. Research has shown a marked difference in microbial dominance between these two regions of the gut. In the colon, families such as *Prevotellaceae*, *Lachnospiraceae* and *Rikenellaceae* are prevalent, whereas in the small intestines of mice, *Lactobacillaceae* are more predominant [77].

## Effect of Diet on Gut Microbiota

Humans have always had a predisposition to sweet foods since the beginning of time [78]. De Filippis *et*

*al*. in 2015 have highlighted the significant impact of dietary changes on the gut microbiota [79]. A diet high in sugars and saturated fats and low in plant-based products and fibre can drastically alter the balance and diversity of key microbial phyla and species in the gut [80]. Such alterations, known as dysbiosis, have been linked to metabolic disorders including obesity, insulin resistance and cardiovascular risk as evidenced in cross-sectional studies. Alterations in the microbiota can also result from mutations and lateral gene transfer [81]. Emerson and Kolm (2005) found that new bacterial functions lead to increased variance and further diversification. The presence of multiple microbial species promotes a broader range of the organisms colonizing an area [82]. Certain gut diseases, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and colorectal cancer, have been linked to sulphate-reducing bacteria, which are influenced by sulphated substances in the large intestine [83]. The distribution of bile acids in the intestines can affect bacterial population dynamics [84, 85]. Browne *et al*. noted that primary bile acids like taurocholate aid in spore germination and microbiota recovery post-antibiotic or toxin-induced dysbiosis, and a decrease in gut bile acid content may promote the growth of pro-inflammatory microbial taxa [86]. The direct relationship between these conditions and the gut microbiota has been further established through faecal transplant experiments in animal models [87, 88]. Moreover, research in 2016 conducted on rats indicates that even intermittent consumption of cafeteria-style food, as infrequent as thrice a week, can induce changes in the gut microbiota [89]. This finding underscores the sensitivity of the gut microbial ecosystem to diet, highlighting that even brief periods of specific dietary habits can have detrimental effects on the composition of the gut microbiota as illustrated in Figure 2.

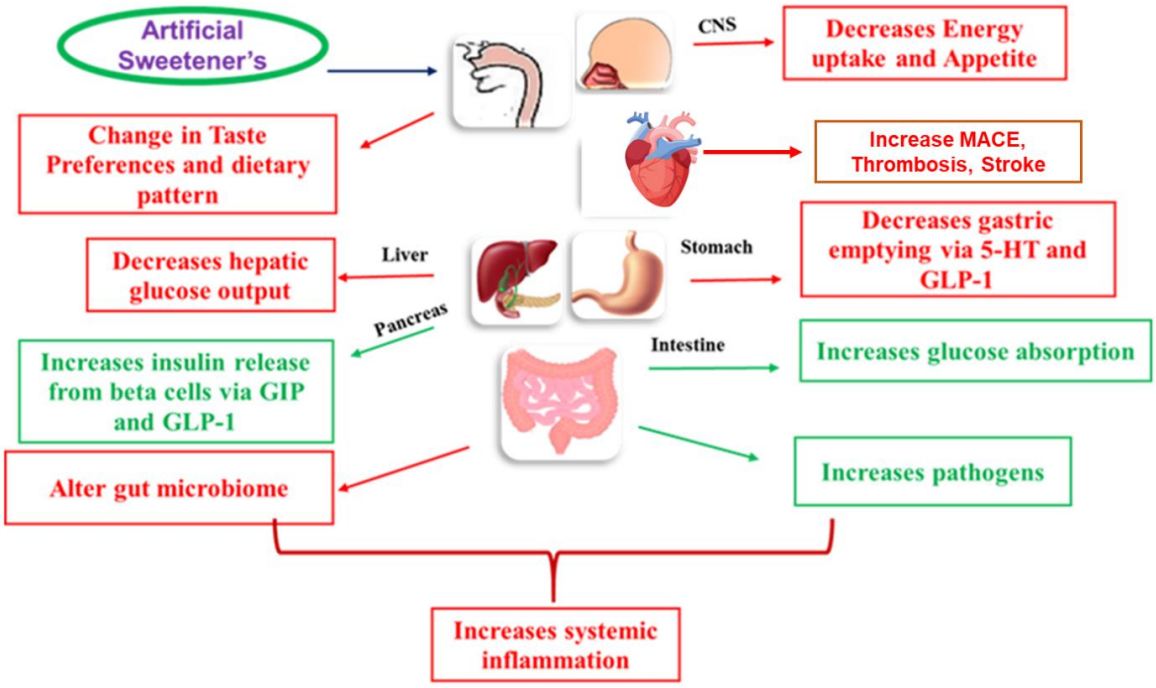


**Figure 2.**

Illustrates the multifaceted impacts of artificial sweeteners on various organs and bodily systems and highlights key areas where sweeteners exert their influence, underscoring the complexity of their effects

Central nervous system (CNS); Gastric inhibitory polypeptide (GIP); glucagon-like peptide-1 (GLP-1); major adverse cardiovascular events (MACE); 5-hydroxytryptamine (5-HT)

An experiment by Anderson and Kirkland in 1980 observed that male rats, after being exposed to 7.5% saccharin for ten days, exhibited a shift in their faecal bacterial balance, showing an increase in aerobic bacteria and a decrease in anaerobic bacteria. This shift in the gut microbiome, or dysbiosis, has been linked to adverse health effects. For instance, inducing cirrhosis in mice was found to alter the aerobic-to-anaerobic bacteria ratio. Research studies have revealed a consistent decrease in bacterial counts in rats after administering various doses of sucralose for 12 weeks [23, 90]. Similarly, Suez and colleagues in 2014 demonstrated through various models and experiments that sweetener consumption disrupts gut microbiota in both mice and humans [26]. Long-term effects of sweeteners on non-diabetic individuals have been a focus of several observational and epidemiological studies, suggesting that sweeteners may exert subtle, cumulative impacts.

These effects are often not evident in short-term intervention studies, randomized controlled trials, or when directly comparing the consumption of sugar-sweetened products. In one study, non-sugar-using adults were given saccharin at a daily dose of 5 mg/kg body weight for a week, leading some participants to develop glucose intolerance [26]. This study also found distinct microbiota profiles between individuals who responded to the sweetener and those who did not. Furthermore, insulin resistance, as demonstrated through faecal transplants into germ-free mice, was found to be transferable *via* gut microbiota only in the responder group. This indicates that the effects of sweeteners might be individual-specific, depending on one's initial gut bacteria composition. In related research, Palmnas *et al*. found similar results in rats given aspartame. This study focused on the impact of aspartame on gut microbiota and glucose intolerance in both lean and diet-induced obese rats, with aspartame doses equivalent to 2 - 3 cans of diet soda *per* day [25]. Other studies have shown significant alterations in gut microbiota in people adhering to strictly plant- or animal-based diets [91]. A research report discovered that diet rich in resistant starch or non-starch polysaccharide fibre lead to a considerable increase in specific bacterial species [92]. A research study in 2011 found that breastfed babies have a more diverse beneficial bacterial population in their guts compared to formula-fed infants [93]. However, formula-fed newborns exhibit a more varied gut microbiota, with different levels of *Lactobacilli*, *Clostridium difficile*, *Bacteroides fragilis* and *E. coli* [93, 94]. Additionally, Kau *et al*. observed higher levels of enteropathogens, like *Enterobacteriaceae*, in undernourished infants' gut microbiota [95].

**Influence of The Immune System on The Gut**

The interaction between the host immune system and the gut microbiota is a complex one. Although the microbiota exhibits a high level of functional redundancy, making species-specific impacts less common, the primary influence of the immune system is on the overall structure and compartmentalization of these microbes to prevent them from attacking host tissues [96]. The gastrointestinal tract is safeguarded by a dynamic, multifaceted intestinal barrier that not only prevents damage but also maintains homeostasis, thus restricting the exposure of the host immune system to the gut microbiota. This intestinal barrier is a coordinated system comprising physical, metabolic and immunological elements [97]. The stability of any specific microorganism within this ecosystem is determined by how much it influences the host's ability to carry out vital functions. Sometimes, the elimination of detrimental bacteria occurs as part of this balancing act [98]. In the context of shaping the gut microbiota, both naturally occurring and externally introduced antimicrobials play significant roles. Antimicrobials produced by Paneth cells in the gastrointestinal tract, such as lysozymes, α-defensins and lipopolysaccharide binding proteins, are primarily found in the mucus layer due to their limited diffusion through the mucus or degradation in the lumen [97, 99]. Another key component of the immune system interacting with the gut microbiota is Secretory IgA. Located in the outer mucus layer where it coexists with gut microbes, Secretory IgA plays a crucial role in reducing the amount of epithelial cell surface area accessible to bacteria [99, 100]. This dynamic between the immune system and the gut microbiota is essential for maintaining a balanced and healthy gut ecosystem.

**Environmental Factors and Microbiota**

Environmental factors, such as living conditions, smoking, mental health and medical interventions, can significantly influence the microbiome profile, as highlighted by research [101, 102]. Medications, particularly antibiotics, are known to alter gut microbiota functioning and genetic expression [103]. Antibiotic treatments can disrupt microbial balance both in the short and long term, often resulting in reduced microbiome diversity and health. Studies indicate that certain antibiotics like ciprofloxacin, metronidazole and clindamycin can have enduring impacts on microbiota composition [72]. The specific effects and the microbiome's recovery post-antibiotic treatment vary greatly between individuals due to pre-existing differences in their microbiota [72, 104]. Furthermore, research by Ng in 2013, suggests that antibiotic use in mice increases susceptibility to microbial infections [105]. This may occur because antibiotics alter carbohydrate availability across mucosal membranes, facilitating the expansion of antibiotic-associated bacteria in the gut. To develop new strategies for maintaining human health, it's crucial to understand the mechanisms behind bacterial growth post-antibiotic use, including changes in metabolic activities and metabolites.

**Gut Microbes and Homeostasis**

The gut microbiota, with its vast metabolic capabilities and genetic content, offers numerous benefits to its host. These include maintaining mucosal barrier integrity, protecting against pathogens and synthesizing essential nutrients like vitamins. The microbiota's interaction with the mucosal immune system is also critical for proper immune functioning. Being a complex community within dynamic ecosystems, the microbiota's balance is sensitive to the loss of any species, particularly those crucial for the survival or metabolic needs of others. Research studies indicates a connection between a less diverse microbiome and various human disorders, including IBD, obesity and arthritis [90, 106]. Metabolic issues induced by sweeteners may stem from disruptions in microbial diversity caused by low-calorie sweeteners like aspartame and acesulfame-K [60]. It's important

to acknowledge significant individual variations in microbiome diversity. Structural and functional shifts in the microbiome lead to the release of various metabolites that communicate with bodily organs [107]. Bacterial fermentation byproducts, such as organic acids, enter the bloodstream and can influence host metabolism. Short-chain fatty acids, for instance, play a crucial role in energy production and intestinal signalling, contributing to glucose production during fasting raised concerns about the effects of sweeteners on fasting glucose and serum levels of short-chain fatty acids [25, 108]. These sweeteners might also impact bile acid metabolism, gene expression and the secretion of hormones like PYY, GLP-1 and GIP [109-111]. Dietary shifts rapidly alter the gut microbiome's composition and function [112, 143]. High fat diet in mice led to metabolic endotoxemia and changes in gut mucous layer thickness, associated with levels of *Akkermansia muciniphila* [18, 112]. Metabolic endotoxemia, linked with obesity and high-fat diets, is characterized by increased serum LPS levels and intestinal hyper-permeability, leading to inflammation and insulin resistance [88].

**Sweeteners Consumption and Homeostasis**

The influence of sweeteners on body homeostasis is diverse and heavily impacted by the gut microbiome, which in turn affects multiple organs [113]. For examples, in the Central Nervous System (CNS), there are noticeable changes regarding increased cravings and altered taste perceptions [114-116]. These effects are intimately connected to the gut-brain axis, where alterations in the gut microbiota due to sweeteners intake influences neural pathways and behaviours [114, 115]. The study by Suez *et al*. was pivotal in demonstrating that artificial sweeteners can induce glucose intolerance through changes in the gut microbiota, emphasizing the indirect but substantial impact on CNS function [117]. Furthermore, other studies have explored how low-calorie sweeteners could affect cognitive functions and mood disorders, further illustrating the complexity of these interactions [118, 119]. In the gastrointestinal system, particularly in the stomach, additive sweeteners can affect hormone secretion such as ghrelin, which is crucial for regulating hunger and satiety [120, 121]. The gut microbiome plays a significant role in the metabolism and hormonal responses to food [40]. Artificial sweeteners may also potentially influence appetite by affecting serotonin (5-HT) and glucagon-like peptide-1 (GLP-1) levels, although the evidence is not conclusive and varies depending on the type of sweetener and individual factors [114]. Furthermore, the role of the liver in metabolizing sugars can be compromised by excessive sweetener intake [122]. Recent studies indicated a connection between non-alcoholic fatty liver disease (NAFLD) exacerbated by gut microbiota dysbiosis and high sweetener use, implying that these compounds may have a role in liver damage [123, 124]. In addition, the role of artificial sweeteners in altering liver enzyme profiles, further complicating the understanding of their metabolic impact [125, 126].

Cardiovascular health is another area may significantly be affected by sweetener consumption. Schulze et al. in 2004 found a correlation between high intake of sweeteners and the risk of cardiovascular diseases, mediated through pathways like obesity and diabetes [127, 128]. The gut microbiome appears to be a key player in this relationship, influencing systemic inflammation and metabolic disorders, which are both risk factors for heart disease [129, 130]. A large prospective cohort study found a link between artificial sweetener usage (particularly aspartame, acesulfame potassium and sucralose) and increased major adverse cardiac events (MACE) risk, thrombosis and inflammatory pathways [131, 132]. High sugar consumption can lead to an increased demand for insulin, contributing to insulin resistance [133]. Pepino (2015) discussed the potential effects of artificial sweeteners on insulin sensitivity and glucose tolerance [17]. Epidemiological and biomedical studies also indicate that prolonged artificial sweeteners consumption may aggravate glucose intolerance and obesity [13, 15, 16]. The evolving understanding of the gut microbiota's role in metabolic processes underscores the connection between changes in the gut microbiome and insulin resistance, which is especially relevant in the development of T2D [134-136]. Similarly, the direct impact of sweeteners on gut microbiota composition in the intestines is profound [137]. The studies by Suez *et al*. and Ruiz-Ojeda *et al*. both highlight how sweeteners can alter the gut microbiota, affecting nutrients absorption, gut barrier function and immune responses [40, 117]. This not only influences metabolic health, but also the overall body homeostasis.

The impact of low-calorie sweeteners on the kidney health are also emerging as there are signs that prolonged use may deteriorate kidney function, particularly in those who already have renal disorders [138]. Moreover, some sweeteners may disrupt hormone balance and affecting the reproductive health. This raises concerns about their potential to cause endocrine disruption [113, 139]. Additionally, preliminary research points to a link between the consumption of sweeteners and a reduction in bone density, raising concerns about an elevated risk of osteoporosis [140, 141]. Sweeteners have psychological and behavioural effects that extend beyond physical health. Studies have revealed that sweeteners may affect mood, desires and eating habits [142, 144]. Understanding these relationships is crucial for maintaining health and preventing metabolic disorders.

In summary, the existing literature emphasizes that the effects of sweeteners extend far beyond simple caloric intake, and involving intricate biochemical and microbial interactions within the body [91, 115, 119,

134, 137]. Therefore, the complex interplay between sweeteners, the gut microbiome and various organ systems underscores the importance of a balanced approach to sweetener consumption.

## Conclusion, Novelty and Future Prospects

A mounting body of research suggests that sweeteners may have contributed to the very epidemic they set out to combat (*i.e.*, insulin resistance and obesity) by disturbing the gut microbiota and metabolic health in susceptible individuals. Consequently, there is an urgent need for studies that examine the metabolic effects of different types of sweeteners and the amounts consumed. Extra research into the specifics of how sweeteners alter the composition and function of the gut microbiota is necessary, along with the creation of personalized dietary plans that account for individual differences. Artificial sweeteners may alter the way drugs are absorbed and processed in the human body; however, data on this is limited. For example, studies did not observe notable changes in metformin distribution in rats [145]. This article provides a novel, comprehensive narrative review that consolidates recent findings on the interaction between artificial and natural sweeteners, gut microbiota and metabolic health, focusing on both *in vivo* and *in vitro* studies. Unlike previous reviews, which have primarily explored general metabolic effects or specific sweeteners in isolation, this review offers an integrated perspective on how various types of sweeteners collectively influence gut microbiota composition and subsequent health outcomes. Additionally, this review places a particular emphasis on the implications of gut dysbiosis, drawing connections to emerging research on obesity, glucose intolerance and cardiovascular risk. By synthesizing this range of evidence, the review underscores gap in existing research and advocates for future studies addressing personalized health impacts of sweeteners based on microbiota profiles. More research is needed to understand sweeteners' metabolic effects, how they modify gut microbiota and the necessity of personalized dietary approaches. Studies on sweeteners' effects on drug absorption and metabolism are limited, with ongoing research in this area (ClinicalTrials.gov Identifier: NCT03407079). Comprehensive research is essential to address gaps in our understanding of microbial dysbiosis and its consequences.

## Conflict of interest

The authors declare no conflict of interest.

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