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Unfolding the Impact of Artificial Sweetners on Gut Microbiome Leading to Metabolic Disease: Obesity & Diabetes Mellitus

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Abstract

Artificial sweeteners (AS) are in high demand due to their low-calorie content. They are used as sugar substitutes by diabetic and obese people, however current research indicates that NSS use may contribute to metabolic disorders. The FSSAI has cleared the use of six artificial sweeteners within acceptable amounts to improve tolerance and ensure safe use. However, many popular nutraceuticals and protein powders contain artificial sweeteners without mentioning how much are used, which may exceed the FSSAI's limits at the expense of the consumer's health.The health effects of these non-caloric sweeteners are still debated since they are metabolized differently, and their metabolic end products have been connected to gut microbiota, glucose intolerance, and weight gain. Although long-term human studies on artificial sweeteners are rare, an effort has been made to analyse previous evidence to consolidate the relation of AS with health issues.

Keywords: Artificial sweeteners; Dysbiosis; Gut microbiome; Diabetes and metabolic diseases.

INTRODUCTION

Artificial sweeteners (AS) are sugar substitutes but are sweeter than sugar, capable of mimicking thse taste of sugar while comprising few or no calories. As Indians are the world's biggest sugar consumers, sweets are an essential part of most Indian communities' daily meals. In such a scenario, the demand for artificial sweeteners is rising rapidly (Paul and Bandyopadhyay 2020). Artificial sweeteners are becoming increasingly popular as health issues like diabetes and obesity are increasing rapidly. As a result, slimming diets and sugar-free diets are burgeoning. These artificial sweeteners are readily available in market and are in reach of common people. However, the breakdown products of these sweeteners continue to have controversial health and metabolic effects since they are not metabolized in the human body. Only when they are consumed in permissible limits, they may be considered to be safe for human consumption.

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The first artificial sweetener to be invented was saccharin. If we compare its sweetness to sugar, its 200-700 times sweeter. On scanning the type of artificial sweetener in 'the added' list in rear of packaged goods, one can 'discover' it in fizzy drinks, candies, condiments, bubblegum, and inedible items such as medical drugs, mouth rinses and toothpastes too. As part a me, acesulfame potassium (acesulfame K) and sucralose, all second generation sweeteners, have 200, 300 and 600 times sweetness effect as compared to sugar respectively and have been given approval in year 1981 by FDA. Latest ones like AS encompass stevia and fermented polyols such xylitol and sorbitol have a purgative effect when ingested in huge quantities (Pearlman *et al*. 2017). There is an approval from FSSAI (Food Safety and Standards Authority of India) for using non-nutritive artificial sweeteners such as sucralose, aspartame (methyl ester), saccharin sodium, acesulfame potassium, neotame, steviol glycosides and iso-maltulose in food articles, but in quantities not exceeding the specified maximum limits (FSSAI 2011). Steviol Glycoside was permitted to be used as a NNS in specific food products under the Food Products Standards and Food Additives Amendment Regulations, 2015 (FSSAI 2015). Steviol Glycoside is recognised by the FSSAI for its light yellow or white powder with an odourless attribute. The primary constituent of stevia is glycosides which generates a sweet taste with no calories. A key ingredient, stevioside in stevia, has a sweetness which is 200-300 times than that of sugar (Chowdhury *et al*. 2022). Stevia is used in many medicines and beverages, but due to conflicting studies, the benefits of stevia still remain controversial.

Table 1: ADI (Acceptable Daily Intake) milligrams per kilogram body weight per day (mg/kg BW/d) given by Joint FAO/ WHO Expert Committee on Food Additives (JECFA)

Sweetener	INS Number (International Numbering System of Food Additives)	ADI mg/ kg body weight per day
Acesulfame Potassium (Ace-K)	950	0-15
Steviol glycosides	960	0-4
Aspartame	951	0-40
Neotame	961	0-2
Saccharin	954 (iv) (iv means subclass of saccharin)	0-5
Sucralose	955	0-15
Iso-maltulose	953	Not specified

Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (2022).

Table 2: Artificial sweeteners and their permissible limits (in parts per million) in food products given by the FSSAI

Name of the sweetener	Chocolate	Sugar based/ sugar-free confectionery	Traditional sweets	Pan masala	Soft drinks	Chewing gum
Saccharin sodium*	500	3000	500	8000	100	3000
Aspartame*	2000	10000	200	_	700	10000
Acesulfame potassium*	500	3500	500	_	300	5000
Sucralose*	800	1500	750	_	300	1250
Neotame*	_	_	_	_	33	------
Isomaltulose*	Isomaltulose is permitted for use in confectioneries, with an upper allowed of 50% (limit) of sucrose without negatively impacting the item's stabilitys	_	_	_	_	_

Food safety and standards (food products standards and food additives) regulations (2011)

Table 3: Maximum concentration of steviol equivalent (mg/kg) specified in the amended regulations.

Name of the sweetener	Yoghurt	Ready to eat cereals	Jams and jellies	Dairy based desserts	Soft drink concentrate	Chewing gum/ bubble gum
Steviol glycosides**	200	350	360	330	200	3500

Food products standards and food additives (amendment) regulations, 2015**

Artificial Sweeteners and Gut Microbiome

The gut microbiota, a diverse and ever-changing population of microorganisms found in the human gastrointestinal (GI) tract, has a significant impact on the host during both health and illness. (Thursby & Juge, 2017) It is a complex and dynamic microbiological biodiversity inhabiting our bodies from womb to tomb, has been associated with an array of biological functions as well as vulnerability to a to variety physiopathology states. Gut microbiota and the host share a symbiotic relationship where microbiota metabolites such as vitamin K and vitamin B provide needy support for growth and development of human. However, diet is also one of the most important factors shaping the gut microbiota. (Shil and Chichger 2021) The connection between nutrition and gut microflora and its possible outcomes of stimulating disease has attracted attention already. A significant role involving the human gut flora is becoming evident in the association between nutrition and metabolic health. ((Fava *et al.*, 2018) The trillions of microorganisms that live in the human intestines depend on dietary nutrients for their survival as well as for human health. The interaction between humans and their microbial inhabitants is largely influenced by diet; gut microorganisms absorb nutrients from food for basic biological functions, and the products of those metabolic processes may have significant effects on human physiology. (Christopher & Tiffany, 2018)

Since there is evidence for the potential effects of NNS on inflammation, obesity, and insulin resistance (risk of diabetes), the influence of NNS on the gut microbiota has recently been studied (Sanyaolu *et al*. 2018) (Ruiz-Ojeda *et al*., 2019) (Walbolt & Koh, 2020). Non-caloric artificial sweeteners, which are commonly used as nutritional supplements, may interact with the microbiota composition and thus impose their effects on the host. Numerous studies have suggested that there may be perplexing links betweenthe illnesses related to metabolic syndrome and non- caloric artificial sweeteners consumption. A wide range of mechanisms associated with body including increased gut sugarabsorption, disruption of sweet taste's ability to indicate calorie implications, a rise in desire to eat, and insulin deficiency responses, explain these phenomena (Suez *et al*. 2015). Artificial sweeteners appear to disrupt basic learned, predictive relationships between sweet tastes and post-ingestive outcomes such as energy delivery. Artificial sweeteners interfere with these relationships by inhibiting anticipatory responses that normally serve to maintain physiological homeostasis, and this interference may have long-term negative health effects (Swithers, 2015).

Dysbiosis refers to changes in the composition and function of gut microbiota. Dysbiosis is typically distinguished by the elimination of many beneficial bacteria and the extinction of bacterial communities which results in decrease of microbial diversification that is linked to a variety of immune-mediated and metabolic disorders and an overabundance of potentially pathogenic commensals (pathobionts). Pathobionts make up a small proportion of the gut microbiota in a healthy gut ecosystem which outgrow other commensals in many diseases (Hrncir *et al*. 2021). In healthy young adults, a study examined the impact of sucrose consumption on the intestinal abundance of bacterial species. The results showed that sucrose consumption changed the abundance of Firmicutes but had no effect on Actinobacteria or Bacteroidetes and concluded that long-term use of sucrose causes dysbiosis in the gut. (Méndez-García *et al*. 2020). Dysbiosis is linked to liver diseases such as non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), cirrhosis, insulin intolerance, obesity and hepatocellular carcinoma.

One study stated that the human gut contains about 100 trillion microorganisms of at least 1000 different species. Xylitol and sucralose have been shown to reduce beneficial microorganisms (Thursby & Juge, 2017). Similarly, research has shown what effects the artificial sweeteners have on the gastrointestinal tract are often related to its interaction with the microbial flora in the human gastrointestinal tract (Gerasimidis *et al*. 2020). In a study it was reported that many pro-inflammatory mediators: tumor necrosis factor-alpha (TNF-α) & interleukin-6 (Bander *et al*., 2020) can be produced

by gut bacteria after consumption of artificial sweeteners, which are associated with other metabolic diseases such as diabetes and obesity (Bian *et al*. 2017). Interestingly, study showed that exposure to artificial sweeteners increases intestinal epithelial apoptosis and permeability, which are associated with inflammatory bowel disease (Shil *et al*. 2020). Despite the controversies in this field, there is strong evidence that artificial sweeteners in the diet can lead to changes in bacterial diversity and potential pathogenicity, possibly with adverse effects on the host, without altering the bacterial composition. Saccharin, sucralose and aspartame increased the ability of common intestinal bacteria to attach to and invade intestinal epithelial cells, except for saccharin, which had no significant effect on *E. coli* invasion. Many studies have reported the negative effects of artificial sweeteners, such as saccharin, sucralose and aspartame on apoptosis and permeability of intestinal epithelial cells which increases the chance of bacteria crossing the intestinal epithelium due to dysregulated apoptosis (Santos *et al*. 2018). As the consumption of artificial sweeteners is increasing in the diet, it is critical to understand their effects on the gut microbiota and how these ill effects can be mitigated.

In contrast to NNS, natural non-calorie sweetener, stevia found to have overall positive effect. In vitro research using specific microbial strains and in vivo studies employing laboratory animals was considered because there were no randomised clinical trials in humans. The findings suggested that using stevia may have a positive impact on the alpha diversity of the microbiota. Modifications in the intestinal microbiome may be influenced by the quantity and frequency of stevia ingestion as well as the concurrent intake of other dietary components. Stevioside's anti-inflammatory capabilities were demonstrated in vitro by reducing the production of tumour necrosis factor (TNF), Interleukin-1 and 6 and inhibiting the nuclear factor B (NF-B) transcription factor, and in vivo by inhibiting NF-B and mitogen-activated protein kinases (MAPK) in lab animals (Kasti *et al*. 2022).

In the human gastrointestinal tract, neither rebaudioside A nor stevioside are absorbed. Microbial metabolism converts both of these steviol glycosides to free steviol. The gut microbiota, which converts glycosides into steviol that the host may absorb, is necessary for the metabolism of steviol glycosides. Steviol glucuronide is rapidly absorbed yet swiftly removed in urine. These pharmacokinetic factors provide a considerable advantage in terms of possible toxicity, because the faster a metabolite is removed, the lower the likelihood of side effects arising. Stevioside is thus a safe, noncaloric, noncariogenic, nonallergenic, and natural alternative to sucrose. According to current research, rebaudioside A and stevioside can be proposed as prospective potential treatments for treating cardiovascular illnesses, diabetes, cancer, inflammation, diarrhoea, and oxidative processes when provided at greater dosages than acceptable daily intake. Stevioside, for example, does not cause hypoglycemia at normal glucose level, whereas stevia does. This result emphasises the importance of continuing to investigate and characterise the biological activity of isolated SGs, because stevia extract contains a wide range of compounds that may have synergistic or inhibitory effects on one another (Pasqualli *et al*. 2020).

Non-Nutritive Sweeteners induced Dysbiosis and its association with Obesity

Firmicutes and Bacteroidetes, the two most significant bacterial phyla in the gastrointestinal tract, have attracted a lot of interest lately. It is generally acknowledged that the Firmicutes/ Bacteroidetes (F/B) ratio plays a significant role in preserving good intestinal homeostasis. Dysbiosis, an increased or decreased F/B ratio; the former is typically associated with obesity, while the latter is associated with inflammatory bowel disease (IBD) and other metabolic disease including insulin resistance. A study conducted on the adult population in Ukraine found that adults who are obese had a considerably higher level of Firmicutes and a lower level of Bacteroidetes in comparison to those who are normal weight and lean. (Koliada *et al*., 2017)NNS use may contribute to the development of these disorders by altering the Firmicutes: Bacteroidetes ratio. (Liauchonak *et al*., 2019)(Stojanov *et al*., 2020) The indigenous microbiota may regulate body weight by modulating host metabolism, immunity, and neuroendocrine activities. The gut microbiota performs metabolic tasks and regulates host gene expression, which influences the body's ability to collect and store energy from food. Obese people with lesser bacterial richness/dysbiosis are also more likely to gain weight. (Lazăr *et al*., 2019)According to evidence from a review paper, an overabundance of saccharolytic gut microbiota may aid in improving food digestion, which raises energy absorption and increases fat deposition, ultimately contributing to the development of obesity(Kho & Lal, 2018). Another review article revealed that regardless of baseline weight or condition, alterations in the gut microbiome towards a more inflammatory pattern of gut microbiota is a concerning result in both acute

and chronic users of NNS. Most notably, number of studies revealed that chronic NNS users had long-term harm to their neurohormonal regulation of satiety and concluded that it cannot be afforded to heedlessly accept NNS use in the fight against obesity and adiposity-related disorders on the basis of a flawed understanding of thermodynamics and the misconception that all people are biologically equal (Christofides E A 2021).

The SCFA acetate, propionate, and butyrate are created through microbial fermentation of indigestible carbohydrates and appear to be essential mediators of the gut microbiome's positive effects. SCFA directly affect host metabolic health via a variety of tissue-specific pathways involving appetite regulation, energy expenditure, glucose homeostasis, and immunomodulation. Thus, increasing microbial SCFA synthesis can be considered a health benefit. (Blaak *et al*., 2020)The study conducted on subjects with morbid obesity (defined as BMI >40 or >35 kg/m^2 with obesity-related comorbidity) indicated a strong correlation between the use of NNSs and a decrease in butyric acid.Butyric acid lowers insulin resistance, improves dyslipidemia, and has antiobesogenic properties.(Farup *et al*., 2019) Using an in vitro model, the study was conducted that ascertained how sweeteners affected the microbiome pattern. Although there was an increase in Bifidobacterium, the total amount of produced short-chain fatty acids (SCFA) and the number of microorganisms were reduced in this investigation, and a detrimental impact on the fermentative profile was noted. Additionally, Additionally, Cyclamate and sucralose caused an effect on the ratio of butyric/propionic acids, suggesting that those SCFA could influence the composition of the gut microbiota.(Vamanu *et al*., 2019) From these studies it may concluded that NNS induces dysbiosis that interrupt the SCFAs ratio that leads to weight gain but more clinical trials on human needs to be done.

Studies have shown that use of AS in drinks increases the risk of obesity(Ruanpeng *et al*., 2017) (Hodge *et al*., 2018)(Malik & Hu, 2022). A meta-analysis of six prospective cohort studies with 26,551 participants discovered that with every 250 mL/day increase in AS soft drink consumption, the risk of obesity rose by 21% (Qin *et al*., 2020).A comparative study between low-calorie sweetener users and non-users revealed that, over a median follow-up of 10 years, the users of low-calorie sweeteners had a higher body mass index, a larger waist circumference (2.6 cm), a higher prevalence of abdominal obesity (36.7%), and a higher incidence of abdominal obesity (53%)(Chia *et al*., 2016).

Non-Nutritive Sweeteners and its association with Diabetes

In recent decades, diabetes has become the most common endocrine disease and the most important comorbidity worldwide. Type 2 diabetes, which accounted for more than 96% of diabetes cases globally in 2021, is virtually exclusively responsible for diabetes prevalence rates. (Ong *et al*., 2023) The main reason is the development of insulin resistance by the body. "High carbohydrate, low fibre, high fat diets" and junk food are the important causative factors related to diabetes (Sylvetsky *et al*. 2012).Higher consumption of artificial sweeteners was linked to an increased risk of type 2 diabetes, according to a study done on participants. (Debras *et al*., 2023) Many studies have observed a positive association between AS and diabetes but this topic is still a controversy.One study done on young adults observed that during an oral glucose tolerance test, prolonged consumption of sucrose causes gut dysbiosis that is linked to altered insulin and glucose levels(Méndez-García *et al*. 2020).

According to one review article, a plausible explanation of this association could be their capacity to trigger the release of GLP-1, a hormone generated in the stomach that encourages fullness and quickens the release of insulin reliant on glucose through its interaction with the small intestine's sweet taste receptors.Results from human in vivo investigations are highly inconsistent, despite the fact that NNS (sucralose, Ace K, and Rebaudioside A) exhibit a high rate of GLP-1 secretion during in vitro trials. (Decker, 2018)In a randomized-controlled trial with 120 healthy adults, the effects of NNS on humans and their microbiomes were evaluated. Saccharin, sucralose, aspartame, and stevia sachets were given for two weeks at doses below the recommended daily allowance, while controls received vehicle glucose or no supplement at all. Each provided NNS individually changed the plasma metabolome, oral microbiome, and stool microbiome, while saccharin and sucralose markedly altered the glycaemic responses.(Suez *et al*., 2022)Another clinical trial assessing sucralose's effects on insulin and glucagon-like peptide-1 secretion in healthy subjects revealed a connection between sucralose and insulin resistance. (Lertrit *et al*., 2018)In a study, people were given either sucralose or water and then given a glucose tolerance test. Those who received sucralose had higher blood insulin levels (Pepino *et al*. 2013) (Romo-Romo *et al*. 2018) and according to one review prior to insulin resistance, hyperinsulinemia may already be present in individuals with normal glucose tolerance(Janssen, 2021). Another study

reported that based on HOMA-IR (Homeostatic Model Assessment for Insulin Resistance), patients who consumed artificial sweeteners had higher insulin resistance than patients who did not consume artificial sweeteners (Mathur *et al*. 2020).

A dose-response meta-analysis of prospective cohort studies revealed that long-term ASB use raised the risk of type 2 diabetes(Meng *et al*., 2021).A meta-analysis of six prospective cohort studies with 26,551 participants discovered that with every 250 mL/day increase in AS soft drink consumption, the risk of T2DM rose by 15%(Qin *et al*., 2020).Another study that examined the relationships between long-term variations in the consumption of artificially sweetened beverages (ASBs) and sugary beverages and associated risk of type 2 diabetes, it was found that an increase in ASB consumption of more than 0.50 servings per day was linked to an 18% increased risk of diabetes. There was a 2–10% reduction in the incidence of diabetes when one serving of sugary beverage per day was substituted with water, coffee, or tea—but not when substituted with ASB(Drouin□Chartier *et al*., 2019). Diet soda that are more in trend nowadays but according to the prospective multiethnic population-based cohort study it was concluded that changing to diet drinks with artificial sweeteners might not be the solution because drinking diet soda may potentially be a risk factor for diabetes on its own(Gardener *et al*., 2018).

NNS consumption, obesity and diabetes

Consumption of artificial sweeteners above the recommended levels of the Food and Drug Administration can have disastrous effects and play a greater role in the development of obesity that leads to diabetes (Tandel 2011). Because obesity results in both insulin resistance and beta cell dysfunction, it is a major risk factor for both type 2 diabetes and prediabetes, especially when it is accompanied by increased intra-abdominal and abdominal fat distribution as well as increased intrahepatic and intramuscular triglyceride content (Klein *et al*. 2022). The most significant risk factor for acquiring diabetes, according to a comprehensive analysis of the researchis adiposity (Ng *et al*., 2020). Adipose tissue in obese people releases increased levels of hormones, pro-inflammatory cytokines, glycerol, and non-esterified fatty acids, all of which may contribute to the development of insulin resistance (Wondmkun,2020). The prevalence of generalized obesity (GO), abdominal obesity (AO), and combined obesity (CO) among T2DM patients was found to be 58.68%, 81.84%, and 53.42%, respectively, in a community-based cross-sectional study. This study concluded that obesity and overweight pose a significant risk for chronic diseases and are thought to be a strong risk factor for the development of T2DM (Vasanthakumar &Kambar, 2020). A higher frequency and longer consumption of artificial sweeteners in packets or tablets was linked to a higher risk of type 2 diabetes, even in the absence of major risk factors. This association was partially mediated by adiposity, according to a study that looked into the long-term use of artificial sweeteners in packets or tablets and its relationship to diabetes risk (Fagherazzi *et al*., 2017).

According to one review article, gut dysbiosis is found to be link between obesity and insulin resistance (Barber *et al*., 2021). There is evidence to suggest that modifications to the gut microbiota may have a role in the pathophysiology of obesity and the development of metabolic illnesses associated with obesity, such as metabolic syndrome, type 2 diabetes, NAFLD, and cardiovascular disease. The gut microbiota has been linked to the pathophysiology of obesity and related metabolic diseases through a number of possible mechanisms. These include: (a) a high abundance of bacteria that ferment carbohydrates, which increases the rate at which short-chain fatty acid (SCFA) is biosynthesised, giving the host an additional energy source that is eventually stored as lipids or glucose; (b) increased intestinal permeability to bacterial lipopolysaccharides (LPS), which raises systemic LPS levels and aggravates low-grade inflammation and insulin resistance; and (c) increased activity of the gut endocannabinoid system(Muscogiuri *et al*., 2019).

CONCLUSION

There is strong evidence that artificial sweeteners have a significant impact on the host microbiome, glucose homeostasis, energy consumption, overall weight gain, and body adiposity, despite the fact that they were created as a sugar substitute to help with weight loss and insulin resistance. From past studies, it is evident that possible linkage of AS consumption with metabolic diseases is dysbiosis. Artificial sweeteners are marketed as a healthier substitute for sugar; however, the vast amount of evidence contradicts this assertion. Artificial sweeteners are added to a huge number of products, and one of the main issues with human research findings that is challenging to analyse is that people's intake of artificial sweeteners is often based on dietary recall and they are unaware of

how much of these sweeteners they are consuming in mouthwash, toothpaste, sauces, chewing gum, protein supplements, and other products. Artificial sweeteners may be harmful to the health if people use them in excess of what is recommended. The choice and use of artificial sweeteners should be done carefully.

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