Supplement
LOW-CALORIE SWEETENERS AND WEIGHT MANAGEMENT

Obesity

Oral and Post-Oral Actions of Low-Calorie Sweeteners: A Tale of Contradictions and Controversies

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Objective: Many scientists and laypeople alike have concerns about low-calorie sweeteners (LCSs). These concerns stem from both a dissatisfaction with the taste of LCSs and reports that they cause metabolic disruptions (e.g., weight gain, glucose intolerance).
Methods: This article provides a critical review of the literature on LCSs from the standpoint of their taste, gastrointestinal, and metabolic effects; biological fate in the body; and impact on ingestion and glucose homeostasis.
Results and Conclusions: Mammals can readily discriminate between LCSs and sugars because both types of sweetener activate distinct oral and post-oral sensory pathways. LCSs differ in their ability to access post-oral tissues, but few studies have incorporated this observation into their design. It is difficult to extrapolate results between mice, rats, and humans because of interspecies differences in the taste and post-oral actions of LCSs and the fact that investigators often use different response measures in rodents and humans. There is confounding in the experimental design of some of the most widely cited studies of LCS-induced metabolic disruptions. The uncritical acceptance of these studies has generated considerable controversy. More work is needed to obtain a clearer understanding of the metabolic effects of LCSs.

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Introduction

Low-calorie sweeteners (LCSs) are a chemically diverse group of molecules that enhance the palatability of beverages, cereals, yogurts, baked goods, and desserts (1). Some LCSs are derived from plant material (e.g., steviol glycosides such as rebiana), while others are synthetized in the laboratory (e.g., saccharin, aspartame, sucralose, cyclamate, and acesulfame potassium). Despite approvals by regulatory bodies (US Food and Drug Administration, European Food Safety Authority, World Health Organization) and extensive research documenting their acute and chronic safety (2–8), LCSs remain controversial. For example, some investigators argue that LCSs increase food intake and body weight (9), whereas others argue that LCSs either have no impact or even decrease food intake and body weight (10).

Here, I examine why the literature on oral and post-oral actions of LCSs contains so many contradictory findings. To this end, I consider LCSs from the standpoint of their taste, gastrointestinal, and post-absorptive effects; biological fate (i.e., absorption, metabolism, and excretion); and impact on ingestion and glucose homeostasis.

Oral Sensory Effects of LCSs

Humans vary greatly in their acceptance of the taste of LCSs (11,12). This stems in part from the fact that LCSs evoke not only a sweet taste but also bitter and metallic off-tastes. Below, I discuss each of these taste attributes separately.

Sweet taste

Like sugars, LCSs bind to T1R2+T1R3, a heterodimeric G protein-coupled receptor that is expressed in a subset of taste cells (13–15). Activation of these T1R2+T1R3-expressing taste cells is thought to stimulate specific taste pathways, resulting in the perception of sweet taste (16).

Because LCSs evoke a sweet taste at lower concentrations than sugars, they are considered more potent. The use of the term "potent," however, has led to the mistaken belief that LCSs elicit a more intense sweet taste than sugars. In fact, LCSs tend to elicit a lower maximal sweet taste intensity than sugars in humans (Figure 1) (17). Likewise, maximally preferred concentrations of sucrose elicit higher rates of licking than do maximally preferred concentrations of the LCS saccharin in rats (18).

Why do LCSs taste less sweet than sugars? One possibility is that LCSs activate T1R2+T1R3 less effectively than sugars (19). A second possibility stems from the observation that some sugars (but not LCSs) activate a T1R2+T1R3-independent taste signaling pathway in mice (20,21) and rats (22). If humans also have a T1R2+T1R3-independent taste signaling pathway that

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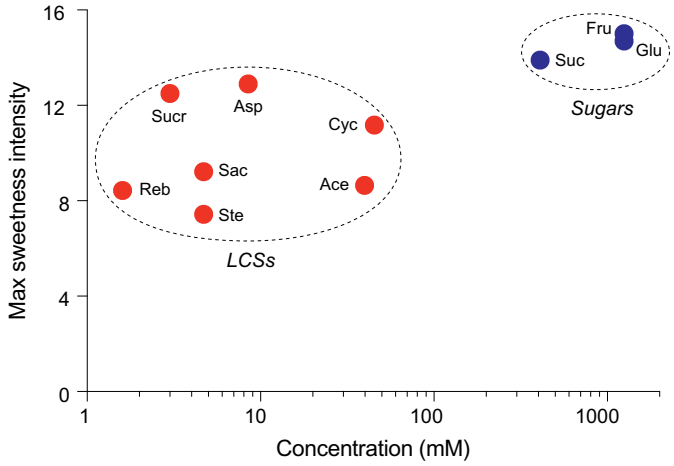

Figure 1 Maximal sweetness intensity of LCSs and sugars varies as a function of concentration in human subjects. LCSs include Ace-K (Ace), aspartame (Asp), Na^+ cyclamate (Cyc), rebiana (Reb), Na^+ saccharin (Sac), stevioside (Ste), and sucralose (Sucr); and sugars include fructose (Fru), glucose (Glu), and sucrose (Suc). The data are taken from (26). [Colour figure can be viewed at wileyonlinelibrary.com]

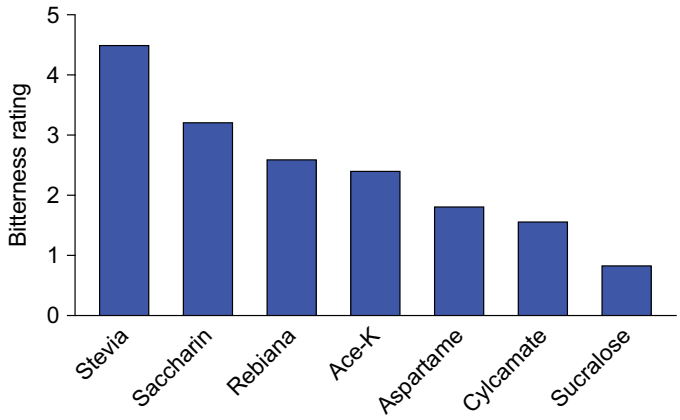

Figure 2 Bitterness intensity of seven LCSs. They were each tested at the concentration that elicited the maximal sweetness intensity in human subjects (see Figure 1). The data are taken from (26). [Colour figure can be viewed at wileyonlinelibrary.com]

responds selectively to sugars, then the simultaneous activation of the T1R2+T1R3-independent and -dependent taste signaling pathways could elicit a stronger taste intensity than either signaling pathway alone. A final possibility stems from the fact that LCSs also evoke a bitter off-taste, particularly at high concentrations (see below). Accordingly, the simultaneous elicitation of sweetness and bitterness could suppress sweetness intensity (23–25).

Bitter off-taste

LCSs evoke a bitter off-taste in most people, even at concentrations that elicit maximal sweetness (26). The intensity of bitterness, however, varies greatly across LCSs (Figure 2). The bitter off-taste stems from the fact that LCSs bind to a class of G protein-coupled taste receptors, called TAS2Rs. Humans express 25 different TAS2Rs, and all of them bind selectively to compounds that humans describe as bitter (27). These so-called bitter taste receptors are expressed in a subset of taste cells, which are distinct from the ones that express T1R2+T1R3 (28). Each TAS2R exhibits a unique molecular receptive range (27), and several of them bind to LCSs at concentrations that elicit bitter taste in humans. For example, in vitro binding studies have implicated TAS2R4 and TAS2R14 in the detection of steviol glycosides (29) and TAS2R43 and TAS2R44 in the detection of saccharin and acesulfame potassium (Ace-K) (30). Further, individual variation in the perceived bitterness of Ace-K has been linked to polymorphisms in TAS2R9 and TAS2R31 (31).

Using a cell-reporter system, Riera et al. (32) discovered that LCSs also activate the transient receptor potential vanilloid-1 (TRPV1). TRPV1 is a gated ion channel that is expressed in both taste cells and trigeminal neurons that innervate the oral epithelium. In addition to evoking a burning sensation, activation of oral TRPV1 may also contribute to the perceived bitterness of LCSs. This is because, under certain experimental conditions, humans confuse the burning sensation of capsaicin (a ligand of TRPV1) with the bitter taste of quinine (33).

Metallic and other off-tastes

Some LCSs (e.g., saccharin, sucralose, rebiana, and Ace-K) also evoke a metallic taste in humans at higher concentrations (12,17,34,35). While the mechanistic basis of metallic taste is unknown, investigators have suggested a role of TRPV1 (32) or inhibition of salivary carbonic anhydrases (36). Stevia and rebiana also evoke a licorice off-taste (37).

Lingering taste

Another distinctive feature of LCSs is that their evoked taste lingers for longer than that of sugars. One can compare sweeteners in terms of the rate at which the evoked sweetness disappears over time. In one experiment, humans were offered isointense concentrations of aspartame, rebaudioside A (rebiana), and sucrose. The sweetness of the aspartame and rebiana took, in respective order, two and three times longer to disappear than did that of sucrose (35). It is not only sweetness that lingers in the oral cavity; the bitter taste of LCSs can persist for longer than 40 seconds (35,38). The length of time each taste quality lingers increases with concentration. The lingering taste may stem from the high lipophilicity of LCSs relative to sugars. A higher lipophilicity would make it harder for saliva to clear LCSs from the oral cavity.

Brain responses to oral sensory input from LCSs versus sugars

fMRI neuroimaging studies have revealed, unexpectedly, that oral stimulation by LCSs and sucrose elicits distinct patterns of brain activation in humans. For example, Frank et al. (39) discovered that iso-sweet concentrations of sucrose and LCSs both activate the frontal operculum and anterior insula, but that sucrose alone activates components of the taste reward circuit (e.g., the left ventral striatum, left dorsal caudate nucleus, bilateral midbrain, and right thalamus). Likewise, when Smeets et al. (40) offered fasted human subjects sucrose or LCSs, the sucrose activated the striatum more strongly, while the LCSs activated the amygdala more strongly. It is remarkable that these two sweetened solutions which were matched for sweetness and pleasantness, could cause such different patterns of brain activation. This observation could

reflect at least two processes: (i) LCSs activated the T1r2+T1r3-dependent taste pathway, while sucrose activated both T1r2+T1r3-dependent and -independent taste pathways; and (ii) LCSs alone activated T2R- and TRPV1-dependent signaling pathways.

Species differences in preference for LCSs

It is often assumed that because LCSs evoke a sweet taste in humans, they will do so in other mammals as well (e.g., (41)). This is true for some but not all LCSs. For example, laboratory mice strongly prefer specific concentrations of saccharin, Ace-K, sucralose, and steviol glycoside over water, but they show a weak to nonexistent preference for a wide range of concentrations of aspartame and cyclamate (42,43). This observation reflects differences between the human and mouse T1R2+T1R3 receptor (44–46). Laboratory rats strongly prefer some concentrations of saccharin, cyclamate, and steviol glycosides over water, but show weak to nonexistent preferences for aspartame (18,43,47,48). The preference for sucralose is highly individualistic across rats, with some preferring specific concentrations of sucralose over water and others avoiding all concentrations (49–51). Failure to appreciate these species differences can confound interpretation of the factors regulating intake of LCSs.

Post-Oral Expression Sites for LCS Receptors

It was originally assumed that LCSs merely activate taste receptors in the mouth. It has since been discovered that the receptors for LCSs are also expressed elsewhere in the body, raising the possibility that LCSs could act post-orally. For example, T1R2+T1R3 (and its T1R2 and T1R3 subunits) are expressed in cells of the gastrointestinal tract, pancreas, adipose tissue, urinary bladder, hypothalamus, and brainstem (52–55). TAS2R bitter taste receptors are expressed in cells of the thyroid gland (56), vascular smooth muscle (57), and airway epithelia (58). Finally, the TRPV1 receptor is expressed in vascular smooth muscle (59) and the A-delta and C-fiber nociceptors that innervate epithelial tissues (60). The next section addresses the likelihood that each LCS would reach these post-oral receptors.

Biological Fate of LCSs in the Body

Figure 3 illustrates the biological fate of the most commonly consumed LCSs. Aspartame, a dipeptide, is rapidly degraded in the small intestine into its constituent amino acids (aspartic acid and phenylalanine) and methanol (8). The amino acids are absorbed and utilized in metabolism and protein synthesis, while the methanol is metabolized in the liver. Given the rapid degradation of aspartame in the small intestine, its low (millimolar) concentration in foods and beverages, and the fact that its constituent amino acids occur at substantially higher concentrations in many common foods (3), it would seem unlikely that it could produce any salient post-oral responses. Nevertheless, it has been proposed that the phenylalanine produced during aspartame digestion inhibits intestinal alkaline phosphatases, which normally detoxify gut bacteria-derived endotoxins; the accumulation of these endotoxins could disrupt metabolism (61).

Sucralose, a chlorinated sucrose molecule, is poorly absorbed from the intestinal tract of humans and mice and is excreted intact in feces and urine (62,63). Because sucralose is not degraded in the intestinal tract, it could interact with receptors in the luminal wall of the gastrointestinal tract or elsewhere in the body.

Steviol glycosides are a group of natural sweeteners (e.g., stevioside and rebiana), which are derived from *Stevia rebaudiana* Bertoni. This is a perennial shrub in the family Asteraceae (Compositae)

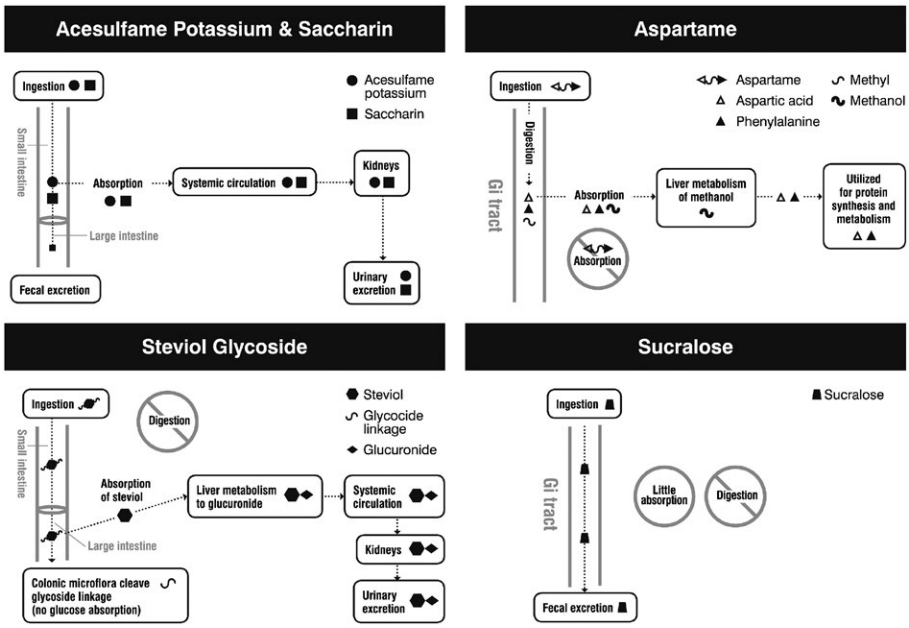

Figure 3 LCSs exhibit different biological fates in the body. Major routes of absorption, digestion, metabolism, and excretion of five LCSs in the human body are illustrated. Figure from Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. Biological fate of low-calorie sweeteners. *Nutr Rev* 2016;74:670-689 (8). CC BY-NC-ND 4.0.

that is native to Paraguay. *Stevia* leaves have been used for centuries by the indigenous peoples of that region to sweeten foods and beverages (64). Because steviol glycosides are not degraded by digestive enzymes, they could potentially interact with LCS receptors in the wall of the small intestine. Once steviol glycosides reach the large intestine, however, they are metabolized by microbes into steviol, which readily diffuses into the bloodstream (65).

Ace-K and saccharin are resistant to the enzymes and microbes in the intestine and are absorbed readily across the gastrointestinal wall. Both sweeteners are excreted intact in urine (8). Thus, ingested Ace-K and saccharin have the potential to interact with tissues throughout the body.

In sum, LCSs have markedly different biological fates in the body. Some are destroyed rapidly in the small intestine, others are converted to metabolites that enter the bloodstream, and yet others enter the bloodstream intact. It follows that one should not assume that all LCSs have the same potential post-oral impact.

Gastrointestinal Responses to Ingested LCSs

If T1R2+T1R3 mediates glucose sensing in gut endocrine cells, then LCSs and sugars should elicit similar physiological responses in the gastrointestinal tract. There are five sources of empirical support for this prediction. (i) Dietary supplementation with sugars or LCSs upregulated expression of the Na^+-glucose cotransporter 1 (SGLT1) in enterocytes of pigs (66) and wild-type but not T1r3 knockout mice (67). (ii) Duodenal infusions of sugars or saccharin increased SGLT1 expression in the proximal small intestine of rats (68). (iii) Dietary supplementation with sucralose, Ace-K, or saccharin (but not aspartame) increased glucose absorption through the small intestine (67). (iv) Direct stimulation of GLUTag cells (a mouse enteroendocrine cell line) with sucralose elicited release of the major incretin hormones, glucagon-like peptide-1 (GLP-1), and gastric inhibitory peptide (GIP) (67). (v) The sucralose-induced release of insulin from GLUTag cells was inhibited by gurmarin, a specific antagonist for the mouse T1R2+T1R3 receptor (67). Taken together, these results directly implicate T1R2+T1R3 in intestinal signaling and glucose absorption.

Several investigators have challenged the hypothesis that T1R2+T1R3 plays a central role in intestinal signaling. For instance, glucose, but not LCSs, elicited incretin release from isolated mouse L or K cells (derived from the duodenum) (69,70). Likewise, intragastric administration of sugars, but not LCSs, altered plasma incretin levels, glucose absorption rates, plasma glucose, and gastric emptying in humans (37,71–74) and rats (75). Whereas one study reported that stimulation of the rat jejunum with LCSs increased SGLT1 and GLUT2 expression in enterocytes (76), a subsequent study failed to replicate this finding (77). Finally, a recent study reported that T1R2+T1R3 activation in the gut not only failed to elicit incretin secretion but also failed to potentiate glucose-stimulated incretin secretion in the rat (78).

There are reports that ingestion of LCSs prior to a glucose challenge enhances either GLP-1 release (79) or the post-absorptive rise in glucose in humans (80,81). These "preload" studies reveal a potential post-oral effect of ingested LCS on gastrointestinal signaling, but only when LCSs are ingested before carbohydrates. Such preloading of LCSs, however, is not common in the real world. People typically ingest LCSs together with food (1). Thus, it is notable that LCSs did not impair glucose tolerance when they were ingested concurrently with a glucose load (82). Clearly, more work is needed to resolve the controversy surrounding the role of T1R2+T1R3 and LCSs in metabolic signaling within the gastrointestinal tract of humans.

Post-Absorptive Responses to LCSs

Responses of pancreatic beta cells to ingested LCS

To understand the impact of ingested LCSs on pancreatic beta cells, investigators examined responses of pancreatic beta cells or MIN6 insulinoma cells to LCSs in vitro. In these studies, the investigators directly stimulated cells with a solution containing glucose alone or a mixture of glucose plus LCS and measured the change in concentration of intracellular messengers or plasma insulin. One set of studies reported that binary mixtures of glucose plus a 40mM to 50mM concentration of saccharin, Ace-K, or sucralose elicited greater production of intracellular messengers than did glucose alone, and that these responses were mediated by T1R2 or T1R3 (83–86). Another set of studies reported that binary mixtures of glucose plus 1mM to 25mM concentrations of different LCSs stimulated more insulin release than did glucose alone (87,88).

Notwithstanding the rigorous nature of the in vitro findings, their physiological relevance is unclear. This is because the investigators used LCS concentrations that were similar to or even higher than those typically found in diet sodas (89). In reality, the concentration of LCSs in foods and beverages would become diluted in the extracellular compartment (ECC) of the body (i.e., blood + interstitial fluid). For instance, if we suppose that an adult woman ingested a 0.355 L (12 fl. oz.) can of diet soda and that her ECC contained 14 L of fluid (90), then the LCS concentration in the diet soda would be diluted 39 times by the ECC. The dilution effect would be magnified by the fact that the LCSs would enter the ECC slowly. As the LCS entered the ECC, some fraction of the LCS already in the ESS would be cleared by the kidney. Thus, the net rise in plasma LCS concentration would depend on the relative rates of intestinal absorption versus clearance.

To better understand the potential effect of ingested LCSs on insulin release from beta cells, investigators should conduct their studies with physiologically relevant concentrations (91). One recent study adopted this approach. The investigators offered mice ad lib access to a highly palatable solution of stevioside (124 μM) (92). Afterward, the authors collected blood samples from the same mice and measured the plasma concentration of steviol, the primary metabolite of stevioside. The plasma steviol concentration (0.396 μM) was 313 times lower than that of the ingested stevioside solution, underlining the extent to which the ECC can dilute plasma concentrations of ingested LCSs (or their metabolites). Despite its low concentration, 0.396 μM steviol enhanced insulin release from pancreatic beta cells by potentiating activity of the TRPM5 (transient receptor potential M5) channel. This finding appears

to constitute the first demonstration of an LCS (or its metabolite) directly modulating insulin release at a physiologically relevant concentration.

The sweet-taste confusion hypothesis

When mammals ingest sweet foods, they activate a series of preemptive cephalic-phase responses (CPRs), including insulin release (93) and postprandial thermogenesis (94,95). Cephalic-phase insulin release Cephalic-phase insulin release (CPIR) improves glucose tolerance after a meal, while cephalic-phase thermogenesis helps compensate for excessive caloric intake. Swithers and Davidson hypothesized that repeated consumption of LCSs would cause sweet taste-induced CPRs to attenuate over time because they effectively uncouple sweet taste from any postprandial elevation in blood sugar (96). They predicted that attenuated sweet taste-induced CPRs would impair glucose homeostasis, reduce energy expenditure, and, hence, promote obesity and diabetes (96). This so-called sweet-taste confusion hypothesis was tested in a series of studies in which rats were maintained on chow diets supplemented intermittently with a saccharin- or glucose-sweetened yogurt (review in (9)). They found that as compared with rats offered the glucose-yogurt, those offered the saccharin-yogurt ingested more calories, expended less energy, gained more weight, and exhibited impaired glucose tolerance.

The studies involving the sweetened yogurts are confounded in three ways. (i) The investigators did not match the sweetness of the two yogurts (97). This is problematic because CPR magnitudes vary as a function of sweetness intensity (21,98). (ii) The investigators neglected to include any control diets in their studies (i.e., unsweetened yogurt or chow alone). This deprived them of a reference point against which to interpret diet-induced differences. For instance, they found that rats offered the saccharin-yogurt cleared glucose from their blood less effectively than rats offered the glucose-yogurt. In the absence of control diets, they have no idea whether the saccharin-yogurt impaired glucose tolerance or the glucose-yogurt enhanced it. In support of the latter interpretation, Teff et al. (99) found that intravenous infusions of glucose over 48 hours enhanced glucose tolerance and CPIR in human subjects. (iii) Finally, the investigators excluded rats from their studies that did not "routinely" consume the saccharin-yogurt (100). This procedure likely biased the sample of rats offered the saccharin-yogurt toward individuals that grow more quickly (97,101). Indeed, when Boakes et al. (97) attempted to replicate the Swithers and Davidson studies using the appropriate control groups and unbiased subject selection, they observed greater weight gain in rats offered the glucose-yogurt. In another study, Foletto et al. (102) supplemented the diet of rats with saccharin-yogurt or plain yogurt. They reported that the saccharin-yogurt caused greater weight gain. However, when Boakes et al. (97) re-analyzed the raw data of Foletto et al., they discovered that "at no point was there a significant weight difference between the groups."

The sweet-taste confusion hypothesis is further undermined by four observations. (i) According to Sylvetsky (1), LCSs are usually consumed together with carbohydrates as part of a meal or snack. Under these circumstances, the sweet taste of the LCS would be associated with a postprandial elevation in blood glucose from the co-ingested carbohydrates. (ii) The sweet-taste confusion hypothesis assumes that sweetness is a reliable predictor of the carbohydrate content of foods. However, if one considers natural sources of carbohydrates, the ones with the highest energy density (roots and tubers) do not taste sweet (103). (iii) There is no evidence that repeated exposure to LCSs attenuates sweet taste-induced CPIR. For instance, when rats were offered a saccharin solution across ten successive trials, there was no decrease in magnitude of the saccharin-induced CPIR (104). Likewise, when humans were exposed repeatedly to an LCS or a sugar, there was no change in CPIR magnitude (105). (iv) A recent meta-analysis compared the effect of LCS versus sugars on food intake and weight gain in rodents and humans (10). The authors found that, on balance, the use of LCS (in lieu of sugars) resulted in lower energy intake and weight gain.

Oral versus post-oral stimulation of intake

The post-oral actions of glucose-containing carbohydrates can stimulate much higher intake than LCSs (106,107). For instance, Figure 4 shows ingestive responses of mice to solutions containing 38mM saccharin or 333mM glucose (108). Panel A shows initial licking responses of the mice to the two solutions during short-term acceptability tests; it reveals that the saccharin solution stimulated higher rates of licking than the glucose solution. Panel B shows daily intake of water versus the two sweetener solutions; it reveals that as compared with water, the saccharin solution stimulated a 2-fold increase while the glucose solution stimulated a 6-fold increase in daily intake. Thus, despite the higher palatability of the saccharin solution, the glucose solution stimulated greater daily intake.

Recent work offers insight into why glucose-containing sugars stimulate higher daily intake than LCSs. The attraction of mice to the taste of LCSs and sugars appears to stem from the activation of dopamine-excitable circuits in the ventral striatum (106,109,110). However, activation of these ventral striatal circuits alone is not sufficient to drive sustained intake. An additional post-oral response pathway needs to be recruited. It consists of nutrient sensors in the small intestine that are selective for glucose (111,112). Stimulation of these intestinal nutrient receptors is thought to activate dopamine-excitable circuits in the dorsal striatum, which in turn drives sustained intake (106,109,110). In fact, optogenetic stimulation of the dorsal striatum can even cause mice to consume unpalatable substances (110).

Finally, one should not always assume that LCSs will necessarily stimulate intake. There is evidence that at least one LCS (sucralose) has the counterintuitive effect of inhibiting food intake based on its post-oral actions. Sclafani at al. (111) attempted to condition a preference for an arbitrary flavor by associating its intake with intragastric (IG) infusions of sucralose. Unexpectedly, the mice developed a weak avoidance of the flavor associated with the sucralose infusions. The authors speculated that the IG sucralose activated bitter TAS2R receptors in the intestine, which in turn generated aversive sensory feedback from the gut. This speculation was based on a study showing that IG infusions of a harmless bitter taste stimulus can condition flavor avoidance in rats and mice (113).

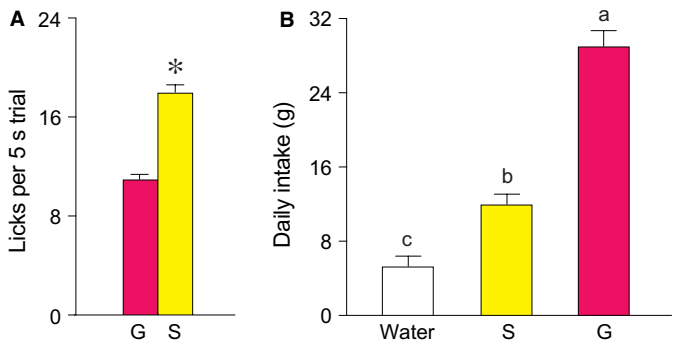

Figure 4 Palatability does not reliably predict daily intake of sweeteners in C57BL/6 mice. The sweeteners included 38mM saccharin (S) and 333mM glucose (G). **(A)** Palatability is represented by initial licking responses, obtained during a no-choice two-bottle acceptability test. Number of licks per 5-second trial (mean ± SE) was compared for each solution using a paired *t* test ($P < 0.05$). In **(B)** Daily intakes (mean ± SE) of water, S, and G. Different letters above bars (a, b, c) indicate means that differ significantly from one another (Tukey post hoc test; $P \leq 0.05$). The data are taken from (108). [Colour figure can be viewed at wileyonlinelibrary.com]

Impact of LCSs on the Microbiome

The oral cavity and gastrointestinal tract contain diverse communities of bacteria and other microbes. LCSs are thought to disrupt these communities by virtue of their antibiotic properties. In the oral cavity, the LCS-induced disruptions appear to be beneficial. This is because LCSs (e.g., sucralose, saccharin, and aspartame) limit growth of periodontal pathogens (114). In the gastrointestinal tract, however, the LCS-induced disruptions are thought to have negative consequences. For instance, Suez et al. (115) examined the impact of LCS consumption on glucose tolerance and gut microbial communities in mice and humans. In the mouse studies, the investigators attempted to manipulate the gut microbiome with antibiotic treatments, fecal transplants, or consumption of binary mixtures of LCSs plus sugars. They found that these manipulations all changed glucose tolerance. In the human studies, the investigators provided seven subjects with saccharin over 6 days. They reported that glucose tolerance deteriorated over the 6-day exposure period in four subjects, but not in the other three. The human studies clearly require replication with a larger number of subjects.

Three other studies examined the impact of LCSs on the physiology and gut microbiome of rodents. Palmnäs et al. (116) offered rats ad lib access to a 0.2 M aspartame solution for 11 weeks. As compared with control rats, those offered aspartame (i) consumed fewer calories per day and gained significantly less weight, (ii) had higher fasting blood glucose levels but lower insulin sensitivity, and (iii) developed an aberrant community of gut microbes. Another study administered Ace-K by oral gavage to CD-1 mice daily over a 4-week period. The Ace-K treatment was associated with an increase in both body weight and activation of bacterial energy harvesting pathways in male (but not female) mice (117). In a third study, male C57BL/6 mice received a sucralose solution as their only source of water for 6 months. The sucralose consumption was associated with changes in bile acid composition and increased expression of proinflammatory genes in both the gut microbiota and the liver (118). Because these studies were all correlational, it is difficult to determine whether the observed changes in gut microbiota were the cause or effect of the other reported changes.

There are two caveats regarding our understanding of how LCS consumption affects the gut microbiome. First, most investigators use the fecal microbiota as a proxy for the microbiota elsewhere in the gastrointestinal tract. This is problematic because the microbial community in feces differs from that in the parts of the digestive tract where digestion and absorption actually occur (i.e., the stomach, small intestine, caecum, and large intestine) (119). Second, most research on the microbiome has been performed on mice. Because rodents (unlike humans) have a large caecum, which contains an extensive microbial community, disruptions to the gut microbiome may have a greater metabolic affect in rodents than humans.

Impact of Fetal and Neonatal Exposure to LCSs

Zhang et al. (120) tested the hypothesis that LCSs would be more palatable to mice who experienced LCS either in utero or during lactation. After offering pregnant or lactating mice a highly preferred solution of Ace-K, the investigators collected samples of amniotic fluid and breast milk. They detected Ace-K in both body fluids. Next, they offered female mice the preferred Ace-K solution during pregnancy or lactation, and then examined ingestive responses of their offspring once they reached adulthood. Remarkably, both fetal and lactational exposure to Ace-K increased preference for suprathreshold concentrations of Ace-K and sucrose in 24-hour tests. In a follow-up study, Chen et al. (121) administered intra-oral infusions of Ace-K twice daily to neonate mice, from postnatal day 4 through weaning. This treatment not only increased preference for suprathreshold concentrations of Ace-K and sucrose during adulthood, but also increased the number of α-gustducin-positive taste buds (and taste cells within a taste bud) in the tip of the tongue. α-gustducin is a G protein that contributes to sweet taste transduction (122).

If pregnant or breast-feeding human mothers ingest LCS-containing foods, do they increase the attractiveness of sweeteners to their offspring? As a first step toward answering this question, Sylvetsky and colleagues (123) collected milk from lactating mothers who had consumed at least one diet soda during the previous 24 hours. They detected extremely low concentrations of Ace-K (≤ 0.011mM), saccharin (≤ 0.007mM), and sucralose (0.0002mM), but no aspartame in the milk samples.

Conclusion

Despite extensive safety testing, many scientists and lay people alike have concerns about LCSs. These concerns stem from (i) persistent complaints about the taste of LCSs, (ii) the increasing use of both artificial and natural LCSs as palatability enhancers in beverages and foods, and (iii) recent observations linking LCS consumption to disruptions in metabolism, blood sugar control, and body weight. While the health concerns about LCSs may be valid, the empirical support for these concerns is contradictory and, in some cases, controversial. In this review, I have highlighted three factors that have hampered progress in studying the health effects of LCSs.

First, LCSs all share the ability to bind to the human sweet taste receptor, T1R2+T1R3. In all other respects, however, LCSs are a functionally heterogenous group of molecules. They differ in their potential to activate post-oral receptors in the gastrointestinal tract, abdominal organs, and central nervous system. Most studies to date have not explicitly incorporated the divergent biological fates of LCSs into their experimental designs. Instead, the investigators appear to have treated LCSs as largely interchangeable.

Second, the idiosyncratic biological effects of LCSs makes it difficult to extrapolate results from rodents to humans. For instance, aspartame tastes sweet to humans but is relatively tasteless to mice and rats. LCSs elicit CPIR in rats (124) and in a subset of humans (105), but not in mice (21). Making sense of these interspecies differences is made even more challenging by the fact that the physiological outcome measures used to assess the effects of LCSs on traits such as appetite, palatability, and taste often differ across species.

Third, some of the most widely cited reports on the negative health impacts of LCSs in rodents are confounded by biased subject selection and a lack of critical control groups. The widespread acceptance of these studies has generated considerable controversy and rancor in the field. Fortunately, investigators have initiated efforts to replicate these studies in ways that address the experimental shortcomings (e.g., (97)). O

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