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Review

Effect of low-and non-calorie sweeteners on the gut microbiota: A review of clinical trials and cross-sectional studies

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ABSTRACT

Use of non-nutritive sweeteners (NNSs) has increased worldwide in recent decades. However, evidence from preclinical studies shows that sweetener consumption may induce glucose intolerance through changes in the gut microbiota, which raises public health concerns. As studies conducted on humans are lacking, the aim of this review was to gather and summarize the current evidence on the effects of NNSs on human gut microbiota. Only clinical trials and cross-sectional studies were included in the review. Regarding NNSs (i.e, saccharin, sucralose, aspartame, and stevia), only two of five clinical trials showed significant changes in gut microbiota composition after the intervention protocol. These studies concluded that saccharin and sucralose impair glycemic tolerance. In three of the four cross-sectional studies an association between NNSs and the microbial composition was observed. All three clinical trials on polyols (i.e, xylitol) showed prebiotic effects on gut microbiota, but these studies had multiple limitations (publication date, dosage, duration) that jeopardize their validity. The microbial response to NNSs consumption could be strongly mediated by the gut microbial composition at baseline. Further studies in which the potential personalized microbial response to NNSs consumption is acknowledged, and that include longer intervention protocols, larger cohorts, and more realistic sweetener dosage are needed to broaden these findings.

Introduction

Increased rates of cardiovascular diseases (CVDs), type 2 diabetes (T2D), obesity, and metabolic syndrome (MetS) have in recent decades become an important public health concern in developed countries [1]. High sugar consumption has been identified as a cause of these diseases, which has led food industries to introduce non-nutritive sweeteners (NNSs) in food and beverages, to reduce the energy intake of consumers and the glycemic index of certain products [2].

Alternative sweeteners contain few or no calories and mimic the sweet taste of sucrose and glucose-fructose syrups [3]. Many have been judged safe by European and international authorities [4,5], and their use has been increasing over time in many countries [3]. According to a study conducted in the United States between 2009 and 2012, around 40% of adults reported consuming alternative sweeteners [6], which was 54% more than those indicating their consumption in previous data from 1999 to 2000 [7]. However, whether the consumption of sweeteners has no harmful effects on the human body is still a matter of debate. Observational and preclinical studies suggest that there is a link between alternative sweetener consumption and shifts in physiologic parameters such as glucose tolerance and insulin resistance (IR) [8]. Growing evidence shows that changes in the gut microbiota might mediate these adverse effects after alternative sweetener consumption [8–10].

Alternative sweeteners are more commonly added to soft drinks, dairy products, sweets such as baked goods, candies and chocolates, jams and jellies, and chewing gum and are also used as table-top sweeteners at home or in cafeterias and restaurants [3]. They can be classified into two main categories: NNSs and low-

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calorie sweeteners (LCSs) [2,5]. More precisely, NNSs have a high sweetening intensity and confer almost no calories to products. They are either artificial (saccharin, sucralose, aspartame, acesulfame-K, advantame, neotame) or natural (steviol glycosides [stevia], Monk fruit, thaumatin). LCSs include polyols, also named sugar alcohols, and other new sweeteners that have fewer calories than table sugar (about half or one-third less), have 25% to 100% of the relative sweetness of sugar, and are converted to glucose more slowly in the body [2,11,12]. Common examples of polyols are xylitol, sorbitol, erythritol, mannitol, isomalt, maltitol, and lactitol. Low and non-calorie sweeteners (LNCSs) approved for dietary use vary across countries, although sucralose, aspartame, saccharin, acesulfame-K, and steviol glycosides appear to be the most consumed worldwide [13].

The gut microbiota is an important ecosystem of thousands of microorganisms living in the intestinal tracts, including bacteria, viruses, and some eukaryotes. Two dominant phyla, Bacteroidetes (Bacteroideta) and Firmicutes (Bacillota), compose >90% of total microorganisms, followed by Proteobacteria (Pseudomonadota), Fusobacteria (Fusobacteriota), Tenericutes (Mycoplasmatota), and Actinomycetota [10]. More precisely, bacteria in the gut can metabolize indigestible carbohydrates and produce metabolites like short-chain fatty acids (SCFAs), which are important for maintaining the host's health [10]. The gut microbiota is implicated in multiple physiologic functions in the human body, such as supporting the host's immunity and bone growth, digesting food, regulating intestinal endocrine functions, providing protection against pathogens, regulating neurologic signals, biosynthesis of essential compounds and other functions that have yet to be investigated [10,14]. Many studies show links between low microbial richness and increases in adiposity, IR, inflammation, and dyslipidemia [15]. Different factors can alter microbial richness, such as genetics, diet, antibiotics, mode of birth, and age [10]. Therefore, dietary patterns modulate the gut microbiota, which in turn positively or negatively influences physiologic parameters linked to metabolic diseases [10].

The effect of LNCS consumption on gut microbiota has been a concern in the past decade. According to recent reviews, studies mainly conducted on in vitro and animal models show that among NNSs, only saccharin, sucralose, and steviol glycosides seem to alter gut microbiota [2,3,16]. As for LCSs, their effect on the gut microbiota is still not completely understood and some might have prebiotic effects [2,3]. A few recent experimental studies have investigated how physiologic parameters vary after changes in microbiota induced by LNCS supplementation. A study conducted by Suez et al. [9] showed that saccharin supplementation in mice, compared with the glucose control group, altered the gut microbiota and, thus, induced glucose intolerance. To test whether impaired glucose tolerance was due to changes in microbiota, the authors performed fecal transplantation on germ-free mice. Germ-free mice that received the transferred microbial composition of saccharin-supplemented mice exhibited glucose intolerance compared with germ-free mice who did not receive the transfer from the control group [9]. Moreover, Li et al. [17] investigated the effect of a 4-wk sorbitol gavage in mice, and the results showed shifts in the abundance of microbiome constituents and glucose tolerance. No causal effect between changes in the microbiota and glucose tolerance was verified. Still, it was observed that bacteria benefiting glucose homeostasis were decreased, whereas bacteria disrupting glucose homeostasis were increased after the supplementation protocol [17].

These examples of previous preclinical studies suggest that LNCS could alter human gut microbiota and therefore affect the health of individuals, which has led research groups to carry out clinical trials on the topic. However, to our knowledge, very few studies conducted on humans have been published so far, which makes it difficult to draw a clear conclusion. In this context, the objective of the present review was to examine the evidence currently available in the scientific literature regarding the effect of sweeteners on human gut microbiota and to discuss the validity of the evidence.

Methods

Search strategy

A bibliographic search was conducted on two online databases, Ovid and PubMed, and was completed by March 2023. The following keywords were used to narrow the search and had to appear either in the title or the abstract of the record: (microbiota OR microbiome OR microflora OR microbial) AND (sweetener* OR "sweetening agent*" OR aspartame OR stevioside* OR cyclamate* OR mannitol OR saccharin OR sorbitol OR stevia OR sucralose OR advantame OR xylitol OR lactitol OR isomalt OR isomaltitol OR maltitol OR erythritol OR hydrogenated starch hydrolysate* OR neotame OR acesulfame-K OR thaumatin OR mogroside*. Additional filters were also applied to the search: language (English) and species (Humans), without any restrictions regarding the publication date. Because we aimed only to include clinical trials and cross-sectional studies, the following keywords were added to the search: NOT (review[Publication Type]) NOT (review[Title]).

Therefore, with this search strategy, the goal was to retrieve clinical trials and cross-sectional studies that investigated the effect of short- or long-term LNCS consumption on gut microbiota or, in the case of cross-sectional studies, the association between both.

Inclusion and exclusion criteria

Only clinical or cross-sectional trials conducted in humans were included in the review. Therefore, in vitro or ex vitro experimentations and studies carried out in animals were excluded. Moreover, because the rationale behind this review was to examine the effects of the widespread use and consumption of LNCSs on the microbiota, only studies carried out in healthy individuals were kept. Studies carried out in participants with a specific health problem were not selected, as they focused more on the cure of specific medical symptoms, which draws away from the main topic of the review. To be included in the review, all studies had to evaluate the effect of a short- or long-term consumption of one or more LNCSs on the gut microbiota. Studies were also excluded if they focused on the oral microbiota instead of the gut microbiota and if the sweetener was combined with another probiotic in the intervention protocol.

Search protocol

Of the 465 records found on Ovid and PubMed (Fig. 1), 157 were duplicates, yielding 308 studies available for title and abstract screening. Following the preliminary screening process of the title and abstract, 294 articles were excluded. Fourteen articles were selected for a full-text review. After the revision of these 14 studies, 3 did not meet the inclusion criteria. More precisely, the outcomes of two studies were not relevant: one studied the effect of one acute dose of polyol on the gut microbiota, and the other did not study the effect of LNCS consumption on the gut microbiota. The third article studied the effect of the combination of a polyol and a probiotic, which did not meet the inclusion criteria.

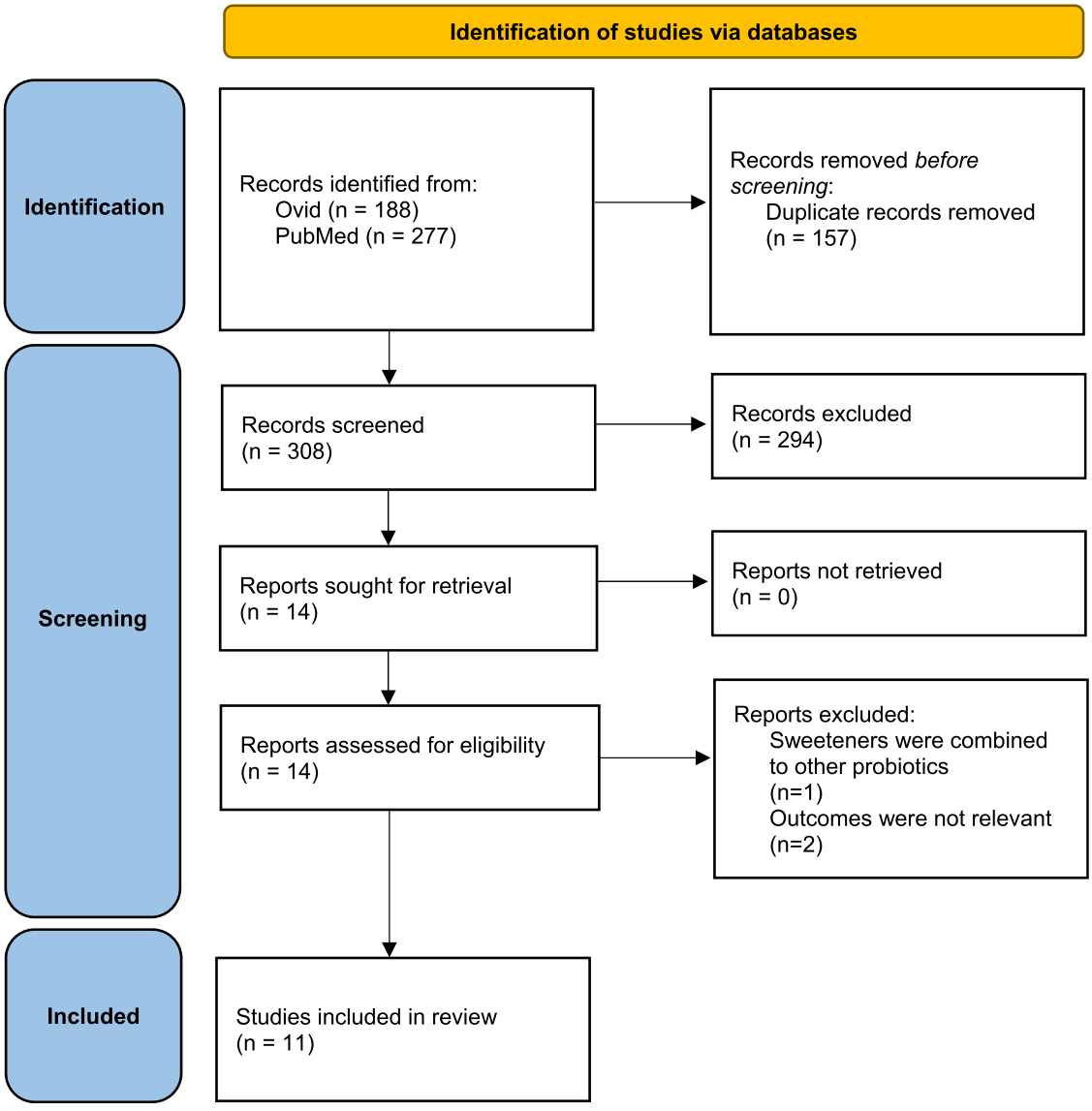

Fig. 1. Diagram of the identification and screening process of included articles.

Therefore, 11 studies were included in the review. One study comprised both a clinical trial and a cross-sectional investigation protocol.

Data extraction

The following information was extracted for each study: author name, publication type, study design, intervention protocol for clinical trials, assessment method for cross-sectional studies, sample size, profile of participants, and main outcomes.

Results

Characteristics of studies

Table 1 presents the details of the selected studies. Regarding study design, eight were clinical trials [9,18–24] and four were cross-sectional [9,25–27]. Of all clinical trials, six were randomized controlled trials [18–23], one was a randomized uncontrolled trial [23], and one was an unrandomized and uncontrolled trial [9]. As for geographic location, two studies were conducted in the United States [19,25], two in Canada [20,27], two in Europe [24,26], two in the United Kingdom [22,23], one in Chile [21] and three in Israel [9,18]. Suez et al. 2014 study [9] conducted in Israel comprised both a clinical trial and a cross-sectional investigation, so it is counted as two studies.

Among clinical trials, two investigated the effect of saccharin only on gut microbiota [9,19], one of sucralose only [21], three of polyols (i.e, maltitol [22], isomalt [24], and lactitol [23]), and the two others focused on multiple NNSs (i.e, one on aspartame, saccharin, sucralose, and stevia [18] and one on sucralose and aspartame [20]). As for cross-sectional studies, two tested the association of artificially sweetened beverages (ASB) consumption with microbial composition [26,27], one focused on global artificial sweetener consumption [9], and one focused on aspartame and acesulfame-K consumption [25]. Publication dates of articles ranged between 2006 and 2022, and studies on polyols had the oldest publication date (2006–2010) [22–24].

Sample sizes in clinical trials varied from 7 to 120 participants, and only two studies were conducted on >50 participants [18,23].

Table 1
Studies included in the review

Reference	Study design	Sweeteners/Intervention protocol or assessment method	Sample size	Profile of participants	Main outcomes
Clinical trials					
Suez et al. [18]	Randomized controlled trial	Aspartame, saccharin, sucralose, stevia 240 mg/d (~8% ADI), 180 mg/d (~20% ADI), 102 mg/d (~34% ADI) and 180 mg/d (~75% ADI) for 2 wk*	120 (6 groups of 20)	Healthy men and women Age 18–70 y old BMI 18–28 kg/m^2	All 4 NNSs had a significant effect on gut microbiome Saccharin and sucralose significantly elevated glycemic responses Microbial composition at baseline influenced glycemic response
Serrano et al. [19]	Randomized double-blind, placebo-controlled study	Saccharin Maximal ADI (800 mg/d) for 2 wk	46	Healthy men and women Age 18–45 y old BMI ≤25 kg/m^2	No changes in microbiota after pure saccharin supplementation No changes in glucose tolerance
Ahmad et al. [20]	Randomized double-blinded crossover and controlled clinical trial	Aspartame, sucralose 425 mg/d (14% ADI) for aspartame followed by 136 mg/d (20% ADI) of for sucralose Two 14-d intervention periods separated by a 4-wk washout period	17	Healthy men and women Age 18–45 y old BMI 20–25 kg/m^2	No changes in microbiota for either treatment No changes in glucose tolerance
Thomson et al. [21]	Randomized double-blind controlled study	Sucralose 780 mg/d for 7 d (75% ADI)	34 (17 in each group)	Healthy men Age 18–50 y old BMI 20–30 kg/m^2	No changes in microbiota No changes in glucose control and insulin resistance Microbial composition at baseline influenced the insulinemic response
Suez et al. [9]	Clinical trial	Saccharin 360 mg/d for 7 d	7	Healthy men and women Age 28–36 y old No details on BMI	The microbiome before and after the intervention differed between responders and non-responders. Responders were individuals who developed an impaired glycemic response after the intervention; whereas the glycemic response for non-responders remained stable
Beards et al. [22]	Randomized placebo-controlled, double-blinded, dose–response human feeding study	Maltitol 22.8, 34.2, and 45.6 g via chocolate bars for each period of 2 wk, respectively, for 6 wk	40	Healthy men and women Age 20–40 y old BMI 18.5–24.9 kg/m^2	Intervention beneficially affected gut microbiota, increasing bifidobacteria
Finney et al. [23]	Randomized uncontrolled longitudinal study	Lactitol 10 g sweeteners in sucrose-to-lactitol ratio of 10:0, 5:5, or 0:10 for 7 d	75	Healthy men and women Age 18–24 y old BMI: men 22.80 ± 3.01 kg/m$^{2;}$ women 22.53 ± 2.88 kg/m^2	Low doses of lactitol (ratio 0:10) beneficially affected microbiota, increasing bifidobacteria and propionic and butyric acid
Gotsner et al. [24]	Double-blind, placebo-controlled, crossover design	Isomalt 30 g/d for two 4-wk periods	19	Healthy men and women Age 21–53 y old BMI 20.8–30.2 kg/m^2	Isomalt beneficially affected microbiota, increasing bifidobacteria
Cross-sectional studies					
Frankenfeld et al. [25]	Cross-sectional	Aspartame, acesulfame-K 4-d food record	31	Healthy men and women >18 y old BMI 24.3 ± 4.1 kg/m^2	Microbiota-abundance profiles and predicted gene function were not associated with recent NNS intake; bacterial diversity differed across consumers and non-consumers
Ramne et al. [26]	Cross-sectional	Any artificially or natural sweetened beverages 4-d food record and FFQ	1085	Men and women Age 18–70 y old BMI of non-ASB consumers: 25.2 ± 4.2 kg/m^2; ASB consumers: 27.1 ± 5.2 kg/m^2	No significant associations observed between NNS intake and gut microbiota composition
Suez et al. [9]	Cross-sectional	Any artificial sweetener FFQ	381	Non-diabetic men and women Age 43.3 ± 13.2 y old No BMI details	Artificial sweetener consumption associated with changes in multiple taxonomic entities and altered glycemic response
Laforest-Lapointe et al. [27]	Cross-sectional	ASB FFQ during pregnancy Fecal samples taken at 3 and 12 mo of age	100	Infants (1 y of age) selected based on maternal ASB consumption during pregnancy (50 daily consumers and 50 non-consumers aged between 20.5 and 42.8 y of age and with a BMI between 17.6 and 42.1 kg/m^2)	Gestational exposure to ASB was associated with one microbiota cluster structure in infants

ADI, acceptable daily intake; ASB, artificially sweetened beverage; BMI, body mass index; FFQ, food frequency questionnaire; NNS, non-nutritive sweetener
*ADI for aspartame, saccharin, sucralose, and stevia: 50, 15, 5, and 4 mg/kg, respectively.

Sample sizes in cross-sectional studies varied between 31 and 1085 participants. Of the 12 studies, 10 were conducted on both healthy men and women [9,18–20,22–26], one was carried out with healthy men only [21], and one was conducted with 1-y-old infants and their mothers [27]. The age of adults ranged between 18 and 70 y, and values of body mass index (BMI) varied between 17 and 32 kg/m^2, approximately.

Clinical trials

Saccharin

To assess the main outcomes from each article, we first compared studies that were conducted on the same type of LNCSs. In Serrano et al.'s study [19], daily saccharin supplementation equivalent to the maximal daily intake (800 mg) was provided for 2 wk to 46 healthy men and women, and it did not alter the gut microbiota in any taxonomic levels. On the other hand, Suez et al.'s 2014 study [9] investigated glucose tolerance and microbial changes after a 7-d saccharin supplementation equivalent to 360 mg/d with seven healthy men and women. The researchers found that microbial composition clustered differently in individuals who had developed poorer glycemic responses before and after supplementation compared with those who maintained normal glycemic parameters [9]. Changes in microbiota were more pronounced in those who developed poorer glycemic responses (i.e, *Bacteroides* were overrepresented, and Clostridiales were underrepresented [9]). Suez et al. [18] investigated the effects of daily supplementation with 180 mg of saccharin (20% of acceptable daily intake [ADI]) on the gut microbiota, blood metabolome, and glucose tolerance of 20 individuals in comparison with five other groups, control and NNSs supplemented, of 20 individuals each. Regarding the effect on gut microbiota, saccharin supplementation significantly altered gut microbiota, increasing levels of *Prevotella copri* and *Bacteroides xylanisolven* [18]. *Prevotella copri* was positively associated with the glucose tolerance test incremental area under the curve (GTT-iAUC) at baseline, whereas *Bacteroides xylanisolven* was negatively associated with this curve. The authors suggested that these changes are detrimental [18]. Butyrate also increased during the trial. Most microbial top loadings were related to glycolysis and glucose metabolism [18].

Sucralose

Thomson et al. [21] studied how high doses of sucralose (780 mg/d) for 7 d, affected gut microbiota and metabolic response in 34 men in intervention and control groups. There were no changes in gut microbiota after the supplementation. Ahmad et al.'s study, evaluating the effect of 136 mg/d for 2 wk on 17 patients who also undertook a protocol with aspartame supplementation intervention protocol 4 wk before, did not show any changes in the microbial composition or fecal SCFAs [20]. In Suez et al. [18], however, a sucralose supplementation of 102 mg/d in 20 individuals for 2 wk did alter gut microbiota, increasing *Eubacterium* and *Dorea longicatena* during the trial. *Eubacterium* was positively associated with the GTT-iAUC at baseline, whereas *Dorea longicatena* was negatively associated with it. These changes were suggested to be detrimental by the authors [18]. Most microbial top loadings were related to purine metabolism [18].

Aspartame and stevia

Ahmad et al.'s study [20] testing the effect of 425 mg/d (14% of ADI) of aspartame for 2 wk on the same 17 individuals as mentioned previously, did not modify gut microbiota. Suez et al.'s latest study [18] tested the effect of 240 mg/d (8% of ADI) of aspartame for 2 wk in 20 healthy individuals and found that it altered gut microbiota. More precisely, during the protocol, abundances of B*acteroides fragilis, Bacteroides acidifaciens,* and *Bacteroides coprocola* increased, and many microbial top loadings were related to the polyamines metabolism [18]. Additionally, in this intervention, the intake of 180 mg/d (75% of ADI) of stevia also altered the gut microbiota: two *Prevotella spp*. were reduced and *Bacteroides coprophilus, Parabacteroides goldsteinii,* and a *Lachnospira spp.* increased during exposure [18]. Several microbial top loadings were related to fatty acid biosynthesis. No other studies eligible for this review focused on stevia.

Polyols

The main outcomes of studies focusing on polyols in this review (i.e. maltitol, lactitol, and isomalt) showed that they beneficially affected the gut microbiota in participants [22–24]. Indeed, Gotsner et al. [24] tested the effect of consumption of 30 g/d of isomalt during 4 wk on 19 individuals in a crossover design with an additional 4-wk placebo-controlled intervention of 30 g/d of sucrose. Compared with the placebo, isomalt significantly elevated bifidobacteria. According to Finney et al. [23], low doses of lactitol (10 g/d) tested for 7 d on 75 individuals increased the bifidobacterial population, which led to increased production of acetic and lactic acid. This fact can contribute to cross-feeding, and other bacteria can produce propionic and butyric acid [23]. Beards et al. [22] tested a progressive supplementation of maltitol via chocolate bars in 40 individuals during 6 wk, reaching 45.6 g/d. The protocol also benefited the gut microbiota, increasing bifidobacteria. All three studies were published between 2006 and 2010, and no other recent human studies regarding polyols were available in the literature.

Cross-sectional studies

Frankenfield et al. [25] concluded that there was a difference in microbial diversity across consumers and non-consumers of aspartame and acesulfame-K among 31 individuals after the assessment of a 4-d food record and the analyses of fecal samples provided on day 5. However, no associations were observed for the relative abundance of bacteria by class or order or for predicted gene abundance [25], findings that opposed results from the clinical trial on aspartame [18]. Suez et al. [9] analyzed the microbiota of 172 randomly selected individuals among the 381 who completed a food frequency questionnaire (FFQ) and found a positive correlation between the consumption of artificial sweeteners and multiple taxonomic entities, such as the Enterobacteriaceae family, Deltaproteobacteria class, and Actinomycetota phylum. Additionally, the Swedish and Canadian cross-sectional studies focused respectively on artificially or naturally sweetened beverage consumption [26] and ASB consumption only [27]. They were carried out with 1085 healthy men and women [26] and 100 infants (12 mo of age) selected based on maternal consumption of ASB during pregnancy [27]. The Swedish study used a 4-d food record and FFQ on visit 1, and participants had to bring back a stool sample on visit 2 [26]. In contrast, the Canadian study conducted with infants and their mothers used an FFQ during pregnancy, and infant stools were obtained at 3 and 12 mo of age [27]. In the Swedish study, no associations were found between high ASB consumption and changes in microbiota after multiple testing corrections compared with the non-consumers [26]. In the Canadian study, maternal ASB consumption was associated with one cluster structure in infants (depletion of *Bacteroides* spp) and urine succinate and spermidine, two metabolites produced by the gut microbiota [27].

Link with glucose tolerance

Many of the mentioned articles also evaluated whether LNCSs are associated with changes in glucose tolerance and if gut microbiota mediates these changes. In fact, all clinical trials published after 2010 assessed the effects of LNCSs on glucose metabolism. Still, only two observed a positive association between LNCS consumption and the development of impaired glucose tolerance [9,18]. In the Suez et al. study [18], only saccharin and sucralose supplementation altered the glycemic response of participants. Those who developed impaired glucose tolerance were defined as "responders," and those who did not were defined as "non-responders"; baseline abundance levels of various bacteria species correlated with the GTT-iAUC [18]. To test whether the NNSs-induced dysbiosis could have a causal relationship with impaired glucose intolerance, the microbiome of participants who presented the most extreme glucose tolerance responses was transplanted to germ-free mice. Mice that were transplanted the microbiome from responders developed a higher glycemic response than mice that were transferred the non-responders' microbiome [18]. Similarly, in Suez et al.'s earlier study, four of seven healthy participants developed significantly poorer glucose tolerance after saccharin supplementation [9]. Their gut microbiota clustered differently from non-responders before and after the supplementation. Stools from two responders and two non-responders were transferred to germ-free mice. Mice that received stools from responders developed impaired glucose tolerance compared with the other mice [9].

Moreover, three clinical trials suggest that the glycemic response to NNSs is, in part, driven by interindividual differences at baseline in the gut microbiota [918,21]. For instance, Suez et al. [18] found correlations between the baseline abundance of species in the microbiota and changes in glucose tolerance after each NNS supplementation. In a previous study by Suez et al. [9], seven individuals underwent a saccharin supplementation protocol. The microbiota of the individuals who presented poorer glycemic responses after the intervention (responders) clustered differently at baseline from the microbiota of those whose glycemic parameters were not altered. On the other hand, Thomson et al. [21] found that, independent of consuming sucralose or placebo, individuals who presented higher insulinemic levels after the intervention had different microbial composition at baseline. These results highlight the possibility that an individual's glycemic response to a sweetener intake might be strongly mediated by baseline microbial composition.

Discussion

This review summarized the main evidence currently available in the literature regarding the effects of alternative sweetener consumption on the gut microbiota. After analyzing the data of all eligible articles, two of the eight clinical trials retrieved concluded that NNS (saccharin, sucralose, aspartame, and stevia) consumption alters gut microbiota [9,18]. These two studies also observed a causal effect between NNS consumption and impaired glucose tolerance in mice [9,18]. The three clinical trials on polyols concluded that polyols may beneficially affect gut microbiota [22–24]. Three of the four cross-sectional studies also observed an association between alternative sweetener consumption and detrimental changes in the gut microbiota [9,25,27], and only one found an association with impaired glucose tolerance [9]. Results from this review also suggest that microbial composition at baseline could, in part, modulate the microbial and glycemic response to an LNCS supplementation.

Besides the lack of studies, the heterogeneity in main outcomes across studies on NNSs could be explained in part by differences in study designs. For example, the intervention protocols differed across the three clinical trials investigating the effect of sucralose on gut microbiota. Suez et al.'s study [18] tested 102 mg/d of sucralose for 2 wk on 20 healthy men and women. It compared changes with five other groups (supplemented either with placebo, glucose, or other NNSs) of 20 individuals [18]. Ahmad et al. [20] carried out a study with 17 healthy men and women who were previously supplemented with aspartame. These participants received 136 mg of sucralose for 2 wk [20]. As for Thomson et al. [21], they studied the effect of 780 mg/d of sucralose for 7 d on the microbiota of 34 healthy men. Only men were selected for this study to avoid any potential interference of menstrual cycle-related changes on insulin sensitivity [21]. These studies thus differed in terms of doses of sucralose administered, duration of the intervention, presence of a control group, profile of participants, and their sex and age. These differences might have influenced the microbial and metabolic response of participants after sucralose supplementation. Similar observations can be made for saccharin and aspartame [9,18,20]. As for stevia, only one clinical trial investigated its effect on gut microbiota, and therefore no comparison can be made [18].

Another possible explanation for heterogeneous results is the relatively small sample sizes in each clinical trial, varying from 7 to 120 in total. However, the study carried out with 120 participants tested four NNSs, and groups comprised only 20 individuals [18]. It is possible that the effects of NNSs on the gut microbiota could not be observed given the sizes of the cohorts or in reverse, positive correlations were observed only by coincidence. Also, smaller sample sizes give less statistical power to studies, therefore decreasing the validity of the results [28]. For example, the study conducted by Suez et al. in 2014 [9] was carried out with seven participants and concluded that short-term saccharin consumption alters the microbiota and impairs glucose tolerance. However, the validity of these results can be questioned because of poor statistical power.

The heterogeneity of results might also be explained in part by individualized microbial responses to LNCSs. In fact, as mentioned in the results section, three articles showed that baseline microbial composition might mediate the microbial and glycemic response to LNCSs [9,18,21]. Therefore, it is possible that the proportion of individuals more susceptible to exhibit an altered microbiota and glycemic response after the LNCS intervention varied across cohorts in each study, therefore creating heterogeneity in results.

In addition to the relatively small sample sizes, another limitation of the studies included in this review is the short duration of the intervention protocols, which varied between 1 and 2 wk. It is highly plausible that a 1- or 2-wk time is not sufficient to observe significant changes in the gut microbiota and, therefore, might not be representative of a long-term LNCS consumption in the general population. In fact, an article analyzing diet, microbiota, and duration of studies has pointed out the fact that habitual diets might have a greater influence on microbial composition than acute dietary strategies [29]. Further research in the field should focus on working on longer clinical trials.

Moreover, doses of LNCSs administered to humans in the studies were often not realistic, especially for saccharin, sucralose, and polyols. Given the fact that a sachet of the commercialized brand *Sweet'N Low* contains 36 mg of saccharin, an individual weighing 60 kg would reach the ADI for saccharin with 25 sachets [30]. Clinical trials on saccharin in this review studied the effects of 180, 360, and 800 mg of saccharin on the gut microbiota (5 to 22 sachets per day), which is relatively high [9,18,19]. The same constatation can be made for studies on sucralose [18,20,21]. Studies on polyols

even tested amounts ≤45 g/d [22–24]. Such quantities are not realistic, even for high LNCS consumers, which might affect the validity of the results [31]. Also, given the fact that LNCSs are present in many food products, another limitation is that it is hard to control the exact amount of LNCSs consumed by the participants during a trial. A way to manage this problem would be to teach participants how to read nutritional labels to avoid products with LNCSs.

Further, an important limitation of cross-sectional studies in this review is that they only allow observation of an association, and not a causal effect, between LNCS consumption and gut microbiota composition. Cross-sectional studies cannot determine whether the microbial composition is directly due to LNCS consumption. It is plausible that overweight or obese individuals in the cross-sectional studies have a higher tendency to consume LNCSs to control their weight or glycemia. In fact, it has been demonstrated that obesity is associated with low fecal bacterial diversity [32]. There is a risk that a positive association between LNCS consumption and microbial composition is due to (or affected by) the association between obesity and microbial composition. Although cross-sectional studies give a good first insight on this subject, clinical trials offer stronger evidence on how LNCSs affect gut microbiota.

Mechanisms by which sweeteners could possibly mediate microbial composition are still not fully understood. However, potential routes have been suggested by experts and by a recent review on the link between LNCS consumption and host physiology [33]. Thus, the first potential mechanism suggested is that LNCSs, which are not always completely absorbed by the gut compared with glucose [34,35], bind to sweet taste receptors in the gut epithelium and affect mucin production and gut barrier function. Both functions have been reported to influence microbial composition [33]. Indeed, it has been reported that mucins (proteins in the inner gastrointestinal tract's epithelium-composing mucus) influence microbial composition [36]. Second, recent evidence shows that several LNCSs might alter bacterial cell membranes and cellular permeability, possibly creating shifts in certain bacterial populations [33].

Certain microbial compositions have been associated with a higher prevalence of diseases, but it is unclear whether they are a cause or consequence of these disorders. A review of the healthy composition of the gut microbiota shows that patients with T2D have higher ratios of Bacteroidota to Bacillota [37,38]. Accordingly, in their article published in 2014, Suez et al. [9] observed an overrepresentation of *Bacteroides* and an underrepresentation of Clostridiales, an order in the Bacillota phylum, in both humans and mice after a saccharin supplementation. In other NNS groups from Suez et al.'s 2022 study [18], *Bacteroides* spp. were also increased. These small similarities between the gut microbial composition of patients with T2D and the microbiota after the NNS supplementation in Suez et al.'s study suggest that NNS supplementation might negatively affect the gut microbiota and increase the risk for T2D. On the other hand, higher levels of butyric acid have been reported to have anti-obesogenic effects, regulate energy expenditure, reduce IR, and decrease dyslipidemia [39]. A study testing the association between NNSs, fecal microbiota, and SCFAs in individuals with morbid obesity found that NNS consumption was associated with lower levels of butyric acid [40]. However, Suez et al. [18] observed an increase of butyric acid after a saccharin supplementation, which is contradictory with the development of the alteration of the glycemic response also observed.

In the three clinical trials on polyols it was concluded that polyols might be beneficial and have a prebiotic action for the gut microbiota by increasing bifidobacteria, which have health-promoting effects [41]. However, no updated studies on these LCSs have been published since 2010 and the actual evidence reviewed in this paper was tested with unrealistic doses. Some evidence shows that excessive consumption of polyols may exert laxative and gastrointestinal symptoms in healthy individuals [3,42]. Therefore, whether polyols are potentially beneficial or not for the gut microbiota is still unknown because of a lack of studies.

Globally, this review of clinical trials and cross-sectional studies on the effect of LNCSs on gut microbiota highlights the heterogeneity in results and the lack of studies in this field. Although results are too heterogeneous to draw a clear conclusion, they also suggest that personalized microbiota-driven effects might mediate the effects of alternative sweeteners on the gut microbiota. Therefore, baseline microbial composition could, in part, influence how an individual's microbiota responds to LNCSs. Other authors in the field support this hypothesis. Bourdeau-Julien et al. [43] tested the effects of an average Canadian diet and a Mediterranean diet on the gut microbiota. They observed that individuals with a higher microbial diversity at baseline had better microbial stability after dietary changes, which supports the rationale behind a personalized response to LNCSs mediated by microbiota at baseline.

Conclusion

Results on how LNCS consumption affects the gut microbiota are heterogeneous. Two of five clinical trials on NNSs (saccharin, sucralose, aspartame, and stevia) concluded that all NNS consumption alters gut microbiota and that only saccharin and sucralose alter the glycemic response to NNSs. Three of four cross-sectional studies observed an association between NNSs and microbial composition. Current evidence suggests that polyols may have a prebiotic effect, but the lack of recent studies limits the understanding of their true effect on the host's physiology. The microbial and glycemic response to sweeteners may be strongly mediated by the individual's gut microbiota composition at baseline. Studies conducted on larger cohorts, with longer durations, more realistic doses of sweeteners, and considering the personalized microbial response to sweeteners are needed.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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