HHS Public Access

Author manuscript

Nutr Today. Author manuscript; available in PMC 2022 May 01.

Published in final edited form as:

Nutr Today. 2021 ; 56(3): 105–113. doi:10.1097/nt.0000000000000481.

An Overview of Current Knowledge of the Gut Microbiota and Low-Calorie Sweeteners

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Abstract

This review provides an overview of the interrelationships among the diet, gut microbiota and health status, and then focuses specifically on published research assessing the relationship of low/no-calorie sweeteners (LNCS) to selected aspects of the gut microbiota. Microbiome research is expanding as new data on its role in health and disease vulnerability emerge. The gut microbiome affects health, digestion, and susceptibility to disease. In the last 10 years, investigations of LNCS effects on the gut microbiota have proliferated, though results are conflicting and are often confounded by differences in study design such as study diet, the form of the test article, dosage, and study population. Staying current on microbiome research and the role of dietary inputs, like LNCS, will allow healthcare and nutrition practitioners to provide evidenced-based guidance to the individuals they serve.

What is the microbiome?

The human body is more than its human components. Trillions of mircroorganisms, termed the microbiome, reside on and within the human body including in the gut, oral and nasal cavities, vagina, and on the skin (Box 1)[1, 2]. The microbiota is comprised of bacteria, archaea, fungi, and viruses and represent a diverse array of species and functional genes[1, 3].

The diversity of the microbiota is represented both within and between individuals, with each person harboring a unique microbial community, the majority of which reside in the colon and are termed the “gut microbiota”[1]. However, these microbes are not passive passengers. The gut microbiome can provide beneficial functions, such as metabolism of undigested food components, vitamin production, and supporting immunity. However, while certain diseases have been associated with abnormal microbiota (i.e. dysbiosis), it is unclear what constitutes a “healthy” gut microbiome[1]. Indeed, the composition of the gut microbiota is variable throughout the gastrointestinal tract (i.e., stomach, ileum, descending colon;

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mucosa to lumen), across geography, with age, and in relation to a host of other lifestyle factors among healthy individuals, demonstrating that the gut microbiota is a dynamic component of human physiology[4, 5].

A variety of approaches are used to determine differences in which microbes are present (composition), their genetic potential (functional potential), and what the microbes are doing (function)[2, 6]. To generate taxonomic classifications of microorganisms (e.g., *Bifidobacterium*, *Lactobacilli*), DNA is isolated from the study samples, typically fecal samples in human studies, and then a specific region of the DNA known as the 16S rRNA gene is amplified so that it can be used to classify the taxa present and characterize the relative abundances of the taxa with the samples. Similarly, metagenomic sequencing creates a sequence database of the full microbial genome so that the genetic potential of the microbiome can be characterized (e.g., the presence of the enzyme used to metabolize a β2–1 linkage, which is found in inulin). A limitation of 16S amplicon and metagenomic sequencing is that it is not possible to determine if the microorganisms are alive or dead at the time of sequencing. To determine what the microbes are actually doing at the time of measurement, RNA gene expression, proteins, and metabolites are measured using metatranscriptomics, metaproteomics, or metabolomics, respectively. Thus, these methods assess outputs or the functions of the microbial community that may affect the host. Therefore, an integrated approach may allow for a more comprehensive view of host-microbe interactions and identification of dietary components that may be used to manipulate the gut microbiota to benefit health and reduce the risk of developing certain diseases.

What is the evidence that diet can influence the microbiome?

Given the role of the gut microbiome in metabolism of dietary components, there is robust evidence that intake of specific food components[7–11] as well as broad dietary patterns[12] influence the gut microbiome over both short[13, 14] and long[12] time scales. Additionally, recent research suggests that food choices may be more important than nutrient profiles in influencing microbiota composition[14]. Indeed, specific foods have been shown to induce transient changes in the gut microbiota composition[7–11] that may then be used to predict intake of those foods[15]. Diet has been shown to outweigh the effect of genetics on the gut microbiome[16], suggesting that the gut microbiota is affected by modifiable lifestyle factors.

However, there is increasing recognition that functional changes in gene expression and metabolite production may be more important than the often transient changes in the taxonomic profile of the microbiota[17]. The gut microbiota metabolizes substrates to produce new bioactive compounds that then may impact host metabolism and immunity[18–20]. For example, some dietary fibers are metabolized by the gut microbiota to produce short-chain fatty acids (SCFAs), including acetate, butyrate, and propionate[18, 19]. In infants, human milk oligosaccharides (HMOs) not only enrich specific bacteria, primarily *Bifidobacteria*, but also result in the production of metabolites such as SCFAs[21]. These metabolites elicit concentration-dependent physiological effects, which are postulated to underly the associated health benefits of dietary fibers including, improved glycemic control, satiety,

weight loss, increased mineral absorption, decreased inflammation, and overall improvement of digestive and intestinal health[18].

Specific dietary components used to target the gut microbiota include prebiotics, probiotics, and synbiotics (Box 2)[22–24]. The benefits of certain probiotics[22] have been systematically reviewed under the auspices of different evidence-based organizations, including American Gastroenterological Association (AGA)[25], Journal of Family Practice[26], World Gastroenterology Organisation[27], European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[28–31], Cochrane[32], and European Food Safety Authority[33]. Notably, the health benefits of probiotic consumption are dependent on the strain(s), dose (>1×10^9 colony forming units (CFU)/serving), and duration of consumption[22]. Similarly, consumption of certain prebiotics is associated with a range of health benefits, including bone, gut, heart, and mood, though the associated benefits are dependent on the type of prebiotic[23, 34, 35]. Currently established prebiotics include certain dietary fibers that impact the abundance and functionality of gut microorganisms, namely *Bifidobacterium* and *Lactobacilli*[23]. A synbiotic may be a combination of a probiotic and a prebiotic (complementary synbiotic), though the individual components do not necessarily need to meet the criteria for pro- and pre-biotics as long as they act synergistically when co-administered (synergistic synbiotic)[24]. As with pro- and pre-biotics, the potential health benefits of synbiotics depend on the duration of use, the strain of microorganism, and the type and amount of nondigestible substrate, as well as factors such as the individual's baseline microbiota, diet, medication, and potentially genetics[24].

How might the microbiome influence health and the response to dietary components?

Increasing evidence suggests that the gut microbiome influences the response to diet and may be a mediating or moderating factor in certain health outcomes[36–39]. The gut microbiome is associated with hallmarks of metabolic syndrome including obesity and type 2 diabetes in humans and these associations are further supported by mechanistic trials using microbiome transplants in animal models[40]. However, it remains challenging to extrapolate the findings from animal models to human health, thus more research is needed before evidence-based recommendations can be made. The gut microbiota may partially mediate the relationship between diet and the development of obesity and type 2 diabetes by metabolite production that affects host energetics or signaling pathways that influence metabolic or inflammatory processes[41]. For instance, SCFAs, namely butyrate, are used as an energy source for colonocytes; SCFAs also interact with G-protein coupled receptors 41 and 43 (GPR41 and GPR43), which induce peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) secretion thereby improving insulin signaling[18]. Increases in lipopolysaccharide (LPS), a component of gram-negative bacteria cell walls, induce low grade, chronic inflammation that may contribute to obesity and type 2 diabetes[41]. Ultimately, the gut microbiota may impact a whole suite of related metabolic conditions by modulation of metabolic and immunologic pathways.

The gut microbiota could also modulate cardiovascular disease risk[42]. SCFAs and LPS modulate blood pressure and vascular function that may influence risk of cardiovascular disease[42]. Also, microbial metabolites trimethylamine (TMA), which is derived from dietary choline, phosphatidylcholine, and carnitine and converted to trimethylamine-N-oxide (TMAO) in the liver by flavin-containing monooxygenase 3 (FMO3), and phenylacetylglutamine (PAG), derived from phenylalanine, have been associated with cardiovascular disease risk[42]. However, associations between TMAO and disease do not indicate causation but rather may be confounded by other factors including kidney function, the gut microbiome, and FMO3 genotype[43]. Furthermore, there is uncertainty about the connections between dietary intake and TMAO concentration. Microbial modulation of the bile acid pool may also influence cardiovascular disease risk[42]. Fat intake (quantity and type), protein source and amino acid composition, as well as fiber and polyphenol intake have an impact upon microbial production of secondary bile acids[7, 44], thereby providing a potential link between diet and microbiota-mediated health outcomes.

Therefore, research suggests that the gut microbiota could mediate diet-induced effects on health outcomes, though more clinical work is needed to substantiate these effects. However, recent research has demonstrated that consumption of the same foods differentially affects the gut microbiome in different people[14] and this variability contributes to interindividual differences in the acute metabolic response to dietary intake[36]. This is the basis for funding for Nutrition for Precision Health, powered by the All of Us Research Program by the National Institutes of Health Common Fund[45]. This initiative provides the opportunity to expand upon the current research to better understand how diet affects individuals differently and how to optimize diet for individual health across the lifespan. From growth and development, particularly of the immune system, during infancy and childhood to mitigation of increases in inflammation and decline of muscle, bone, and brain integrity with age, the diverse and dynamic gut microbiome may contribute a variety of health outcomes in humans[46].

Considerations for future diet-microbiome studies

Increased interest in diet-microbiome interactions and advances in the molecular and computational approaches used to study the microbiome has resulted in an explosion of research in this area[47]. However, a lack of standardization or recommendations for microbiome and dietary data collection has led to potential risk of confounding with other factors, as well as a high degree of heterogeneity between study designs, data collection, and analysis limiting the ability to compare results and conclusions between studies[6, 17, 47].

Potential for confounding by both inter- and intra-individual variability may be minimized by stratifying participants by potential confounders such as baseline microbiota, age, gender, diet, lifestyle factors, and medications; collecting multiple microbiome samples per assessment timepoint coupled with multiple days of dietary history prior to each sample; standardizing collection times; and increasing sample size[17, 47, 48]. The potential confounding factors may change based on the intervention, the research question(s), and/or the population, so participant demographics, metabolic features, longitudinal and cyclical

considerations, supplement and medication use, bowel habits, and environment should be considered[47].

Dietary intervention descriptions and methods used to assess habitual dietary intake must be well documented to ensure replication and comparisons among studies[49]. When complete feeding studies are not feasible or appropriate for the hypothesis being tested, stabilizing diet (i.e. having participants maintain their habitual dietary intake)[47] should be considered. Dietary intake aspects beyond nutrient composition, such as intake of specific foods, cooking, and food matrix must also be considered as these factors affect the type and amount of nutrients, particularly fibers, available to the gut microbiota due to changes in digestibility and absorption[8, 14, 15, 50, 51].

What are low- or no-calorie sweeteners and why is there interest in the gut microbiota?

Low/no-calorie sweeteners (LNCS) are compounds that provide sweet taste without the calories or carbohydrates associated with table sugar (i.e. sucrose) or other caloric sweeteners. Common LNCS include acesulfame potassium (acesulfame K), advantame, aspartame, monk fruit extract, neotame, saccharin, sucralose, and steviol glycosides (e.g. rebaudioside A)[52]. LNCS have risen in popularity as the food and beverage industry has shifted to reducing added sugars in their products[53]. Prior to reaching the market, all permitted LNCS have undergone extensive safety evaluations by scientific and regulatory agencies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA), and/or the U.S. Food and Drug Administration (FDA), resulting in the establishment of Acceptable Daily Intakes (ADI) for each sweetener[52, 54, 55] (Table 1).

LNCS types vary in their digestion, absorption, metabolism, and excretion[56–58], meaning that the effects of one isolated LNCS on the gut microbiome cannot be extrapolated to all LNCS. For instance, while both saccharin and sucralose are not metabolized, saccharin is rapidly absorbed and excreted in the urine while sucralose is poorly absorbed and is excreted in the feces[56, 58]. Conversely, rebaudioside A is hydrolyzed by the gut microbiota to the parent compound steviol that is subsequently absorbed and excreted in the urine[56, 58]. Therefore, differences in absorption and chemical conversion of these sweeteners or their components may lead to differences in their ability to interact with the gut microbiota throughout the intestinal tract. A 2014 study[59] reported a link between LNCS exposure, the gut microbiota, and glucose intolerance that spurred intense interest in this field. Additionally, in vitro evidence suggests that LNCS may also promote the transfer of antibiotic-resistance genes between microbes[60]. However, despite continued research, there remains a great deal of uncertainty regarding the effects of LNCS on the gut microbiome and any resulting impacts on human health[55–57, 61, 62].

Considerations for LNCS-microbiome studies

Several study design elements should be considered when evaluating the results and conclusions of LNCS-microbiome studies. These include the study diet, the form of the test

article, the dose and exposure, and the study population[55]. Both short- and long-term dietary patterns affect the gut microbiota. Therefore, studies must control for or record dietary intake to ensure that any dietary impact on the gut microbiota is accounted for. Also, commercial LNCS formulations typically contain small amounts of the sweetener molecule itself and primarily consist of carbohydrate bulking agents, such as maltodextrin[55]. Therefore, studies should be conducted with the pure, unadulterated sweetener as well as with the bulking agent to ensure that effects are not solely due to the bulking agent. Sweeteners should also be investigated individually, as differences in their chemical structures lead to differences in their metabolism and potential to affect the gut microbiota[55–58]. Importantly, to ensure relevance to human health, LNCS doses should not exceed the sweetener's ADI.

The study population is another consideration to ensure relevance to human health. Rodents colonized or transplanted with defined microbes (gnotobiotic) that are hypothesized to play a role in the metabolism of certain nutrients or from human donors with a specific phenotype (e.g., disease) are useful in illuminating potential modes of action of the connections between diet, the gut microbiota, and health[63]. However, differences in gastrointestinal physiology, microbiota compositions, effects of genetic background in mice, coprophagy, housing conditions and feeding all limit the translation of rodent research[64]. Most studies have been conducted in animals and in vitro models, limiting biological relevance due to differences in the rodent gut microbiome[6, 65, 66] and limitations in extrapolating tested concentrations in vitro to human exposure levels from the diet.

Using a combination of in vitro, animal, and human models will enable the determination of both clinical effects on health and the gut microbiome as well as mechanisms by which the gut microbiome may mediate the effects on health. For instance, while taste receptors are expressed throughout the gut and may be activated by LNCS, it is unknown whether activation of these receptors may also modulate microbial composition or function[61, 67]. It is postulated that activation of these receptors may be an important mechanism by which LNCS could modulate the gut microbiota since the extremely small doses used (e.g. milligram amounts) are lower than the 3 g/day dose required for most compounds to elicit a direct effect on the gut microbiome[23, 61]. Mechanistic studies will therefore complement the findings of clinical trials on the effects of LNCS on human health and the gut microbiome. Human studies, preferably randomized controlled trials, are necessary to be able to make evidence-based recommendations.

What does the literature report on the effects of LNCS on the gut microbiota?

Briefly, a literature search identified relevant articles on LNCS and gut microbiota using the following inclusion criteria: 1) in vivo studies conducted in animals and/or humans (in vitro studies excluded); 2) testing one or more orally administered LNCS; and 3) evaluation of the gut microbiota[55].The summarized results of the literature search are presented in Table 2[59, 68–89].

The majority of studies were conducted in animal models, with sucralose being the most commonly investigated LNCS. The effects of LNCS on the gut microbiota reported in the scientific literature are unclear in humans and experimental data are needed that control for confounding factors. Of the identified nonclinical and clinical studies, only four remained after removing those with confounding factors, such as dose in excess of human ADI or dose not reported, diet not equivalent between groups or controlled, small sample size of one subject per group, or use of non-equivalent control group or no control group. However, it should be noted that even doses at or below the ADI in animal models may not be relevant to humans due to differences in gastrointestinal physiology and digestion as described above. All human clinical trials contained at least one confounding factor that disqualified them from the final analysis. For instance, habitual diet was not controlled in any of the four clinical studies. One study was cross-sectional and therefore could not report the dose or amount of LNCS consumed[71]. Additionally, two of the studies did not have a control group[59, 73].

The results of investigations of several LNCS were inconclusive after removal of confounded studies, including results for aspartame, cyclamate, neotame, and saccharin. Of the remaining studies, Uebanso et al. investigated the effect of both acesulfame K (15 mg/kg/d, 8 weeks) and sucralose (low dose: 1.5 mg/kg/d, high dose: 15 mg/kg/d, 8 weeks)[68]. Studies by Li et al. (low dose: 5.5 mg/kg/d, high dose: 139 mg/kg/d, 4 weeks) and Nettleton et al. (2–3 mg/kg/d, 9 weeks) investigated the effects of rebaudioside A[88, 89]. The high dose of rebaudioside A in the study by Li et al. is in excess of the ADI (~10x) and therefore was excluded from the analysis. Both acesulfame K and rebaudioside A show no effects on the gut microbiota in mice[68, 88], though rebaudioside A did alter the composition of the gut microbiota in rats[89]. These changes in the gut microbiota composition were accompanied by an increase in the cecal concentrations of acetate and valerate, which were positively correlated with fat mass and total weight[89]. The one study of sucralose found a dose-dependent decrease in fecal *Clostridium IVXa* in mice[68]. Thus, the literature shows marginal effects of LNCS on the rodent gut microbiota at doses relevant to human consumption. The implications of LNCS consumption on the human gut microbiome and effects on health outcomes are therefore unclear. The ability to draw conclusions from the literature is hampered by the limited number of studies without confounding factors.

Conclusions

Microbiome research is an emerging area of science, with many new research opportunities arising as novel links between the gut microbiota and different dietary components or aspects of health are investigated. Continued research is critical as the gut microbiome is an integral part of human physiology that is impacted by diet as well as other factors such as age, physical activity, genetics, health status, medication, and environmental exposures. A crucial component of this relationship is dietary intake. The two-way relationship between diet and the gut microbiome has implications for human health and disease. Future research should focus on establishing links between specific changes in the gut microbiome and human host health effects, as well as dietary components that may contribute to or reduce the risk of such effects via microbial modulation while controlling for potential confounders in study design.

There is no clear evidence that LNCS adversely impact the gut microbiota when consumed by humans at approved levels [55]. However, gut microbiota changes as a result of LNCS consumption have been demonstrated in some animal studies[56, 57], warranting further investigation into the potential effects of long-term exposure in humans. Unfortunately, due to the popularity of LNCS, media headlines often overstate the study implications and should be interpreted with caution. Confounding and study design limitations make it difficult for researchers and clinicians to interpret study results. Future studies should reduce confounding factors by controlling the diet, using pure forms of LNCS and investigating the effects of bulking agents, administering doses below the ADI, and selecting a relevant study population. Further research will help elucidate the effects of LNCS on the gut microbiota and human health.

Acknowledgements

RLH and HDH received funding to write the manuscript from the Institute for the Advancement of Food and Nutrition Sciences (IAFNS) through an ILSI North America Low-Calorie Sweeteners Committee grant. IAFNS is a is a nonprofit science organization that pools funding from industry collaborators and advances science through the in-kind and financial contributions from public and private sector participants. Drs. Cindy D. Davis and Alexandra Lobach approved the final version of the article with respect to representation of their webinar content. All contributors read and approved the final manuscript.

Conflicts of Interest and Funding Disclosure: This work was supported by the Institute for the Advancement of Food and Nutrition Sciences (IAFNS) through an ILSI North America Low-Calorie Sweeteners Committee grant. IAFNS is a is a nonprofit science organization that pools funding from industry collaborators and advances science through the in-kind and financial contributions from public and private sector participants.

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Box 1. Definitions of the microbiome

Microbiome: the collection of microbial genomes[1].

Microbiota: the collection of microbial organisms[1].

Gut microbiome: the collection of microbial genomes inhabiting the gastrointestinal tract[1].

Metagenome: the collection of all of the genetic material in a sample[2].

Metagenomics: the study of collected genomes from an ecosystem to understand the taxonomic and functional properties of microbial communities[2].

Metatranscriptomics: the study of RNA gene expression from microbial communities[2].

Metaproteomics: the study of proteins from microbial communities[2].

Metabolomics: the study of small molecules (i.e., metabolites) within a sample[2].

Box 2. Definitions of pre-, pro-, and syn-biotics

Probiotic: live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[22].

Prebiotic: a substrate that is selectively utilized by host microorganisms conferring a health benefit[23].

Synbiotic: a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host[24]. May be a combination of a probiotic and prebiotic (complementary) or individual components that act synergistically when co-administered (synergistic)[24].

Table 1.

Currently permitted low/no-calorie sweeteners in the U.S.[52]

Low/no-calorie sweetener	ADI (mg/kg bw/d)	Sucrose sweetness equivalence	Max daily mg intake based on 60kg person
Acesulfame K	15	200 x	900
Advantame	32.8	20000 x	1968
Aspartame	50	200 x	3000
Monk fruit extract	NS*	100–250 x	--
Neotame	0.3	7000–13000 x	18
Saccharin	15	200–700 x	900
Sucralose	5	600 x	300
Steviol glycosides	4	200–400 x	240

*Not specified

Abbreviations: ADI, Acceptable Dietary Intake; mg, milligrams; kg bw, kilograms bodyweight; d, day

Table 2.

Results of literature search on the effects of LNCS on the gut microbiota

Low/no-calorie sweetener	Nonclinical studies*	Clinical studies*	Studies without any confounding factors*	
			Number	Gut microbiota findings (compared to control)
Acesulfame K	• 3 studies in mice[68–70]	• 1[71]	1[68]	• No change reported in mice[68]
Aspartame	•1 study in mice[59] • 1 study in rats[72]	• 2[71,73]	0	• Inconclusive
Cyclamate	• 1 study in monkeys[74]	• None	0	• Inconclusive
Neotame	• 1 study in mice[75]	• None	0	• Inconclusive
Saccharin	• 3 studies in mice[59,79] • 1 study in rats[76] • 2 studies in piglets[77,78]	• 1[59]	0	• Inconclusive
Sucralose	• 9 studies in mice[59,68,70,80–85] • 1 study in rats[86]	• 2[73,87]	1[68]	• Dose-dependent ↓in fecal *Clostridium IVXa* in mice[68]
Rebaudioside A	• 2 studies in mice[85,88] • 1 study in rats[89]	• None	2[88,89]	• No change reported in mice[88] • ↓ Clostridiales family XIII, *Ruminococcaceae UCG 005*; ↑ *Akkermansia muciniphila*, *Bacteroides goldsteinii*, *Bacteroides thetaiotaomicron* in rats[89]

*Confounding factors may include: dose in excess of human ADI or not reported, diet not equivalent between groups or controlled, small sample size of one subject per group, or use of non-equivalent control group or no control group. Table adapted from ILSI webinar slides.