REVIEW

Adaptation of the Gut Microbiota to Modern Dietary Sugars and Sweeteners

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ABSTRACT

The consumption of sugar has become central to the Western diet. Cost and health concerns associated with sucrose spurred the development and consumption of other sugars and sweeteners, with the average American consuming 10 times more sugar than 100 y ago. In this review, we discuss how gut microbes are affected by changes in the consumption of sugars and other sweeteners through transcriptional, abundance, and genetic adaptations. We propose that these adaptations result in microbes taking on different metabolic, ecological, and genetic profiles along the intestinal tract. We suggest novel approaches to assess the consequences of these changes on host–microbe interactions to determine the safety of novel sugars and sweeteners. *Adv Nutr* 2020;11:616–629.

Keywords: dietary sugars, artificial sweeteners, gut microbiome, evolution, adaptation

Introduction

Sugar is ancient. Its earliest harvest from domesticated sugarcane dates to between 9000 and 6500 BC in Oceania (1). The word sugar derives from the Sanskrit word *sarkara*, a reference to the granulated texture of sugar (2). Its cultivation later spread to China and India and subsequently imported into Europe. While originally limited to medicinal applications and reserved as a luxury item of nobility, the rise of slave-operated sugar plantations in South America and the Caribbean in the 17th century reduced the cost of sugar and increased its availability (3). Thereafter, sugar was added to everyday foods—tea, fruits, breads, and meats—available to the working class and became a necessity of Western life (3).

Today in the United States, added sugar consumption is >100 g per person per day (4), 4 times the recommended maximum intake (5). Current sugar consumption reflects an increasing trend. American sugar consumption 100 y ago is estimated at 62 g per person per day, and for 1813, the estimate is <10 g per person per day (6). The excess sugar in diets has been hypothesized to be causative of numerous modern diseases prevalent in Westernized cultures. These diseases include metabolic syndrome and its component diseases obesity, diabetes, cardiovascular disease (7–9), liver disease (10), tooth decay (11), and cognitive diseases, including Alzheimer disease (12, 13).

The link between sugar and these diseases has been postulated to be at least partially through the gut microbiome (14–16). The implication is that increased consumption of existing sugars and novel sweeteners has altered the carbohydrate pools available to the microbiome, creating distinct environments in the gutthat are filled by exogenous microbes or endogenous microbes that have undergone adaptation, some of which are pathogenic.

This review provides an overview of how diet defines the gut habitat of microbes and a preview of what may occur with dietary change. The discussion includes both compositional and transcriptional changes in the gut microbiome and evolutionary changes that lead to strain variations. Here we do not discuss the (nearly compensatory) loss of complex carbohydrates and fiber from Western diets but instead refer readers to other reviews (17, 18). In this review we consider the role of added sugars and sweeteners in shaping the microbiome. Our discussion highlights the demonstrated malleability and evolvability of microbes to changing environments, the potential dangers of pathogenic

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.

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Abbreviations used: GLUT, glucose transporter; PTS, phosphotransferase system; TLR, toll-like receptor.

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TABLE 1 Usage and absorption of common dietary sugars and sweeteners in the United States[1]

Sugar/sweetener	Date first approved for use in the US	Single-serving amount	Daily consumption per kilogram body weight	Percentage (%) absorbed or consumed in small intestine	Percentage (%) absorbed or consumed in large intestine	Percentage (%) in feces
Sucrose	NA	35 g	1–2 g	>95	<5	<1
High-fructose corn syrup-55	1970	30 g	1–2 g	See fructose and glucose; fructose absorption increases with glucose co-consumption	See fructose and glucose	See fructose and glucose
Glucose	NA	25 g	1–2 g	>95	<5	<1
Fructose	NA	25 g	1–2 g	90	10	<1
Trehalose	2000	3 g	0.5 g	>20	Not reported	Not reported
Sorbitol	1972	2–90 g	<1 g	25	75	<1
Erythritol	1996	500 mg–10 g	<1 g	90	10	<7
Xylitol	1960	300 mg–1 g	<1 g	50	50	1
Mannitol	1950	40 mg	35 mg	25	75	<3
Stevia (Rebaudioside A)	2008	30 mg	2 mg	60% between the small and large intestine	60% between the small and large intestine	5% as steviol
Aspartame	1981	120 mg	8.7 mg	70% of methanol; 85% of phenylalanine; >95% of aspartic acid	30% of methanol; 13% of phenylalanine; <3% of aspartic acid	0% of methanol; 2% of phenylalanine; 2% of aspartic acid
Saccharin (benzoic sulfimide)	1959; usage limited 1972–1977	30 mg	<5 mg	95	Not reported	3
Sucralose	1998	40 mg	1.6 mg	10–30	Not reported	70–90
Acesulfame potassium	1988	30 mg	5 mg	95	Not reported	Not reported

[1]Full information and references are provided in Supplemental Table 1. NA, not applicable.

microbes utilizing novel sugars and sweeteners to enhance their colonization and virulence, and approaches to studying the impact of dietary sugars on the microbiome. As well, we introduce the concept of microbial biogeographical identities to describe how the same microbe can be functionally distinct when residing in different gut environments and how these differences can promote genetic adaptation and strain divergence.

Current Status of Knowledge

How do changes in dietary sugars and sweeteners redefine the microbial habitats of the gut?

The gut microbiome is defined as the assemblage of microbes and their habitat (19). For the gut, this environment is established by the host's genetics and external environmental factors, which includes diet. While the host's species identity exercises significant control over the microbiome (20, 21), within a specific host, environmental factors are the dominant controllers of microbiome composition (22, 23). Diet has been demonstrated to be capable of restructuring the microbiome within days, yet is typically reversible on a similar timescale (22, 24–26). Of the macronutrients, carbohydrates and nitrogen sources have been demonstrated to be the most influential (18, 27–29), and simple sugars can override host genetic effects on the microbiome (30).

Roughly 48% of the caloric intake in the American diet is carbohydrates (31), with 13% coming from added sugars (32). Modern types of added sugars and sweeteners in the Western world are naturally occurring oligosaccharides, sugar alcohols, and glycosides as well as synthetic sugars. Additionally, some natural and artificial sweeteners do not contain sugar moieties but comprise peptides or other molecules. Termed low-calorie sweeteners, these are nonnutritive or low calorie due to a combination of being poorly metabolized (sugar alcohols and some of the intense sweeteners) in the human body and/or providing the effective sweetness of sucrose at very low doses (intense sweeteners). For simplicity, we refer to compounds with sugar moieties as sugars (oligosaccharides, sugar alcohols, and glycosides) and others as sweeteners. The major sugars and sweeteners consumed in America today are displayed in **Table 1**, and further details are shown in **Supplemental Table 1**. Notably, in the past 50 y, several novel sweeteners have been created and other natural sugars have been supplemented into foods (**Figure 1**).

These additions not only changed the types and amount of the sugars and sweeteners Americans consume, but also reduced the consumption of sucrose. Concurrent with research in biochemistry and the nutritional sciences, the microbiome field must assess the effects of these sugars and sugar substitutes on human physiology via alterations in the structure and/or function of the gut microbiome.

The effect of sugars on microbial physiology has been a cornerstone of microbiology. Catabolite repression was initially discovered through the observation that in the presence of glucose, some nonglucose metabolizing enzymes

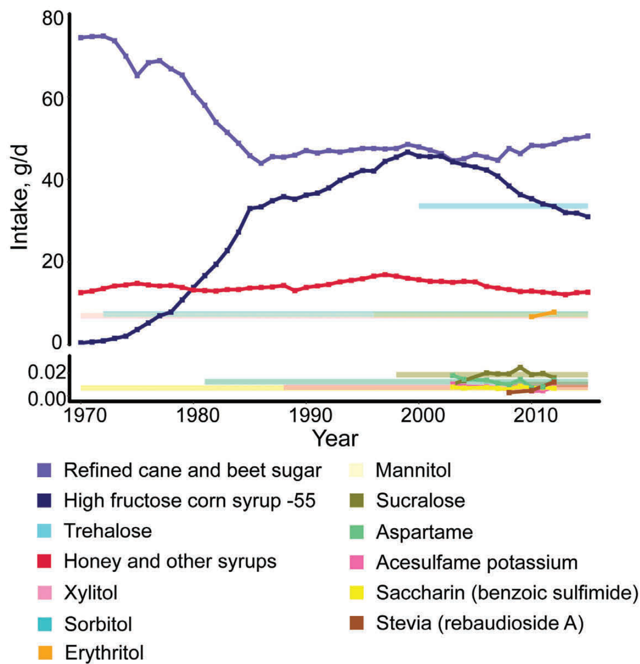

FIGURE 1 Consumption trends for common dietary sugars and sweeteners in the United States. Food manufacturers are not required to report the amount of sweeteners in food. Here, intake amounts are estimated where limited data are available (dark lines and points) and hindcasted (transparent lines) where no data are available. Consumption data for refined cane and beet sugar, high-fructose corn syrup, and other sugars/sweeteners were acquired from the US Food and Agriculture Economic Research Service (33). Estimated consumption for acesulfame potassium, aspartame, erythritol, saccharin, stevia, and sucralose were calculated from the percentage of adult Americans consuming low-calorie products (34, 35) multiplied by the market share of the sweetener (36–43) multiplied by the daily amount consumed for a 62-kg individual (Table 1). The hindcasted amount for trehalose was based on anticipated usage (44). All other hindcasted values are the average of estimated intake to illustrate the years these products were used as food additives.

are suppressed (45). This observation was later extended to other carbohydrate sources (46, 47), with the overall assumption that in the presence of a more energy efficient nutrient source, cells conserve energy by turning off other costlier metabolic pathways. Recent single-cell studies have expanded this concept and demonstrated that this metabolic switch is determined on a per cell basis rather than at the community level (48). These foundational works imply that microbial metabolic activities can change immediately in response to the introduction of a novel sugar. As we discuss subsequently, this immediate response posits microbes for additional avenues of adaptation, whether by changing their population size or by genetically diversifying.

The behavior of microbes in response to sugar is more complex and difficult to predict and study in the gut than in culture. Microbial exposure to sugars and sweeteners varies along the intestinal tract as a result of how readily each sugar/sweetener is absorbed by the host (Table 1, Supplemental Table 1). Most sugars and sweeteners are actively absorbed in the small intestine through sugar transporters, resulting in only 5–30% of these sugars and sweeteners reaching the large intestine (49). Consequently, the small intestinal gut environment is enriched ∼10-fold in sugars and sweeteners compared with that in the large intestine. These available sugars appear to be important substrates for microbes in the small intestine as small intestinal microbes are enriched in carbohydrate uptake and utilization genes and transcripts with respect to microbes in the large intestine (50).

Sugars and sweeteners are not absent in the large intestine, however. Fructose, sugar alcohols, and some sweeteners (e.g., sucralose) are passively, slowly, or very poorly absorbed in the small intestine (Table 1, Supplemental Table 1). Up to 30–90% of these sugars and sweeteners pass into the large intestine. Furthermore, overconsumption of these sugars and sweeteners readily results in malabsorption and overflow to the large intestine. The exact amounts of sugars/sweeteners that reach the large intestine, however, is difficult to generalize across individuals. Individuals (and females compared with males) display considerable variation in their absorptive capacity for a sweetener or sugar (51–56). Notably, the fructose transporter termed glucose transporter (GLUT) type 5 (GLUT5) is not present in infants (57), and in adults, its absorption is enhanced in a dose-dependent manner in the presence of glucose (58). Moreover, the small intestine adapts to repeated sugar ingestion by increasing the expression of sugar transporters, hydrolases, and other catabolic enzymes (59–61). In addition, complex sugars/sweeteners are broken down into simpler compounds by the host or by gut microbes, thereby adding an extra layer of complexity to the total profile of nutrients available to gut microbes. As a result, the concentration of sugars/sweeteners in the gut is not a simple product of what the host consumes, but rather is dependent on the individual host's absorptive capabilities and the metabolic activities of gut microbes. Therefore, we cannot assume a single profile of sugars/sweeteners in the gut but must instead consider concentration gradients of each sugar/sweetener throughout the gut (**Figure 2**).

How are microbes altered by changes in dietary sugars and sweeteners?

Summing over the complexity of host sugar/sweetener absorption, microbial products, and varying intestinal conditions produces a surfeit of gut microenvironments along the intestinal tract. In the Restaurant Hypothesis (62), each of these environments can be thought of as a restaurant serving different foods. A microbe thrives in the environment that best meets its nutrient and environmental needs, its niche [as defined by Hutchinson (63)], and presents the least competition from other microbes. The gastrointestinal tract is not a homogenous microbial culture, but rather

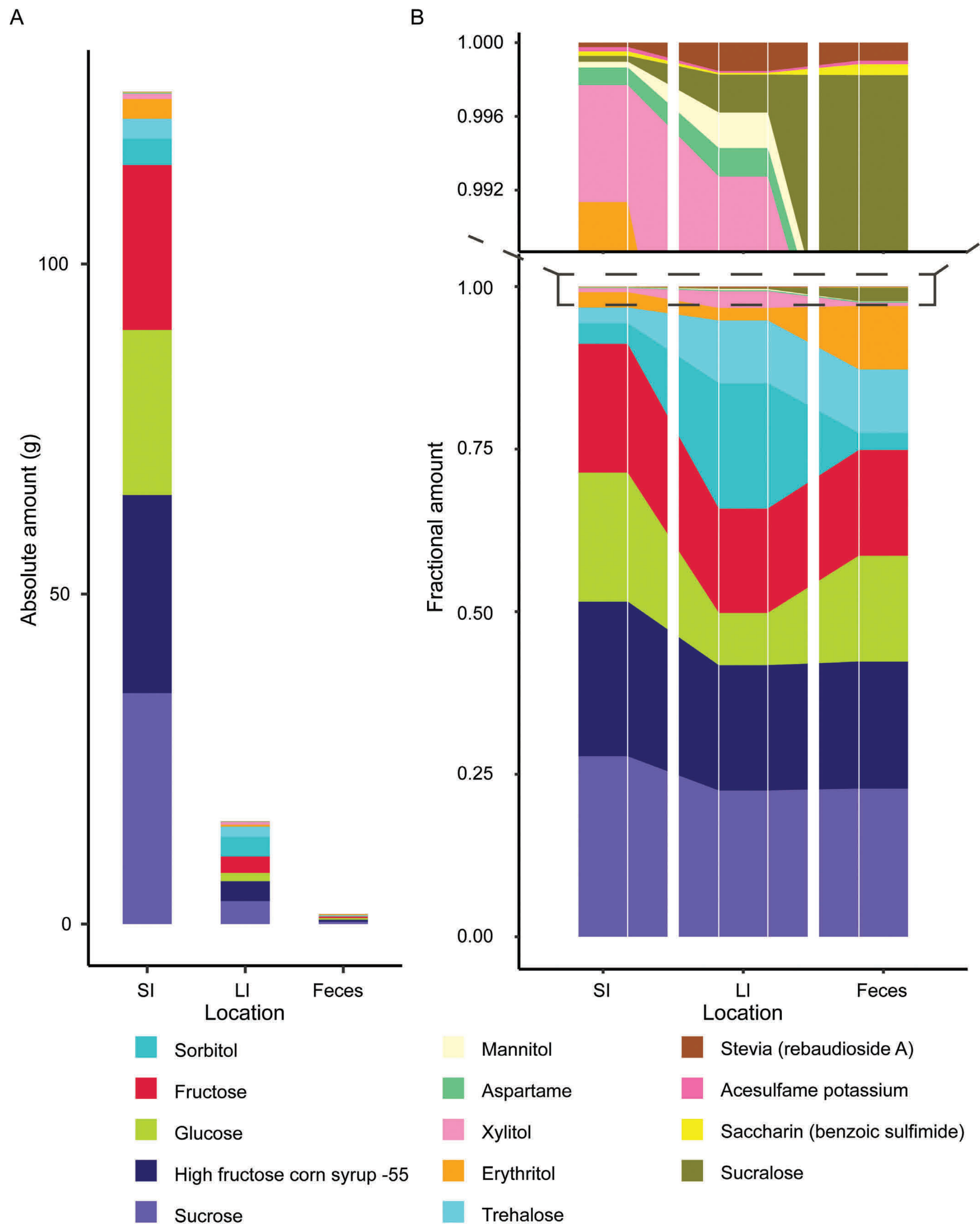

FIGURE 2 Absolute (A) and fractional amounts (B) of common dietary sugars and sweeteners in the SI and LI and in feces. Data assume all sugars/sweeteners are consumed at once in the amounts estimated for a single serving for a 62-kg individual. The amount/fraction of trehalose, erythritol, and acesulfame potassium in feces is unknown. We predict that 10% of these sugars/sweeteners are present in the LI and 90% in the SI. For (B), the stacked bar plots for SI, LI, and feces directly above the label are calculated fractional amounts. The additional bar plots on one or both sides of these central bar plots illustrate gradients of sugars/sweeteners in the intestinal tract. These gradients are estimated amounts in the transition between the different regions of the intestinal tract, which are separated by thick white lines. Differences among the SI, LI, and feces reflect differences in the host absorption and microbial consumption of these sugars/sweeteners along the intestinal tract (Table 1). LI, large intestine; SI, small intestine.

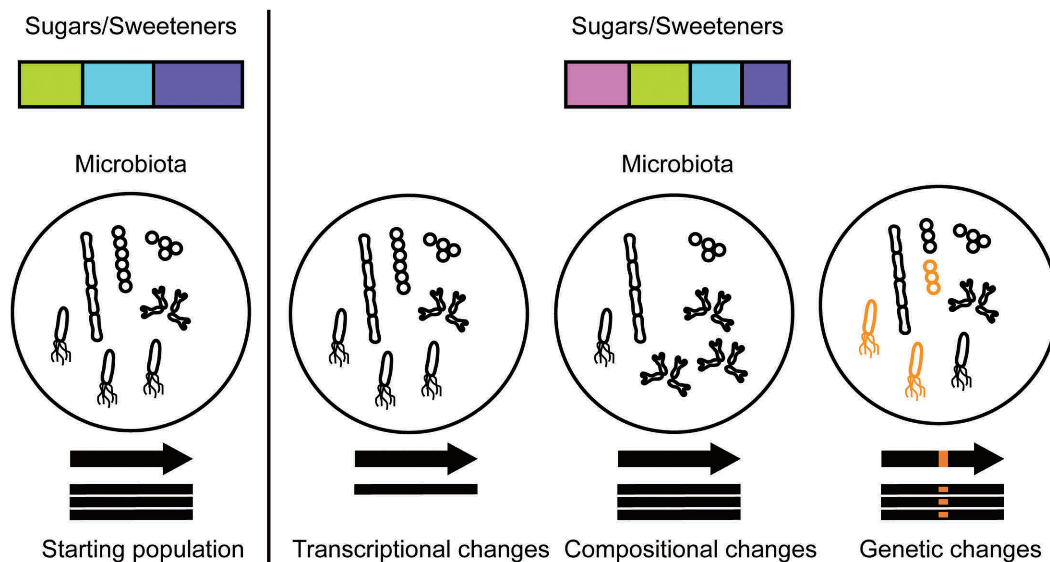

FIGURE 3 Changes in sugar and sweetener consumption lead to *1*) transcriptional, *2*) compositional, and/or *3*) genetic changes in gut microbes. The colored bar plots represent illustrative fractional compositions of sugars/sweeteners in the gut. *1*) In response to a changed set of dietary sugars/sweeteners, microbes can alter their transcriptional profiles to best utilize the new nutrient pool. These changes can manifest as extensive metabolic alterations and lead to *2*) changes in microbiome composition, whereby microbes whose niches are best filled increase in abundance. *3*) Alternatively or in addition, strain diversification within a species (indicated by the orange microbes) could occur allowing existing microbes to alter their niches and utilize the new compound. This later scenario is reflected in genetic changes to the microbiome. A combination of all scenarios is expected in real microbiomes.

displays biogeography (64), whereby different microbial communities exist at distinct gut locations. We propose that microbial habitation in these different microenvironments (restaurants) contributes to the observed biogeography of the gut. In other words, the variation in microbes found along the gut is dependent on the variation in sugars/sweeteners present along the gut.

Importantly, this hypothesis leads to an additional postulate, namely that the same microbe could exist in multiple, different, spatially separated microenvironments. To best utilize each microenvironment, the same microbe may require different regulatory, metabolic, and genetic adaptations. Pathobionts may be the result of such adaptations: these are commensal organisms that, while normally benign, become pathogenic under specific host conditions or at specific gut locations (65). As any microbe can be functionally altered by the local gut environment, we suggest the term "microbial biogeographical identities" as a generalization. Here we use this concept to discuss the mechanisms of adaptation, the resulting microbial products, and the potentially altered host interactions resulting from different microenvironments.

Specifically, changes to the sugar/sweetener pool in the gut lead to 3 mutually inclusive predictions regarding the effect on microbes (**Figure 3**). First, microbes can differentially regulate their metabolic networks to accommodate altered microenvironments via catabolite repression or other regulatory pathways. Transcriptomics and metabolomics have the capacity to capture these signatures of dietary change. Second, transcriptional changes and the availability of carbon substrates can alter which microbes are present and their abundance in each microenvironment. Such changes manifest as microbial community compositional shifts typically analyzed by 16S ribosomal RNA gene sequencing. Third, microbes can genetically adapt to altered conditions to maximize use of the new microenvironments, akin to the selection of antimicrobial resistance. As we discuss in the following, strain variation is rarely studied, but in studies designed to detect such events, genetic adaptation is observed.

Regarding the first prediction, that microbes can differentially regulate their metabolic networks, the first route available to a microbe for adaptation to an altered nutrient pool is by altering the transcription and protein levels of relevant metabolic and transport proteins. It has been observed that glucose and fructose suppress polysaccharide utilization genes (66) by catabolite repression. These sugars suppress the regulatory protein of polysaccharide utilization genes in *Bacteroides thetaiotaomicron* in the presence of other nonsuppressing sugars and negatively impact the colonization ability of this bacterium in mice (66). As well as affecting metabolism inside the bacterial cell, external metabolites present in the gut are also altered by dietary sugar. In mice fed diets where the sole carbohydrate source was either glucose or fructose, the fecal levels of several short-chain fatty acids, amino acids, and sugars differed in the following ways: butyrate and propionate were lower, while succinate, lactate, taurine, tyrosine, threonine, phenylalanine, and xylose were

higher in fecal samples from mice fed a fructose diet than in mice fed a glucose diet (67).

These transcriptional changes pave the way for the second predictionof global microbiome compositional changes in the gut. While compositional changes in the microbiome due to dietary change have been observed in an abundance of studies, few studies have compared diets that vary only in the presence of a particular sugar. One successful study added 30% fructose (by weight) to the drinking water of mice on either a high-sugar diet (by energy: 26% sucrose, 44% starch, 12% fat, and 18% protein) or a high-sugar/high-fat diet (by energy: 30% sucrose, 13% starch, 42% fat, and 15% protein) (68). This addition of fructose altered the microbiome, albeit modestly, in animals on both diets, demonstrating that the addition of a single sugar can impact microbial abundances. Similarly, in rats fed combinations of high-/low-fat (by energy: ∼12% compared with 45%) and high-/low-sucrose (by energy: 3.2% compared with 17%) diets, the animals fed high sucrose, irrespective of fat content, displayed a reduction in fecal microbial diversity and compositional changes in select gut microbes (69).

The aforementioned studies concern sugars consumed at the gram level per serving. What of the intense sweeteners that are consumed within the milligram range? Can even lower amounts (tenths or thousandths) of a novel compound introduced to the diet affect the gut microbiome? The work of Suez et al. (70), while not without limitations, provides some indication that the answer is yes. In mice fed saccharin at levels expected to be a 5-fold excess from what people actually consume [5 mg/(kg per d) compared with a predicted 1.6 mg/(kg per d)], the abundances of several taxa were altered (70). Moreover, saccharin, though not a carbohydrate, appeared to select for microbes with differential gene content for carbohydrate and other macromolecule biosynthesis pathways. While transcriptional changes were not measured, these gene content changes suggest that regulatory events like catabolite repression may be occurring (70). It should also be noted that the host was impacted by the saccharin-altered gut microbiota. Germfree recipient mice receiving stools from saccharin-fed animals displayed glucose intolerance similar to that of the donor mice (70). These phenotypes could be derived simply by colonizing germfree mice with mouse fecal microbes that had been cultured with saccharin (70). The authors were also able to observe similar findings in people. In a cohort of nondiabetic people, several clinical markers indicated worse glucose handling in individuals consuming saccharin than in controls (70). As well, these investigators fed saccharin for 1 wk to individuals who did not normally consume saccharin. Some of these individuals (4 of 7) had increased blood glucose concentrations and developed a microbiome that when transferred to germfree animals elevated the blood glucose concentrations of the mice (70). While further clinical research is needed in this area, this work suggests that consumption of milligram amounts of a sweetener may induce functional changes in the microbiome and that such changes are dependent on the microbes present.

The third prediction, regarding genetic adaptation and strain emergence, has been more elusive to address. Improved detection methods in strain isolation and metagenomic sequencing have verified that strain variation exists in the human microbiome and within single individuals (71–76). However, only recent publications have been able to provide direct evidence for dietary sugars driving strain variation.

Sousa and colleagues were able to demonstrate strain emergence and coexistence with the original bacterial population in response to dietary galactitol, a sugar alcohol derived from galactose. When these investigators inoculated mice with an *Escherichia coli* strain with a mutation disrupting the ability to make galactitol, part of the *E. coli* population reverted and regained galactitol metabolism, leading to the coexistence of both galactitol-negative and galactitol-positive strains (77). Importantly, the abundances of galactitol-positive *E. coli* strains in mice co-colonized with both strains were dependent on the amount of galactitol in the mouse diet, while the abundances of galactitol-negative strains were dependent on the total microbiome composition. These findings thus implied that the galactitol-positive strains were able to utilize a poorly exploited galactitol microenvironment, whereas galactitol-negative strains competed with the collective microbiome for other carbon sources.

A similar finding was observed from the paradoxical results of Yin and colleagues. In their study, they tested the survival of *Lactobacillus plantarum scrB* mutants, incapable of metabolizing sucrose, and their wildtype counterparts (78). The strains were singly inoculated into mice fed a high-sucrose diet (by energy: 30% sucrose, 13% starch and maltodextrin, 40% fat, and 17% protein). A day following inoculation, *L. plantarum* sucrose mutants were cultured from fecal samples at 115% higher levels than wildtype *L. plantarum* (78). These results demonstrate that *L. plantarum* can shift its metabolic needs to other resources in the gut and, in doing so, it adapts to a less competitive niche. If we use the Restaurant Hypothesis as an analogy, this shift would be akin to *L. plantarum* deciding between eating in a restaurant with excellent food but booked to capacity or going to a less crowded restaurant with worse food. Given the competition for the first restaurant, the second restaurant is a better choice. Interestingly, the addition of the *scrB* mutant induced only modest changes in the total gut microbiome. This observation suggests that adaptation to a different sugar source could lead to strain diversification and potentially replacement within a single species without impacting the gut microbiome composition. Such an event would be undetectable if only the microbial community structure was profiled.

Remarkably, these studies demonstrate that diverged strains, originating from the same parental population, can coexist. Coexistence in these cases can be explained by negative–frequency-dependent selection (79). As one strain increases in abundance, it depletes the nutrient pool and subsequently reduces the collective fitness of all organisms

dependent on that nutrient. This depletion is analogous to a restaurant running out of pie, but still having cake. If some of the patrons diversify to preferring cake, then the restaurant is able to achieve maximal capacity, serving both pie and cake consumers. Similarly, strain diversification in a microenvironment prevents exhaustion of and complete reliance of the local microbial population on one nutrient by shifting at least part of the microbial population to using a different nutrient. As a result, resources are partitioned in the microenvironment and closely related strains coexist.

Evidence supporting multiple and spatially distinct strains was recently found in the human gut. In looking at 602 isolates of *Bacteroides fragilis* from 30 fecal samples belonging to 12 individuals, Zhao et al. observed variations in genes, including the homologs *susC* and *susD* (80). These genes are predicted to function in importing polysaccharides, and variation in these genes may reflect a selective pressure on utilization of the host rather than of dietary polysaccharides. Importantly, the evolutionary divergence of coexisting isolates from a single individual suggests niche differentiation. Moreover, the lineages of those isolates remained stable over 1.5 y despite evidence of selective sweeps within the lineages. The authors note that these observations suggest the lineages are spatially separated, in support of our concept of microbial biogeographical identities.

Recently, Collins and colleagues illustrated the possibility of strain selection due to an added dietary sugar occurring in humans (81). These authors observed that the hypervirulent RT027 and RT078 *Clostridium difficile* ribotypes are able to utilize low levels of trehalose effectively through 2 different mechanisms (81). RT027 strains contain a mutation in the repressor gene *treR*, which regulates expression of phosphotrehalase (*treA*) involved in the catabolism of trehalose-6-phosphate to glucose and glucose-6-phosphate. This mutation results in higher expression of the phosphotrehalase at low trehalose concentrations. RT078 strains, meanwhile, have acquired a second genetic locus that includes homologs of *treA* and *treR*, a putative trehalose-specific phosphotransferase system (PTS) system IIBC component transporter gene (*ptsT*), and a putative glycan debranching enzyme gene. In an in vitro model of *C. difficile* infection, the RT078 strains with a functional *ptsT* were able to outcompete strains deleted for this gene. Moreover, the authors were able to demonstrate that the ability of the RT027 strain to utilize low amounts of trehalose increased *C. difficile* virulence in mice.

While the *C. difficile* RT027 and RT078 strains existed prior to the introduction of trehalose as a food additive, the emergence of these strains as "hypervirulent" follows the introduction of trehalose in the global food market. This study illustrates a paradigm of microbial adaptation to a modern dietary sugar impacting human health. It should be noted that where selection for these strains first occurred may not have been in humans. The RT078 ribotype is prevalent in livestock, and direct transmission between livestock and humans has been demonstrated (82). Consequently, the reservoirs of strain diversity may exist in nonhumans and the environment, while selection occurs in humans.

C. difficile is unlikely to be the only microbe that has undergone selection due to dietary sugars. Over evolutionary history, enterococci host adaptation and speciation is correlated with acquisition of carbohydrate transporters and other utilization genes for dietary sugars (83). Moreover, metagenomic data suggest that variation in sugar metabolism is present in many coexisting strains in the gut microbiome (72).

Are there other mechanisms by which dietary sugars and sweeteners can affect gut microbes?

So far, we have limited our discussion on the effects of dietary sugars/sweeteners to their roles as nutrients for the microbiome. It must be considered, however, that some of these compounds may inhibit or be toxic to the gut microbiome. Indeed, for several dietary sugars and sweeteners, this situation has been demonstrated to be the case. Xylitol was introduced to foods for this very functionality. In the 1970s, it was observed that oral bacteria are unable to utilize, grow, or adapt to xylitol (84, 85), and hence xylitol is frequently added to oral products and gums. Similarly, some strains of *Lactobacillus reuteri* are growth inhibited by stevia glycosides (86); and sucralose inhibits growth and activates heat stress, DNA damage, and membrane damage responsive promoters in *E. coli* (87). Nevertheless, it must be kept in mind that these sugars and sweeteners are not universally toxic and microbes capable of metabolizing these compounds exist (88, 89).

While it is straightforward to hypothesize that the effects of dietary sugars and sweeteners on gut microbes are solely direct, it would be naïve to ignore concurrent changes in the host environment. Thaiss et al. observed that when at least 8 g/L (44 mM) glucose is applied to Caco-2 cells, the cell–cell junctions become more curvy and sinuous after at least 24 hours (90). These changes are hypothesized to cause the reduced mucus thickness and impaired barrier integrity observed under hyperglycemia, which in turn leads to endotoxin translocation, an immune response, and alterations in the gut microbiome (68, 90). It should be noted, however, that luminal concentrations of glucose in the small intestine are not regularly this high. Glucose concentrations of 25–100 mM can be observed in rats consuming a 67% by energy glucose diet, but these concentrations are only observed in the upper small intestine and for <8 h (91). Normal concentrations of glucose in the mammalian small intestine range from 0.2 to 48 mM (91). Additionally, hyperglycemia is defined as a fasting blood glucose concentration >11 mM (92). Therefore, the specific cellular effects of glucose on intestinal cells leading to loss of barrier integrity need to be further investigated.

What pathways could be affected in adapted microbes and how might these adaptations affect the host?

One way to conceptualize the microbial and host processes affected by microbial adaptation is to follow the sugar/sweetener from the gut lumen to inside the microbe

and back out (**Figure 4**). The first line of interaction between microbes and a dietary sugar/sweetener is typically an import system. These include phosphotransferase systems (phosphate dependent), major facilitator superfamily transporters (ion coupled), ATP-binding cassette transporters (ATP dependent), sodium–glucose linked transporters (Na^+ coupled), and glucose uptake transporters (facilitated diffusion) (93). The type of transport system varies by microbe and type of sugar (93). As well, some sugars are imported by multiple transport systems in a given organism (93–95). Following import, the sugar/sweetener enters into metabolic pathways. An example of selection acting on these processes in microbes was observed in *E. coli*. *E. coli* adapted in vitro to sucrose display mutations in or affecting the regulation of sucrose transport (a permease) and metabolism (a fructokinase and a sucrose hydrolase) (96). Furthermore, in metagenomic studies of mice fed a high-sugar/high-fat diet (by energy: 16–30% sucrose, 10–25% starch and maltodextrin, 40–45% fat, 15–19% protein), genes involved in sugar transport and metabolism were enriched (15, 22).

Whether changes in microbial sugar/sweetener transport and metabolism directly affect the host is uncertain. These changes, however, are associated with effects on the host. In several different human populations, increases in microbial gene content for sugar metabolism and transport are associated with type 2 diabetes and obesity (97–99). Similar microbial metabolic profiles were enriched in the previously mentioned study by Suez and colleagues, in which saccharin promoted a glucose intolerance promoting microbiome (70). As previously mentioned, comprehensive clinical studies coupled with microbiome analyses using nutritionally relevant amounts of saccharin or other specific sugars/sweeteners need to be performed to fully address this question.

Continuing inside and back out of the microbial cell, dietary sugars are incorporated into glycoconjugates, including peptidoglycans, capsular polysaccharides, exopolysaccharides, and lipopolysaccharides, in the cell wall and membrane (100). These glycoconjugates serve a variety of functional roles for microbes, including strain identification among microbes and by the host (101). For example, DC-SIGN (dendritic cell–specific intercellular adhesion molecule-3–grabbing non-integrin) lectins (100) and immunoglobulin A (101) actively promote colonization of microbes in the gut by recognizing displayed microbial glycoconjugates. Specific exopolysaccharides can promote interleukin-10–mediated anti-inflammatory immune responses (65). On the other hand, lipopolysaccharide stimulates a proinflammatory immune response through toll-like receptor (TLR) 4 (TLR4) signaling (102) and is associated with metabolic syndrome (103). Therefore, microbial sugar adaptation can significantly affect host–microbe interactions as well as determine whether the immune system actively removes, promotes, or ignores a microbe.

Available sugars also impact the formation of biofilms (104, 105), flagellar structures (100), and motility (106). Biofilms and flagella can alter microbial persistence in the gut, interact with the immune system, and have strong impacts on host disease states. In particular, biofilms have been implicated in colorectal cancer (107) and antibiotic resistance (108). While flagella normally stimulate the immune system through TLR5, there is some evidence suggesting that glycosylation of flagella reduces TLR5 recognition in the opportunistic pathogen *Burkholderia cenocepacia* (109) and enhances *C. difficile* adhesion to epithelial cells (110).

Moving further outside the cell, microbial metabolites can be formed from sugar/sweetener metabolism. For example, microbially produced short-chain fatty acids may link microbial carbohydrate metabolism and host obesity by providing an additional energy source to the host (111) or by activating anti-inflammatory pathways (103). Microbially produced lactate has also been demonstrated to stimulate intestinal stem cell proliferation and epithelial cell development, thereby protecting the small intestine against chemical- and radiation-induced injury (112). Stevia is broken down by microbes to steviol, which is processed in the liver and converted to steviol glucuronide (113); this process is not known to cause any effects on the host. Other links between dietary sugars/sweeteners and microbial metabolites remain to be understood. For example as mentioned earlier, *C. difficile* adaptation to trehalose is linked to increased toxin production in mice consuming trehalose (81).

Finally, there are likely many other mechanisms by which microbial interactions with sugars and sweeteners impact the host. There is evidence in humans and in animal models that sugar alcohols promote several beneficial microbes (114, 115). Whether this interaction occurs simply by promoting the growth of these microbes or through a more complex interaction is unknown. A new finding indicates that fructose increases phage production in *L. reuteri* through a stress–response pathway and thus alters microbiome composition in a novel manner (116). As mentioned earlier, the transport and metabolic genes involved in sugar metabolism are regulated by the presence of other sugars, leading to preferential metabolism of one sugar over another (117). Thus, in systems where multiple sugars are present, the effect of adding a novel sugar to a system may not directly impact the genes involved in transporting and metabolizing that sugar or may only affect a subpopulation of cells (48), potentiating strain diversification.

What approaches can be used for studying microbial adaptation to human sugar and sweetener consumption?

How do we move forward in trying to understand mechanistically how dietary sugars and sweeteners reshape the gut microbiome and affect host physiology? Research needs to identify not only the sugars that can be used by microbes, but also what metabolites and biological processes are affected by a given sugar, how microbes can adapt to altered carbohydrate pools, the existing variation in sugar metabolism within the human microbiome, and the effect of such adaptation and variation on human physiology. Here

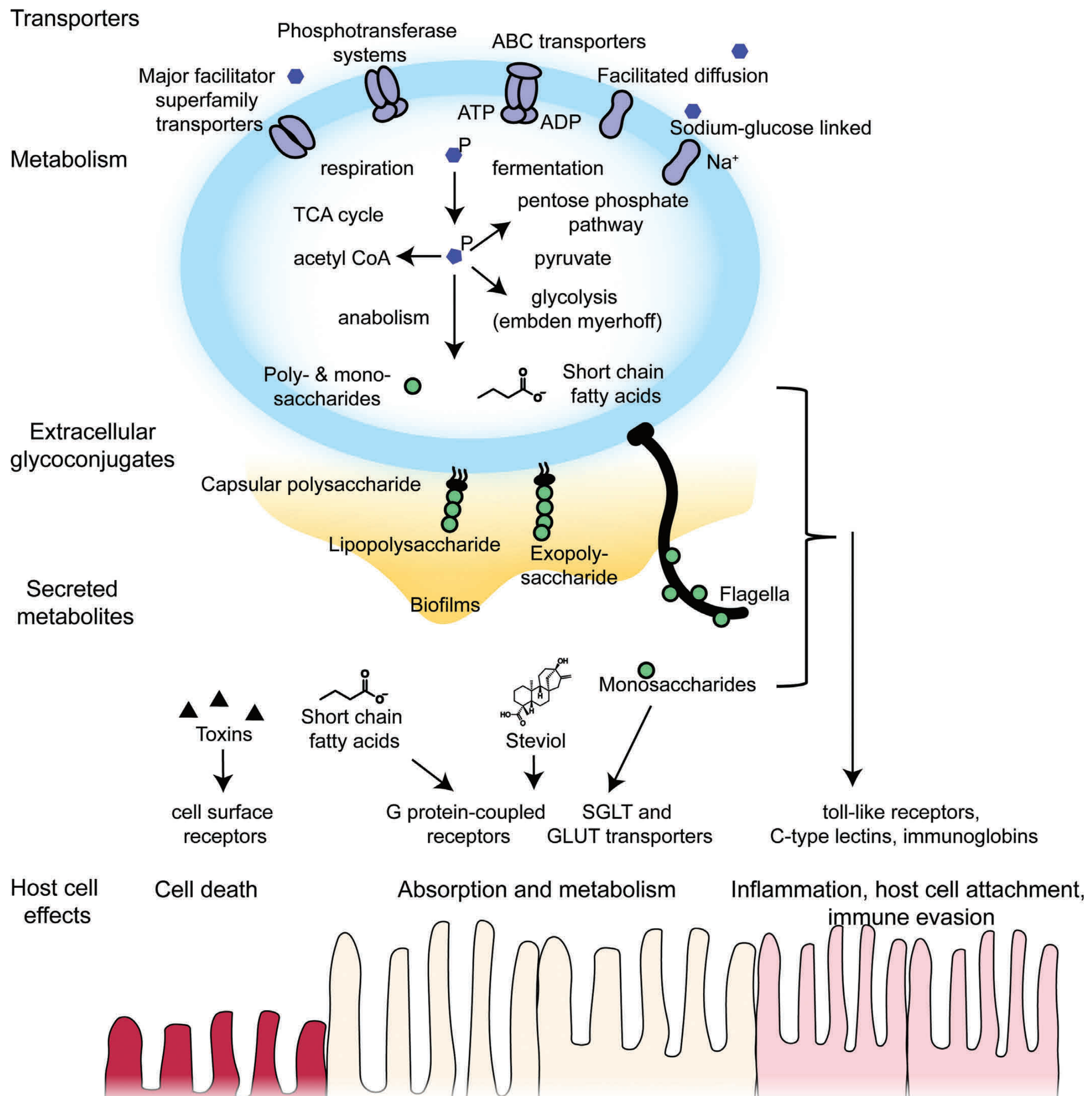

FIGURE 4 Host effects resulting from microbial adaptation to dietary sugars. Microbial adaptation to sugar is observed in sugar transporters and metabolic genes and produces changes in microbial proliferation and behaviors. These changes also lead to differential microbial primary and secondary metabolites including polysaccharides and monosaccharides, short chain fatty acids, and toxins. Secondary metabolites including toxins can lead to destruction of the epithelium and subsequent sepsis. Polysaccharides and monosaccharides, short chain fatty acids, and other sweetener metabolites can be absorbed, metabolized, and taken up into host circulation via G protein–coupled, SGLT, GLUT, and other cell surface transporters. Polysaccharides and monosaccharides can also become incorporated into extracellular glycoconjugates, including lipopolysaccharides, exopolysaccharides, capsular polysaccharides, biofilms, and flagella. These structures are recognized by the host immune system through toll-like receptors, C-type lectins, immunoglobins, and other molecules. Recognition can lead to an anti-inflammatory or proinflammatory response. Specific glycoconjugates can block this recognition, allowing the microbe to evade the immune system. These structures also permit attachment to the host epithelium. ABC, ATP-binding cassette; GLUT, glucose transporter; SGLT, sodium–glucose cotransporter; TCA, tricarboxylic acid cycle.

we discuss the use of experimental evolution, organoids, and host genetic background to address these topics.

Experimental evolution is a powerful and underutilized technique to explore the adaptive capability of microbes to a novel nutrient pool. To model the gut, various culture systems (bioreactors, fermenters, and chemostats) have been developed with varying levels of complexity, ease of use, and scalability (118–123). Experimental evolution can be accomplished in live hosts as well, by passaging communities from host to host. This is best achieved using gnotobiotic animals where the microbial community can be controlled (124, 125). Evolved microbes and their metabolites can be

tested for their interaction in hosts or in cell culture models (see below).

Gut organoids are an advanced cell culture methodology that allows for determination of events that occur at the epithelial layer of any region of the gut (126). While still more simplified than a complete host, variations on these systems allow for magnification of gut events. For example, enteroendocrine-cell–enriched enteroids allow for the ability to detect changes in gut hormones due to a microbial metabolite (127, 128). As well, these systems can be used to study how changes in a microbe's physiology changes its adhesion to gut cells (129) or other host–microbe interactions.

Analysis of the microbiomes of individuals avoiding particular sugars because of health preferences or as restricted by a genetic condition is a viable method to provide insight into how dietary sugar shapes the human microbiome. In the latter case, strong mechanistic support was provided for the positive correlation between *Bifidobacteria* and lactose through the finding that individuals who do not produce lactase in adulthood have higher levels of *Bifidobacteria* (130, 131), implying that individuals not producing lactase, yet still consuming lactose, have more free lactose available to the gut microbiome, which promotes the growth of lactose-utilizing *Bifidobacteria*.

Similar observations may be true for individuals with other metabolic deficiencies. Individuals with mutations in SLC5A1 are unable to transport glucose and galactose via SGLT1 (132, 133). These individuals experience life-threatening diarrhea on consumption of lactose, glucose, and galactose, but not fructose. However, certain individuals gain tolerance to these sugars as they age (134), perhaps through adaptations of the microbiome. Fructose intolerance (135) has been traced to a lack of aldolase B, fructokinase, or FBPase (136), deficiencies in the fructose transporter GLUT8, or simply by overloading the transporter GLUT5 (137, 138). These individuals suffer from irritable bowel syndrome symptoms and/or hypoglycemia. Removal from their diet of fructose and other sugars sharing the same pathways/enzymes improves their condition (136, 138, 139). Trehalase deficiency has been documented in Finnish individuals, causing affected individuals to suffer from abdominal pain on consuming trehalose (140). At least 8% of Greenland's population is reported to have trehalase deficiency via autosomal dominant inheritance (141). These groups of people represent rare opportunities to assess in humans the lifelong effects on the microbiome of the absence or reduction of a single sugar.

Conclusions

Humans are born with a preference for sugar (142). Despite the documented health problems associated with the consumption of sugar, people will continue to look for ways to include sugar and sweeteners in their diet. Attempts to lessen the metabolic burden of sucrose has led to the increased use of natural but otherwise infrequently consumed sweet-tasting compounds and the development of novel sweeteners. However, by changing the suite and amounts of sugars and sweeteners we consume, we create novel gut habitats that alter our microbial community, change microbial metabolism and excreted metabolites, and select for novel microbial strains.

The health consequences of adding sugars and sweeteners to our diet has yet to be fully understood. While the increase in simple sugars and the reduction in dietary fiber from our diet is believed to have lasting and detrimental effects on our microbiome (18, 143), the health impact resulting from switching from one sugar or sweetener to another is less clear. Nevertheless, we must acknowledge that any change in the profile of sugars/sweeteners we consume redefines the nutrient environments in our gut. How indigenous and exogenous microbes use these environments can result in benign, harmful, or beneficial effects on the host. However, any microbe that rapidly expands in the gut, like a pathogen, is expected to have the upper hand in using and adapting to novel nutrient environments.

Therefore, research must be forward thinking. What are the new generally recognized as safe products entering the food market? For example, the sweeteners alitame (a dipeptide), neohesperidin dihydrochalcone (a flavanone glycoside), and thaumatin (a protein) are not available in the United States but are in other countries. How will the microbiome be affected by these products in both the short and long term? How can we balance host and microbial metabolism of these products? What nutritional advice do we give to children, adults, and the elderly, and healthy compared with sick individuals?

The primary goal of these studies should be to address the safety of dietary sugars and sweeteners. In addition, a simple permutation in study design can shift the scope of such research towards developing beneficial gut microbes that thrive in our modern diet full of sugars and sweeteners. Furthermore, these studies present fortuitous examples of host–microbe co-evolution without the need to predict the historical diet and lifestyle of the host. Given the ubiquity of sugars and sweeteners in our culture, studies on the host–sugar/sweetener–microbe triumvirate are central to supporting and improving our health.

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