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Synthetic sweeteners and their impact on the gut microbiota - current state of knowledge

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Summary:
Introduction and purpose:
The aim of artificial sweeteners is to replace the sweetness of sugar without contributing to the higher energy consumption or unfavorable metabolic effects conventional sugar causes. Synthetic sweeteners - one of the most popular groups - are labeled as safe for use in foods and represented by acesulfame K, aspartame, neotame, saccharin, sucralose, cyclamate and advantame. Although they underwent safety procedures, their possible unbeneficial effects are still being evaluated. In this paper we focus on their impact on the gut microbiome.
Brief description of the state of knowledge:
For now, the advantage of search conducted on animals is observed. Those papers show the possibility of a relationship between artificial sweeteners and composition of the gut microbiota. Changes that were observed affected the gut microbiota in a negative way as they may be responsible for causing disbalance in the conglomerate of microorganisms.
Studies on humans are limited. Long term consumption of acesulfame K and sucralose is linked with modulation of the composition of the microbiota. It has also been shown that synthetic sweeteners are able to increase the ability of bacteria to form a biofilm.
Conclusions:
Heretofore published paperworks about the impact of synthetic sweeteners on the gut microbiota are not sufficient to conduct valuable and unambiguous conclusions. Evidence collected until now is leading to a deduction that gut microbiota can be affected by the consumption of the sugar surrogates although more data is needed to clearly determine their possible role.
Key words: gut microbiota; synthetic sweeteners; health

1.Introduction

Nowadays, when the prevalence of obesity worldwide has been continuously growing, different solutions are being researched. As higher consumption of simple sugars is identified not only with obesity, but also with diseases such as metabolic syndrome with impaired glucose tolerance or diabetes, elevated blood, lipids arterial, hypertension, hepatic steatosis and cardiovascular morbidity, limiting consumption of those carbohydrates may be one of the best health-promoting activity [1]. In the light of this information non-caloric artificial sweeteners, which are defined as food additives that aim to mimic the taste of sugar, may represent a valuable tool to not only reduce calorie intake, but also other side effects table sugar causes. Synthetic sweeteners are one of the group of sugar surrogates that consist of acesulfame K, aspartame, neotame, saccharin, sucralose, cyclamate and advantame [2]. Extensive scientific research came to the conclusion that synthetic sweeteners currently approved for use in foods in the United States and Europe are

safe for human health and well tolerated if taken in recommended daily doses [3]. However, they may not be without caveats as their impact on the human's microbiota is being questioned.
The gut microbiota is a variable constantly changing ecosystem made of trillions of microorganisms that live in the intestinal tract. Microbiome is proven to have a pleiotropic impact on human's health - it modulates immune response, metabolic pathways [4]. This conglomerate is incessantly modified by many factors such as lifestyle, medicaments, diseases and also dietary patterns [5]. When it comes to individual's diet it is known that carbohydrates play a crucial role in modifying the microbiota - an excessive consumption of fructose promotes the increase of proinflammatory bacteria, endotoxin levels therefore inducing gut dysbiosis [6], so shifting to non-caloric sweeteners could be potentially helpful, therefore in this review we discuss how gut microbes are affected by consumption of synthetic sweeteners.

2.State of knowledge

2.1. Aspartame

Aspartame (E-951) was invented in 1965 by James Schlatter, who obtained this compound as part of research into antiulcer drugs. By accident, after licking it off his finger, Schlatter discovered the sweetness of aspartame [7]. Its large-scale production began in 1981 and nowadays aspartame is one of the world's widely used artificial sweeteners and is an ingredient in more than 5000 food products globally [8]. Aspartame is dipeptide, which consists of two amino acids (L-phenylanine and L-aspartic acid). It is hydrolyzed and absorbed in the gastrointestinal tract through the action of enzymes such as esterases and peptidases. Aspartame is broken into phenylalanine (50%), aspartic acid (40%) and methanol (10%) during metabolism in the body, which are absorbable in intestinal mucosa [9]. It is interesting to note that safety studies have found the metabolic products of aspartame to be more toxic than the original substance itself [2,7]. Aspartame is about 200 times sweeter than sugar, while its calorific value is almost zero [7]. According to EU regulation, all food that contains aspartame has to have a visible label with words "contains aspartame (source of phenylalanine)" to warn people with phenylketonuria, for whom dietary restriction of phenylalanine is the mainstay of treatment [2]. The acceptable daily intake (ADI) of aspartame is 40 mg/kg bodyweight in Europe and 50 mg/kg bodyweight in the United States for both adults and children [7].
The study in rats with diet-induced obesity has examined the impact of low-dose aspartame consumption on anthropometric, metabolic, and microbial variables. Changes in fecal samples, which were observed, include increase of total bacteria and abundance of Enterobacteriaceae and Clostridium leptum. In rats consuming high-fat diet aspartame also attenuated the typical high fat-induced increase in the Firmicutes:Bacteroides ratio and enriched Roseburia species [10].
In another study with estrogen-defficient rats, aspartame did not alter serum-acetate levels produced by gut microbiota, although it increased insulin level and lowered serum glucose concentration. The findings suggest aspartame with meal may be preferable sweeteners to fructose and sucrose in estrogen deficient rats, and possibly post-menopausal women, however it need to be confirmed in human studies [11].
The work conducted by Wang et al. has sought to examine the intergenerational impact of sweeteners on offspring, as they can be detected in milk as well. The research performed on 3-week-old offspring of obese dams consuming high fat/sucrose diet with aspartame showed that maternal consumption of sweeteners alters cecal microbial composition and metabolism of propionate/lactate in the offspring [12]. Nettleton et al. also presented an association between maternal low dose aspartame consumption and the gut microbiota of both groups of dams and offspring. There were minimal differences observed within the fecal microbiota of these animals, relative to the control group: an increased abundance of Clostridium leptum and enrichment of the family Porphytomonadaceae [13].
Serum metabolomics analysis in humans revealed that aspartame is rapidly metabolized in the small intestine and associated with elevations in the short chain fatty acid propionate, which is a bacterial end product and highly glucogenic substrate. However, because of this rapid hydrolyzation it is hard to understand how aspartame influences the gut microbiota [2].
The study which sought to investigate the role of consuming artificial sweeteners on gut bacterial pathogenicity and gut epithelium-microbiota interaction, using models of microbiota (Escherichia coli and Enterococcus faecalis) and the intestinal epithelium, showed that sweeteners are able to increase the ability of bacteria to form a biofilm. The exposure of model gut bacteria to different concentrations of artificial sweeteners such as saccharin, sucralose, and aspartame increased the ability to adhere to, invade and kill the host epithelium [14]. Aspartame and other examined sweeteners (sucralose and saccharin) are also suggested as responsible for changes in balance of the gut microbial community via QS - inhibition, which is a molecular system termed quorum sensing and used by microorganisms to communicate within and regulate group behaviors [15].
The recent finding reports also promoted growth of Bifidobacterium, but it was the result of fermenting fecal samples collected from healthy volunteers in batch cultures with many different food additives among others aspartame-based sweetener, sucralose and stevia. Likewise propionic acid increased and branched-chain fatty increased with aspartame-based sweetener [16].
Apart from that there is no data on the potential impact of aspartame on the human gut microbiome changes. The recent double-blinded crossover trial analyzing fecal samples collected from seventeen healthy adults consuming realistic doses

of aspartame and sucralose confirmed no differences in the gut microbiome and fecal short-chain fatty acids (SCFAs) before and after treatments [17].

2.2. Sucralose

Sucralose (E-955) is a non-caloric sweetener derived from sucrose by the selective replacement of three hydroxyl groups by chlorine atoms. This process intensifies the sweetness of sucralose to about 600 times of sucrose, thus its small amounts are widely used in foods and beverages as a sugar substitute [18]. The acceptable daily intake of sucralose is 5 mg/kg bodyweight [2]. After intake, sucralose is poorly absorbed and undergoes little metabolism. It enters unchanged into the lower gastrointestinal tract and more than 85% of the consumed sucralose reaches the colon, hence it potentially might be a microbiota perturbing agent [19].

Once absorbed, it is bound to plasma proteins, distributed throughout the body and finally eliminated by urine through active tubular transport [20]. In 2008 there was performed the first study determining the effect of sucralose on the intestinal microbiota with the use of fecal samples from rats that received the sweetener for twelve weeks. This supplementation caused the decreased total number of anaerobic and aerobic bacteria, bifidobacteria, lactobacilli, Bacteroides and Clostridium [21]. Escoto et al. also proved that chronic consumption of sucrose and sucralose in the group of mice causes a reduction in bacterial communities, negative alterations in bacterial diversity and immune parameters [22]. In the other study mice were given different doses of sucralose. What is crucial one of them (0,3 mg/ml of sucralose) was equal to the ADI and in this group a reduction of probiotics abundance (Lachnoclostridium and Lachnospiraceae) was found in cecum compared to the control group. On the other hand, Allobaculum, which was reported as positively correlated with diabetes, was increased [23]. The effects of various consumed sucralose doses were compared in rats, which were also fed a high-fat diet. Zhang et al. proved that the richness and diversity of fecal microbiota were not changed by sucralose, but some doses tended to reduce beneficial bacteria, Lactobacillaceae and Akkermansiaceae [24]. On the other hand Sánchez-Tapia et al. found that sucralose leads to the lower α-diversity of the gut microbiota and increases the abundance of Bacteroides fragilis in particular [25].

It is also proved that even maternal diet in pregnant mice shapes the microbial communities of neonates and its effect continues in later life. Maternal sucralose intake during gestation and lactation caused in 3-week-old offspring inhibition of intestinal development and disruption of the barrier function. It also induced intestinal low-grade inflammation, significantly changed the compositions and diversity of the gut microbiota including reducing butyrate-producing bacteria and cecal butyrate production [26].

In humans data are inconsistent when it comes to the impact of sucralose intake on the composition of the gut microbiota. The randomized double-blinded crossover clinical trial examining fecal samples from healthy volunteers after sucralose treatment showed no differences in the median relative proportions of the most abundant bacterial taxa, the microbiota community structure and fecal SCFAs before and after treatments [17]. On the other hand, there are at least two studies assessing the effects of sucralose consumption in humans. The latest work revealed that long-term sucralose intake could induce gut dysbiosis evidenced by alteration in abundance of Firmicutes without affecting Actinobacteria and Bacteroidetes. The study also showed a 3-fold increase in Blautia coccoides and a 0.66-fold decrease in Lactobacillus acidophilus compared to the controls [27]. Although there are no conclusive studies in humans, current evidence indicates that sucralose could change the composition of the gut microbiota.

Sucralose, like aspartame, might increase the ability of bacteria to form a biofilm [14]. The other already quoted study revealed that addition of sucralose to fecal samples received from healthy volunteers and their fermentation shifts microbiome community structure by increasing the abundance of Escherichia/Shigella and Bilophila [16].

2.3. Acesulfame K

Acesulfame K (E-950) is the potassium salt of acesulfame used as one of the major low-calorie artificial sweeteners in the modern diet. It undergoes metabolization by the human body, which is found as innocuous, although previous studies have described acesulfame K as genotoxic and inhibiting glucose fermentation by the intestinal bacteria [2,28]. Acesulfame K belongs to sulfonamides, which is a chemical class known from its antimicrobial features. It is about 200 times sweeter than sucrose and has an ADI of 15 mg/kg bodyweight [28].

The study in mice which received acesulfame K turned out that the total bacteria, Firmicutes, Bacteroidetes, and several other genera were similar in comparison to the control group [29]. In contrast, the gut microbiota of CD-1 mice was perturbed after 4 weeks of acesulfame K intake. Following changes were highly gender-specific: in males Bacteroides, Anaerostipes, and Sutterella were increased, while in females Mucispirillum also increased, but Lactobacillus, Clostridium, an unassigned Ruminococcaceae genus, and an unassigned Oxalobacteraceae genus were depleted [28]. In another study, in female mice, acesulfame K intake caused decreased relative abundance of Lactobacillus and Clostridium [2]. The study conducted by Wang et al. in adolescent mice showed that acesulfame K possesses a bacteriostatic effect evidenced by a strong inhibition on the growth of Escherichia coli [30]. Recent studies in rats indicate that acesulfame K intake during mice pregnancy has consequences on the progeny, causing an increase in Firmicutes and a depletion of Akkermansia muciniphila [31].

In the human trial, after 4-day acesulfame K treatment, there were no differences in median bacterial abundance between consumers and nonconsumers of the sweetener. However, bacterial diversity changed compared to the control group [32].

2.4. Cyclamate

Cyclamate (E-952) is an artificial sweetener which has an intensely sweet taste. It was discovered in 1937 by Michael Sveda. It is interesting to note that not all humans have the ability to convert cyclamate to cyclohexylamine. This process takes place in the gastrointestinal tract by intestinal flora [33].

In 1970 cyclamate was entirely outlawed by the FDA. The main reason for such a decision was the result of an experiment on rats, where after providing a diet made of cyclamate-saccharin and cyclohexylamine the rats ended with the bladder tumors [34]. This experiment was criticized by several medical representatives providing that it was not relevant enough to state that it causes the bladder tumors. Therefore the cyclamate is still available and admitted in the European Union, with daily doses 7 mg/kg bodyweight, whereas in the USA such sweetener is forbidden, after the FDA's decision [35].

The study from 2019 made by Emanuel Vamanu has sought to examine the sodium cyclamate impact on the gut microbiome and metabolomic response in vitro. In order to compare the effects on the microbiota pattern that was altered by the in vitro treatment of various sweeteners (sodium cyclamate was used in one group), healthy untreated microbiota were used as the control sample. This result obtained from this study showed that groups with the sodium cyclamate resulted in higher levels of the ammonia and acetic acids compared to the control sample. However, the level of the butyric and propionic acids decreased after in vitro sodium cyclamate treatment. It is interesting to note the effect of sodium cyclamate on the pattern of the microbiota where the number of copies of bacteria such as: Bifidobacterium, Enterobacteriaceae, Lactobacillus were higher than in the control group. Hence, cyclamate has a significant impact on gut microbiota [36].

On the other hand, another study contributing to a similar experiment on monkeys, ended with different results. The experiment was prosecuted in order to evaluate the effect of oral consumption of cyclamate on fecal flora of monkeys investigating the presence of Catenabacteria, Peptostreptococci, Clostridia, Bacteroidaceae, Bifidobacteria, lactobacilli, streptococci, enterobacteria, staphylococci and Veillonella. The constitution of the monkeys' fecal flora that was treated with cyclomate was not quite different from that of the normal monkeys from the control group [37,38].

Mallett et al. who conducted the experiment on rats were focused on evaluating the effect of the metabolic cyclamate on the gut microbiota. This was in vitro study which analyzed the anaerobic culture system. The maximum formation of cyclohexylamine, which corresponded to a 2-3% molar conversion of cyclamate to cyclohexylamine, was reached in about eight weeks. Although any gross taxonomic changes were not visible and present in microflora [39]. However the study concluded that numbers of clostridia increased in the feces of rats treated by cyclomate. The research made by Drasar et al. concluded that in humans, dietary cyclamate did not appear to alter the numbers of different fecal microbes [40].

2.5. Saccharin

Saccharin (E-954) was discovered in 1879 by Constantin Fahlberg. It is an artificial, non-nutritive sweetener. It has 200–700 times more sweetness compared to table sugar. In the 1970s saccharin was associated with the emergence of bladder cancer during the studies on rats. Although in 2000 The National Institutes of Health's National Toxicology Program came to the conclusion that saccharin should be taken off the list of probable carcinogens.

One of the first studies that analyzed the impact of saccharin on the gut microbiome was conducted by Anderson et. al in 1980. This study analyzed the effect of sodium saccharin on the rat's caecal microflora which contained aerobic and anaerobic populations of bacteria. Despite observing increased ammonia production by Proteus vulgaris the results show that even high doses of the saccharin in the caecal did not change the total numbers of anaerobic microorganisms. However, the numbers of some bacteria which are sensitive to saccharin were lower than in the control group in vitro [41].

In order to determine the impact of saccharin on the gut microbiome, Jotham Suez and other researchers carried out the research on mice. This particular research relied on culturering fecal matter in vitro from naive mice under the anaerobic conditions. Such experiment resulted in increase of the Bacteroidetes phylum and significantly resulted in reduction of Firmicutes [42]. However other studies showed different results. For example, the study by Kristian Daly et al. conducted an experiment on piglets. During this experiment, the numbers of Lactobacillus located in caecal increased after intervention with saccharin.

Moreover, another experiment conducted by Suez et al. found out that a saccharin diet can help to reduce the lactobacilli from metabolism [43,44,45]. The study from 2021 made by Shil et al. focused on the models of microbiota with Escherichia coli and Enterococcus faecalis. As the results changes in these models were found after the saccharin intervention. The ability to be invasive of E. faecalis increased [14].

In recent years the topic about the impact of saccharin on the gut microbiomes has gained popularity. A good example of this trend is the double-blind study in humans made by Serrano et. al from 2021. The participants were randomized to the one of four groups which were different only by substances that were put in oral capsules (placebo, saccharin, lactisole, saccharin with lactisole). Members achieved the maximum ADI of substances for two weeks. The results did not reveal any changes in taxonomic levels of microorganisms or metabolomics profiles compared to the control group [43].

2.6. Neotame

Neotame (E-961) is a safe and well tolerated sweetener, which is sweeter than sucrose from 7000 to 13000 times. Its chemical structure is very similar to aspartame. The ADI of neotame is 0,2 mg/kg bodyweight. The appearance of the sweetener is white powder and it has a sugar taste with licorice aftertaste. Thanks to its properties, it can be used in baking as substitutes of table sugar [46]. Although neotame has been approved for general use since 2002 the effect on the gut microbiome is still unclear. The study in mice from 2018 shows that a four-week neotame consumption led to decreased Firmicutes and increased Bacteroidetes. Likewise the difference in metabolic patterns and fecal metabolite profiles was observed [47]. Due to the fact that neotame is allowed to be used for a relatively short time the number of studies is extremely limited.

3.Conclusions

The increasing prevalence of obesity and its metabolic comorbidities have led to a growing replacement of simple sugars by artificial sweeteners. Nowadays, whereas sweeteners are used in many food processes, the potential modifications of the gut microbiome caused by artificial sweeteners in healthy adults and children are a matter of concern. Carried out animal trials allows us to conclude that these sugar alternatives may induce many compositional and functional alterations in the gut microbiota. It is interesting to note that even maternal intake of some sweeteners such as aspartame and sucralose during gestation and lactation influences the microbiome communities of neonates.

However, understanding how consumption of synthetic sweeteners affects the gut microbiota in humans remains challenging. Based on the analyzed literature we conclude that long-term intake of sucralose could shift the composition of the human microbiota. Furthermore, acesulfame K may be responsible for changing bacterial diversity, although it did not cause differences in median bacterial abundance between consumers and nonconsumers of this sweetener. Additionally, sucralose, saccharin and aspartame might increase the ability of bacteria to form a biofilm and are also suggested as responsible for changes in balance of the gut microbial community via QS-inhibition. Apart from some in vitro studies in simulation systems results, we have no data determining the effects of cyclamate on the human gut microbiota so far.

Mentioned results are still insufficient and further research is needed to elucidate whether the changes observed in the intestinal microbiota in animals are present in humans as well. Due to the many variables that may change the composition of the gut microbiome it is difficult to design and conduct studies with repeatable results, but understanding the effects of sweeteners for which evidence is not available so far could have in the future a dramatic impact on public health in a multitude of ways.

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