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Review

Low calorie sweeteners and gut microbiota

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HIGHLIGHTS

- The low calorie sweetener, NHDC, enhances the population abundance of gut *Lactobacillaceae*.
- NHDC reduces lag phase of *Lactobacillus* growth and enhances expression of its sugar transporters.
- A bacterial plasma membrane receptor is required for the sweetener-induced proliferation of *Lactobacillus*.
- Fecal specimens are poor representatives of the microbial content of the large intestine.

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ABSTRACT

Studies dating back to 1980s, using bacterial cultures, have reported associations between low calorie sweeteners (LCS) and alterations in bacterial composition, raising the potential that LCS might exert effects on the host via interactions with gut microbiota. However, the results of a few recent studies carried out in this area have produced controversies. There is evidence that human fecal samples, used in most human microbiome studies, may provide a poor representation of microbial contents of the proximal intestine. Furthermore, fecal short chain fatty acid levels do not exemplify the amount of short chain fatty acids produced in the intestine. Short chain fatty acids are largely absorbed in the intestine by a tightly regulated mechanism. Here we present an exemplar study showing that the determination of the molecular mechanism(s) underlying the precise mode of action of a LCS on gut microbiota allows for rational and scientifically-based recommendations.

Contents

1. Gut microbiota

The human body is inhabited by a vast number of microorganisms that live in harmony with the host. The majority reside in the large bowel. The gut microbiota performs an essential role in maintaining

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health, by being a major contributor to key processes such as nutrition, immunity and protection against harmful microbes [1]. Disruption in the stable establishment of commensal gut microbiota, allowing pathogenic bacteria to flourish, is an important factor in the development of a number of metabolic diseases [2].

The composition and activity of the gut microbiota is shaped by a number of factors including diet, environmental elements and the host's genetic background. Most notably, diet and dietary factors are major determinants of gut microbiota composition and activity [3]. The recognition that gut microbiota plays an essential role in conferring health benefits on the host has recently generated tremendous interest in designing dietary approaches to enhance the growth of beneficial gut microbiota [4]. Such strategies will be greatly benefited by furthering our understanding of the underlying mechanisms by which dietary components influence the diversity and dynamics of gut microbial communities. This will facilitate the development of scientifically based strategies to assist in the establishment and maintenance of a beneficial gut microbiota.

Traditional culture-based approaches to characterize microbial communities can only recover a small fraction of the total diversity. However, the advent of next-generation sequencing technologies has transformed the way in which microbial ecosystems are studied. Comparative analysis of 16S rRNA gene sequences provides the phylogenetic framework for describing community structure, allowing characterization of microbial ecosystems in unprecedented detail and resolution. This characterization is fundamental to understanding the dynamics of bacterial community structure, identifying predominant populations and determining how these may be influenced by environmental factors such as dietary composition.

Defining the interrelationships between gut composition, microbial community structure and the functional operations of the gut microbiome is a key step to advancing our understanding of the efficacy of feed components and dietary supplements. This will allow the design of new dietary approaches to manipulate the composition and activity of the gut microbiota in order to enhance gastrointestinal health and well- being of individuals.

2. Use of sweeteners

Evidence from large epidemiological studies and randomized trials has implicated excessive sugar consumption to adverse health consequences, prompting leading healthcare professionals to recommend population-wide reductions in the intake of refined sugars [5]. The substitution of caloric sugars for low calorie sweeteners (LCS) (also referred to as non-nutritive sweeteners or artificial sweeteners) in foods and beverages is one approach to promote adherence to these recommendations. LCS are highly potent sugar substitutes that permit reductions in the energy density of foods and beverages, while maintaining high palatability. There is, however, a great deal of controversy regarding the health consequences of LCS consumption, with conflicting studies suggesting beneficial [6–8] harmful [9,10] or trivial [11,12] outcomes. These controversies have resulted in major misunderstandings amongst the public regarding the use of LCS in foodstuffs.

3. Low calorie sweeteners and gut microbiome

Studies dating back to 1980 have reported associations between LCS exposure and alterations in bacterial dynamics in vitro [13–15] raising the possibility that LCS might exert effects on the host via interactions with gut microbiota. However, relatively little detailed and well-controlled studies have been carried out to assess the direct effect of sweeteners on human gut microbiota.

It has been shown that intake of LCS, such as lactitol or maltitol, increases the population abundance of some beneficial bacteria (lactobacilli and bifidobacteria) in human fecal samples and thus demonstrating prebiotic effects [16,17]. It has also been reported that xylitol consumption by mice positively affected the metabolic activity of a number of gut microbial populations [18]. However apart from the knowledge that these LCS sugar alcohols are metabolized by the gut microbiota, nothing is known about the molecular basis of their effects.

A recent study by Suez et al. [19] describes a series of observations concluding that artificial sweeteners induce glucose intolerance via changes in gut microbiota. Giving mice artificial sweeteners, saccharin, sucralose and aspartame in drinking water, they suggest that these sweeteners reach the large intestine and directly interact with the microbial community, provoking changes in the population dynamics of gut microbiota, leading to metabolic derangements. These conclusions, especially with regard to the effect of aspartame, which is completely hydrolyzed in the small intestine to its constituent amino acids and therefore would not reach the microbiota intact, are highly debatable. In further work, adding commercial saccharin to the drinking water of mice (at maximal accepted daily intake levels recommended by the Food and Drug Administration), they report that this induces glucose intolerance in both low and high-fat diet-fed obese mice, with antibiotic treatment seemingly reversing this metabolic disorder. Analysis of fecal microbiota composition revealed noticeable differences in microbial composition and function, and also concentrations of fecal short chain fatty acids between saccharin-treated mice and controls. Saccharin consumption led to compositional alteration in bacterial taxa that had previously been linked to type II diabetes in humans. In the study of Suez et al. [19] no information on the potential molecular basis of saccharin induced-dysbiosis leading to glucose intolerance was reported. It was also described by these authors that four out of seven human volunteers consuming saccharin developed poorer glycemic response, with three showing no difference. It was further stated that the fecal microbiome of the four individual responders clustered differently from the three non-responders after consumption of saccharin. However the microbiome configurations of the responders also clustered differently from non-responders before saccharin consumption. Moreover, microbial taxa identified by Suez et al. [19] to be altered in a cohort of human sweetener consumers, compared to non-consumers, were not reported to be altered in the mouse models. It is worth noting that these studies were carried out using fecal samples, with interpretation of data extrapolated from rodents to humans. Additionally, concentrations of short chain fatty acids in the feces, reported by these authors to be characteristic of increased microbial energy harvest, do not mirror that in the intestinal contents; normally a large proportion of produced short chain fatty acids are absorbed across the intestinal epithelium.

Altogether, these findings should be interpreted with caution until large scale randomized controlled trials, using more biologically relevant doses of sweeteners, are carried out. Furthermore, there is a need to assess the underlying molecular mechanisms in order to determine the specific effect of a sweetener on gut microbiota composition.

3.1. Systems to study diet-gut microbiota interactions

Most human intestinal microbiome studies have relied on the use of fecal specimens, or on extrapolation of data from investigations employing rodents as models. Although easily acquired, fecal specimens are increasingly being recognized as poor representatives of the microbial content of more proximal regions of the intestine and of the mucosal-associated microbiota [20]. Furthermore, fecal short chain fatty acid levels do not exemplify the amount of short chain fatty acids, SCFA, produced in the intestine. SCFA are largely absorbed in the intestine by a tightly regulated mechanism. High fecal concentrations of total or individual SCFA may be the result of low intestinal absorption, the rate of transit or shifts in microbial cross feeding patterns [21]. There are also physiological and metabolic differences between rodents and humans, and, depending on the type of studies, inferring the results of investigations using rodent models to that in humans may lead to misleading scientific interpretations. Moreover, in investigations using mice and human subjects for assessing potential relationships between