Supplement
LOW-CALORIE SWEETENERS AND WEIGHT MANAGEMENT

Obesity

Metabolic Effects of Low-Calorie Sweeteners: A Brief Review

Allison C. Sylvetsky[1,2]

Low-calorie sweeteners (LCS) are found in a variety of foods and beverages, yet their role in diet, weight, and obesity-related chronic disease is controversial. This article summarizes proceedings from one of four presentations during a preconference session titled "Low-Calorie Sweeteners and Weight Management," which took place at the 2017 Obesity Society Annual Meeting in Washington, DC. The objective of this brief review is to summarize findings of observational and interventional studies of LCS effects on weight and metabolic health and to provide potential explanations for their discrepant results. Key research priorities for advancing the understanding of the role of LCS in weight and chronic disease are also discussed. The existing literature suggests that LCS consumption is consistently associated with obesity, diabetes, and related cardiometabolic conditions in observational studies. Although several plausible mechanisms have been proposed to explain these associations and have received considerable support in cellular and rodent models, the relevance of these mechanisms to humans has yet to be confirmed. Meanwhile, randomized controlled trials have demonstrated that LCS may benefit weight loss and weight maintenance. This is the case particularly when LCS are administered in the context of behavioral weight loss support and are consumed knowingly by habitual LCS consumers. Although these findings suggest that LCS may be useful for weight control among those cognitively engaged in weight loss and who are aware of their LCS consumption, LCS administration in these studies does not reflect typical consumption. Furthermore, few interventional studies have assessed the role of LCS on metabolic outcomes other than body weight. Additional factors must be considered before recommending LCS for weight management and chronic disease prevention, and further study of LCS effects on a variety of cardiometabolic outcomes, including visceral adiposity and glucose homeostasis, is warranted.

Obesity (2018) **26**, S25–S31. doi:10.1002/oby.22252

Introduction

Low-calorie sweeteners (LCS), such as acesulfame-potassium, aspartame, saccharin, sucralose, and steviol glycosides (e.g., stevia) provide sweetness with no or few calories. LCS are present in products such as diet beverages and sugar-free condiments as well as in a plethora of grains, snack foods, yogurts, desserts, and breakfast cereals, which consumers often do not realize contain LCS (1,2). Given current public health emphasis on lowering added sugar intakes, in parallel with widespread incorporation of LCS into a variety of packaged foods and beverages, consumption of LCS has increased in recent years (3). Approximately 25% of children and 41% of adults reported LCS consumption in the United States on a given day in 2009 to 2012, based on data collected from the National Health and Nutrition Examination Survey (4). Although the safety of LCS for human consumption is well established from a toxicological perspective, their effects on metabolism, weight, and health are not fully understood. Despite their increasing use, recommendations for LCS consumption remain inconclusive (5,6).

According to a joint position statement released by the American Diabetes Association and American Heart Association, "at this time, there are insufficient data to determine conclusively whether the use of LCS to displace caloric sweeteners in beverages and foods reduces added sugars or carbohydrate intake, or benefits appetite, energy balance, body weight, or cardiometabolic risk factors." The 2015 Dietary Guidelines Advisory Committee Scientific Report was similarly cautionary, in stating that "added sugars should be reduced in the diet and not replaced with LCS, but rather with healthy options, such as water in place of sugar-sweetened beverages."

Given the uncertainty surrounding the utility of LCS consumption for weight management and metabolic health, the objective of this review is to briefly summarize findings from observational and interventional studies of LCS effects and to discuss potential explanations for seemingly discrepant findings, with specific focus on the research design and populations studied. Key knowledge gaps requiring further study are also highlighted.

Human Observational Literature

The discussion of the observational literature will focus on a growing body of prospective cohort studies, which have been comprehensively reviewed in a recent meta-analysis by Azad

[1] Department of Exercise and Nutrition Sciences, Milken Institute School of Public Health, The George Washington University, Washington, DC, USA. Correspondence: Allison C. Sylvetsky (asylvets@gwu.edu) [2] Sumner M. Redstone Global Center for Prevention and Wellness, Milken Institute School of Public Health, The George Washington University, Washington, DC, USA.

Funding agencies: This work was funded by the Department of Exercise and Nutrition Sciences at the George Washington University.
Disclosure: The author declared no conflict of interest.
Received: 7 March 2018; **Accepted:** 7 June 2018; **Published online 2 October 2018. doi:10.1002/oby.22252**

et al. (7). Although limited by their inability to determine causality, prospective cohort studies have the advantage of following large sample sizes over an extended period of time. The majority of prospective cohort studies demonstrate that LCS consumption is associated with adverse cardiometabolic health outcomes (8-14); however, there are also several reports that do not observe adverse effects on metabolic disease risk (15-18).

One of the most notable studies linking LCS use to obesity was conducted by Fowler et al. (13), who demonstrated that participants reporting consumption of diet beverages were more likely to gain weight over the 7- to 8-year follow-up period, even after adjustment for baseline BMI. A dose-response relationship was observed as compared with nonconsumers; consumption of 3 to 10 diet beverages per week (approximately 1 per day) was associated with increased BMI, whereas consumption of 22 diet beverages per week (approximately 3 per day on average) predicted significantly greater weight gain. The same group observed a similar dose-response relationship between diet beverage consumption at moderate doses (0-1 and > 1 serving per day) and visceral adiposity in a separate cohort of older adults, independent of their BMI at baseline and despite little change in body weight (14). The lack of an increase in body weight may be attributable to the fact that this latter study enrolled older adults, who were 65 years of age or older at baseline (14). Positive associations between LCS intake, weight gain, and adiposity have also been documented in numerous other studies (7).

Associations between LCS and type 2 diabetes (11), metabolic syndrome (8), and nonalcoholic fatty liver disease (12) have also been reported in large and long-term prospective cohort studies. In some cases, associations remained statistically significant even after adjustment for potential confounders, including weight and adiposity (11). For example, in a study by O'Connor et al. (11), type 2 diabetes incidence was 22% higher among LCS consumers. Although the association was attenuated after adjustment for adiposity, the elevated risk among LCS consumers remained statistically significant. Similar findings have been shown for nonalcoholic fatty liver disease (12), stroke (10), and dementia (10) incidence, although associations were no longer statistically significant after adjustment for weight and adiposity. Given that weight may be in the causal pathway linking LCS intake to unfavorable metabolic outcomes, whether adjustment for weight and adiposity is appropriate has been challenged (19).

Despite findings of epidemiologic studies linking LCS consumption at doses reflective of real-life consumption with weight gain and the development of metabolic disease (7), several limitations inherent to prospective cohort studies must be mentioned. A key consideration is residual confounding, defined as bias that remains after adjustment for relevant covariates. Dietary assessment methods used to assess LCS consumption are often flawed (20,21), and it is also not possible to determine how LCS are used by study participants and for what purpose. Nonetheless, several plausible physiologic mechanisms have been proposed (22,23) and support a potentially causal role of LCS in promoting the onset of obesity, diabetes, and related conditions (24). Although several of these mechanisms have received considerable support *in vitro* (25,26) and in rodent models (27,28) and are described in the "Proposed Biologic Mechanisms" section below, the dosages tested in these mechanistic studies often far exceed levels of reasonable human consumption and thus should be interpreted cautiously.

Human Intervention Studies

In contrast to findings of observational studies, intervention studies investigating LCS effects on body weight predominantly report beneficial (29) or neutral (7) effects. The majority of intervention studies studying LCS effects on body weight have used the gold standard randomized controlled trial (RCT) design and most have tested LCS effects administered at clinically relevant doses, reflecting reasonable levels of human consumption. The largest of these RCTs to date was recently conducted by Peters et al. (30). In this study, 308 subjects who were habitual LCS beverage consumers at baseline were enrolled in a comprehensive weight loss intervention for 12 weeks and assigned to consume either diet soda or water. Greater weight loss was observed in the diet beverage group compared to the water control group. Although these findings are indeed promising, it is crucial to consider the population studied, which was composed of habitual LCS consumers. Participants randomly assigned to the water group therefore underwent a more demanding behavior change, in having to cease LCS use and begin drinking water. This challenges the applicability of these findings to LCS-naive individuals who may begin using LCS as a weight management approach.

Another concern noted across numerous intervention studies testing LCS effects is a lack of an appropriate control (31). For example, de Ruyter et al. (32) conducted the largest and longest duration study in children to date, wherein school-aged children were randomly assigned to covert replacement of one sugar-sweetened beverage (SSB) per day with a sugar-free alternative or continuation of SSB during their usual snack time for 18 months. Although less weight gain was observed in children randomly assigned to sugar-free beverages compared to those who continued consuming SSB, it is unclear how the weight trajectory in the sugar-free group would have compared to a true control group: for example, plain water, unsweetened seltzer, or nothing.

Although there are notable exceptions (32,33), the majority of intervention studies investigating LCS effects are conducted within the context of behavioral weight loss support (30,34) and often involve calorie-restricted diets (35). These studies quite convincingly demonstrate that when used among individuals actively engaged in weight loss and who are receiving behavioral support, LCS offer a viable strategy for adhering to prescribed weight management regimens (36). The extent to which these findings can be extrapolated to support benefits of LCS use for weight management in the general population has been debated (37), as the manner in which LCS are consumed likely plays a key role in mediating their effects (31). Critical considerations for interpreting the results of RCTs investigating LCS effects on body weight are summarized in Table 1.

Few RCTs have investigated effects of prolonged LCS consumption in humans on outcomes other than body weight (33,38). However, given that LCS have been shown to promote metabolic dysregulation in rodent models, it is critical to assess biomarkers of metabolic disease in human intervention studies, in addition to assessing weight and adiposity.

TABLE 1 Key considerations for the design and interpretation of randomized controlled trials evaluating effects of LCS on body weight

Consideration	Explanation
Selection of study population	How might the age, weight status, race/ethnicity, sex, and metabolic health of the participants enrolled in the study affect the outcomes observed?
Inclusion of control group	What are LCS compared to? Is there an appropriate control?
Habitual exposure to LCS	Do study participants already consume LCS? Is typical LCS use an inclusion or exclusion criteria for study participation? Is baseline LCS use assessed and/or reported?
Specific LCS tested	What LCS are being studied? If diet beverages are administered, what LCS do they contain? Can findings with one LCS be generalized to other LCS?
Vehicle of LCS administration	Are the LCS provided through beverages, foods, or packets? How might this influence their use and resulting effects, and to what extent do findings of studies using diet beverages apply to LCS ingestion via foods, condiments, or packets?
Selection of study outcomes	What was the time period over which outcomes were assessed? Is this duration likely sufficient to observe changes in study outcomes? Are metabolic outcomes other than body weight evaluated?
Behavioral context	Are study participants receiving behavioral weight loss support? Does the intervention involve calorie restriction?
Controlled setting vs. free-living use	Are LCS administered as a replacement for or in addition to sugar-sweetened beverages? Does LCS administration in the study closely mirror consumption in real life? Are study participants aware that they are consuming LCS? Are dietary habits or lifestyle practices related to study participation likely to influence effects observed?

LCS, low-calorie sweetener.

Proposed Biologic Mechanisms

Several biologically plausible mechanisms, which are likely not mutually exclusive (22,23), have been proposed including activation of sweet taste receptors located in the oral cavity and throughout the human body, acceleration of glucose absorption, promotion of adipogenesis, alteration of the gut microbiota, and disturbance of the expected relationship between sweetness and calorie ingestion. These mechanisms involve numerous pathways and tissues and therefore may have wide-ranging potential effects on metabolism and health. Evidence in support of each of these mechanisms has been detailed previously (24,39) and is briefly summarized below. It is important to note that some may be compound specific, whereas others may relate to sweet taste and may therefore likely be generalizable across different sweeteners. Additionally, most mechanistic studies have tested LCS effects at supraphysiologic doses (26), whereas others have documented LCS effects at doses theoretically reasonable for human consumption (40).

Activation of sweet taste receptors

Sweet taste receptors are located throughout the human body, in addition to on taste buds within the oral cavity. As opposed to in the oropharynx where activation of sweet taste receptors allow the sensation of sweetness to be perceived by the brain, activation of extra-oral sweet taste receptors triggers physiologic responses (41). Sweet taste receptors are activated by a wide variety of sweet-tasting compounds, including nutritive sweeteners, LCS, and sweet-tasting proteins (41). Although LCS were once thought to be metabolically inert (39), LCS-induced activation of sweet taste receptors leads to the release of metabolic hormones including glucagon-like-peptide 1 (activation of sweet taste receptors in enteroendocrine cells) (42) and insulin (activation of sweet taste receptors in pancreatic beta cells) *in vitro* (26), albeit at supraphysiologic doses (39). The potential importance of sweet taste receptors in eliciting metabolic effects has been further demonstrated in mechanistic studies (42,43), in which the observed metabolic effects diminish when sweet-tasting compounds are administered along with sweet receptor inhibitors, such as lactisole or gurmarin, or when testing LCS effects in sweet taste receptor knockout models (40,44). The proposed roles of extra-oral sweet taste receptors in glucose homeostasis (41) and gut hormone release (39) have been reviewed in detailed previously (39).

It is also important to note that sweet taste receptors have also been identified in other tissues, including adipose tissue (45), testes (46), and bone (47), yet their respective roles in metabolism and health have yet to be elucidated. Although sweet taste receptor–mediated physiologic effects such as higher insulin release (48,49) would be expected to promote food intake, fat storage, and weight gain, the extent to which this augmentation would be clinically relevant is not presently clear (49). It is also difficult to discern whether acute increases in insulin reported in human studies (39,48) are due specifically to activation of sweet taste receptors.

Glucose absorption

Administration of sucralose in combination with glucose has been shown to increase the rate of glucose absorption in rodents (43) and may be due to LCS-induced upregulation of the two main intestinal glucose transporters, SGLT-1 and GLUT2 (50). However, no differences in glucose absorption as a result of LCS ingestion have been observed in humans to date.

Promotion of adipogenesis

As discussed above, sweet taste receptors are located outside of the oral cavity, including in adipose tissue (23,45). Although their role in adipose is not well understood, incubation of preadipocytes with LCS including acesulfame-potassium, saccharin, and sucralose has been shown to promote adipogenesis (25,51). Inhibition of lipolysis in mature adipocytes has also been reported (25). If LCS exposure promotes adipogenesis *in vivo*, this would lead to greater fat accumulation, adiposity, and weight gain. A

study to investigate whether prolonged LCS exposure alters metabolic pathways in humans is currently under way (ClinicalTrials.gov identifier NCT03125356, unpublished).

Alteration of gut microbiota

Several rodent studies have reported LCS-induced changes in the gut microbiome (27,52-56). Suez et al. demonstrated that several LCS, including aspartame, sucralose, and saccharin, altered the gut microbiota, leading to the development of glucose intolerance (27). Saccharin, which had the most robust effects, was administered for 11 weeks at a dosage reasonable for human consumption (5mg/kg) and compared to glucose or water controls. Saccharin-exposed mice developed glucose intolerance, but this effect disappeared when saccharin was administered along with antibiotics (27). LCS-induced changes in the gut microbiota promoted glucose intolerance were further supported by findings of fecal transplant studies, in which germ-free recipients of microbiota from saccharin-exposed mice, which were not themselves exposed to saccharin, also developed glucose intolerance. Similar findings have been reported with other LCS in rodent models (56).

Suez et al. also included a small human trial, in which seven healthy volunteers consumed 5 mg/kg saccharin for 1 week and underwent daily oral glucose tolerance tests to assess alterations in glucose tolerance (27). Following the intervention, four of the volunteers had decreased glucose tolerance and were characterized as "responders," whereas no differences in glucose tolerance were observed among nonresponders. Microbial composition differed among responders and nonresponders following the intervention, and transplantation of microbiota from responders into germ-free mice induced glucose intolerance in the rodent recipients. Although these findings in a small sample of human subjects supports the possibility that LCS may induce changes in the gut microbiota in humans (57), several additional methodological concerns beyond the small sample size (58) have been raised. It is therefore necessary to evaluate potential effects of LCS on the human gut microbiota in a longer-term and larger human RCTs (59) because although the rodent evidence is fairly robust, there are several concerns regarding the interpretation of study findings and their relevance to humans (58).

Disturbance of relationship between sweetness and calories

LCS are sweet but contain no or few calories, which has been proposed to represent a novel stimulus involving uncoupling of the expected pairing between sweetness and calories (60). This has been hypothesized to interfere with learned responses to nutrient ingestion, leading to impairments in appetitive and metabolic regulation (24). In several experiments, Swithers et al. have provided rats with intermittent access to yogurt or water containing either saccharin or glucose (3 days of the week) and plain yogurt or water on the remaining days of the week (28). In these experiments, rodents intermittently exposed to saccharin at doses reflective of human consumption (in which sweetness is uncoupled with calories) have higher energy intakes, gain weight, and develop relative hyperglycemia compared to rodents exposed to glucose (in which sweetness is paired with calories) (28). Higher weight gain in rodents following exposure to LCS-sweetened yogurt compared to sucrose-sweetened yogurt was also reported in an analogous experiment by Feijo et al. (61), who exposed rats to saccharin, aspartame, or sucrose in addition to ad libitum chow and water for 12 weeks. However, Boakes et al. (62) did not observe weight gain or metabolic impairments after intermittent exposure to saccharin, using a similar paradigm, which may be explained by differences in the chow and/or yogurt varieties administered (62). Swithers et al. 28,60 also excluded animals that did not consume at least 70% yogurt, whereas Boakes et al. (62) retained these animals in the analysis. Although unfavorable metabolic effects are consistently observed following intermittent administration of LCS in yogurt, the translatability of this experimental design to the context of human consumption has been challenged (63). Notably, the majority of rodent studies in which LCS are delivered continuously and/or in a vehicle other than supplemental yogurt do not consistently report weight gain after LCS exposure (36). It is therefore important to determine whether the timing and form of LCS administration may influence their effects (31).

Conclusion

Findings of prospective cohort studies, by and large, demonstrate that LCS consumption is associated with increased risk of obesity and related chronic diseases. In contrast, RCTs support beneficial or neutral effects of LCS on weight management, with little available data evaluating metabolic biomarkers, such as glucose tolerance, satiety hormone responses, and changes in inflammatory cytokines and fat-derived hormones. Several contextual factors likely contribute to the reported discrepancies in findings of observational and interventional studies investigating LCS effects (Figure 1). Although considered to be the gold standard, RCTs typically do not reflect usual LCS consumption behaviors in the general population. In particular, free-living individuals consuming LCS are often not cognitively engaged in weight loss efforts and are not provided with extensive behavioral resources for lifestyle modification, as is the case in some, but not all, RCTs (31). Meanwhile and as described above, prospective cohort studies are subject to numerous inherent limitations, and findings may be influenced by residual confounding (64).

It is therefore critically important to design future studies in a manner that best captures free-living LCS consumption at doses reflective of human consumption. This may include investigating LCS effects when consumed in addition to added sugars rather than only as a one-to-one replacement, when administered in vehicles other than diet beverages, and when used among population subgroups such as pregnant women, children, and individuals with metabolic disease. It is also paramount to experimentally investigate the role of LCS on outcomes other than body weight and to differentiate between effects that may be sweetener specific versus generalizable across compounds.

In addressing these outstanding research questions (59), consideration of contextual factors such as habitual consumption, prior LCS exposure, motivation for LCS use, overall dietary patterns, and other factors that likely to contribute to differences across individuals and between studies is critical. As the debate surrounding role of LCS consumption in weight and metabolic disease lies at the intersection of physiology and human behavior,

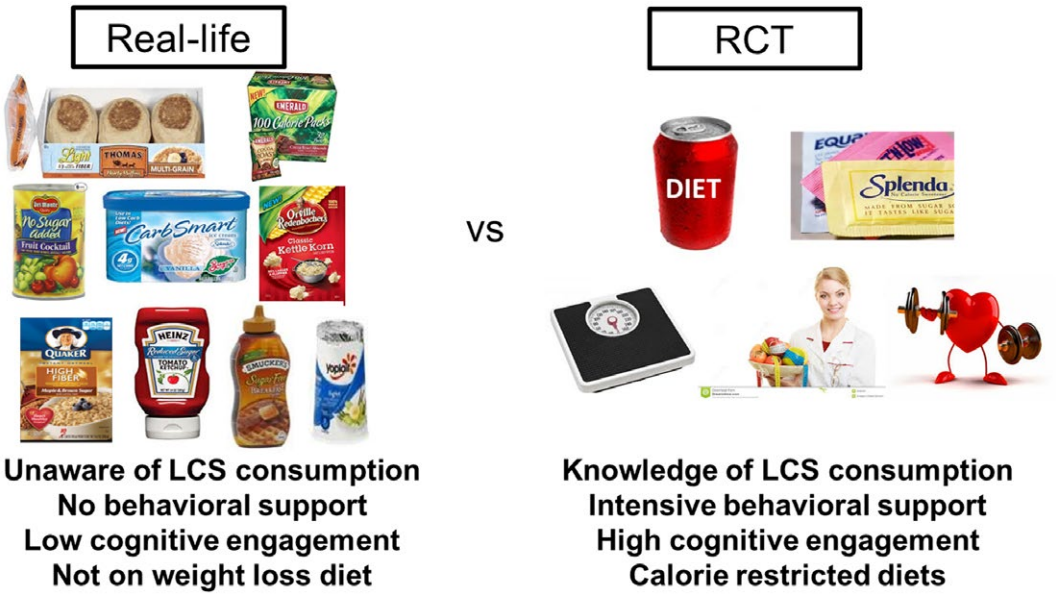

Figure 1 Contextual factors may explain discrepancies between findings of prospective cohort studies versus randomized controlled trials (RCTs). Administration of low-calorie sweeteners (LCS) in RCTs differs from the way LCS are consumed in free-living individuals. Whereas RCTs typically involve administration of a predetermined quantity of diet beverages or sweetener packets and often involve other behavioral lifestyle changes, LCS are found in a wide variety of foods and beverages and are often consumed inadvertently and without concomitant weight management support.

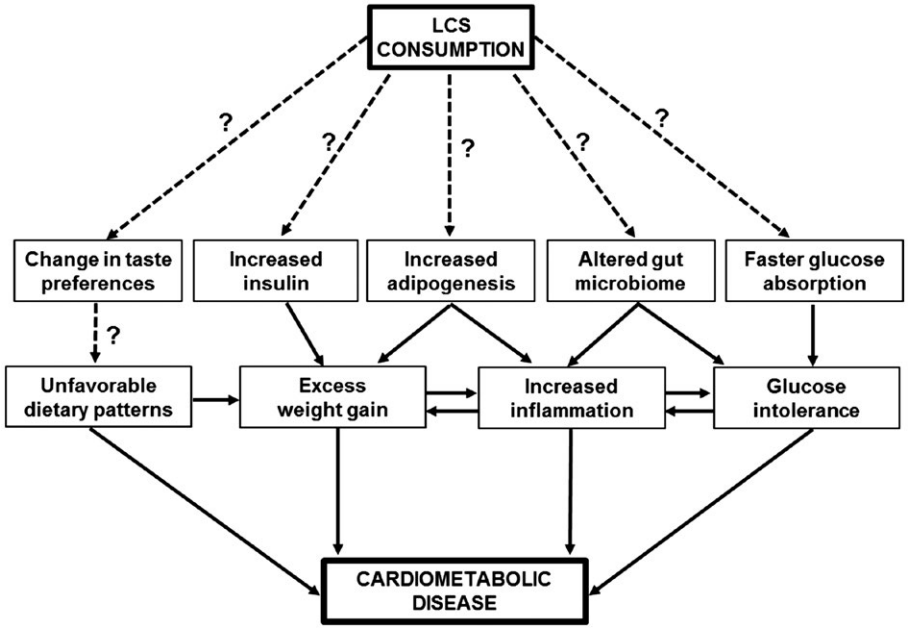

Figure 2 Proposed biological mechanisms are interrelated and may contribute to observed associations between low-calorie sweeteners (LCS) and metabolic disease. Several biological mechanisms have been proposed, which may in part explain positive associations among LCS, weight gain, diabetes, and cardiovascular disease (CVD) reported in epidemiologic studies. Although these mechanisms have not been confirmed in humans, they have received support in cellular and rodent models, are interrelated, and are likely not mutually exclusive.

a complex and multifaceted approach is necessary to generate conclusive data and meaningfully advance the field. **O**

References

1. Sylvetsky AC, Dietz WH. Nutrient-content claims—guidance or cause for confusion? *N Engl J Med* 2014;371:195-198.
2. Sylvetsky AC, Greenberg M, Zhao X, Rother KI. What parents think about giving nonnutritive sweeteners to their children: a pilot study. *Int J Pediatr* 2014;2014:819872. doi:10.1155/2014/819872
3. Sylvetsky AC, Welsh JA, Brown RJ, Vos MB. Low-calorie sweetener consumption is increasing in the United States. *Am J Clin Nutr* 2012;96:640-646.
4. Sylvetsky AC, Jin Y, Clark EJ, Welsh JA, Rother KI, Talegawkar SA. Consumption of low-calorie sweeteners among children and adults in the United States. *J Acad Nutr Diet* 2017;117:441-448.
5. Gardner C, Wylie-Rosett J, Gidding SS, et al. Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care* 2012;35:1798-1808.
6. Sylvetsky A, Rother KI, Brown R. Artificial sweetener use among children: epidemiology, recommendations, metabolic outcomes, and future directions. *Pediatr Clin North Am* 2011;58:1467-1480, xi.
7. Azad MB, Abou-Setta AM, Chauhan BF, et al. Nonnutritive sweeteners and cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials and prospective cohort studies. *CMAJ* 2017;189:E929-E939.
8. Nettleton JA, Lutsey PL, Wang Y, Lima JA, Michos ED, Jacobs DR Jr. Diet soda intake and risk of incident metabolic syndrome and type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* 2009;32:688-694.
9. Lutsey PL, Steffen LM, Stevens J. Dietary intake and the development of the metabolic syndrome: the Atherosclerosis Risk in Communities study. *Circulation* 2008;117:754-761.
10. Pase MP, Himali JJ, Beiser AS, et al. Sugar- and artificially sweetened beverages and the risks of incident stroke and dementia: a prospective cohort study. *Stroke* 2017;48:1139-1146.
11. O'Connor L, Imamura F, Lentjes MA, Khaw KT, Wareham NJ, Forouhi NG. Prospective associations and population impact of sweet beverage intake and type 2 diabetes, and effects of substitutions with alternative beverages. *Diabetologia* 2015;58:1474-1483.
12. Ma J, Fox CS, Jacques PF, et al. Sugar-sweetened beverage, diet soda, and fatty liver disease in the Framingham Heart Study cohorts. *J Hepatol* 2015;63:462-469.
13. Fowler SP, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP. Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain. *Obesity (Silver Spring)* 2008;16:1894-1900.
14. Fowler SP, Williams K, Hazuda HP. Diet soda intake is associated with long-term increases in waist circumference in a biethnic cohort of older adults: the San Antonio Longitudinal Study of Aging. *J Am Geriatr Soc* 2015;63:708-715.
15. Chen L, Hu FB, Yeung E, Willett W, Zhang C. Prospective study of pre-gravid sugar-sweetened beverage consumption and the risk of gestational diabetes mellitus. *Diabetes Care* 2009;32:2236-2241.
16. Bomback AS, Derebail VK, Shoham DA, et al. Sugar-sweetened soda consumption, hyperuricemia, and kidney disease. *Kidney Int* 2010;77:609-616.
17. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. *Circulation* 2012;125:1735-1741, S1.
18. Fung TT, Malik V, Rexrode KM, Manson JE, Willett WC, Hu FB. Sweetened beverage consumption and risk of coronary heart disease in women. *Am J Clin Nutr* 2009;89:1037-1042.
19. Sylvetsky Meni AC, Swithers SE, Rother KI. Positive association between artificially sweetened beverage consumption and incidence of diabetes. *Diabetologia* 2015;58:2455-2456.
20. Subar AF, Freedman LS, Tooze JA, et al. Addressing current criticism regarding the value of self-report dietary data. *J Nutr* 2015;145:2639-2645.
21. Lopes TS, Luiz RR, Hoffman DJ, et al. Misreport of energy intake assessed with food records and 24-h recalls compared with total energy expenditure estimated with DLW. *Eur J Clin Nutr* 2016;70:1259-1264.
22. Pepino MY, Bourne C. Non-nutritive sweeteners, energy balance, and glucose homeostasis. *Curr Opin Clin Nutr Metab Care* 2011;14:391-395.
23. Pepino MY. Metabolic effects of non-nutritive sweeteners. *Physiol Behav* 2015;152:450-455.
24. Swithers SE. Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. *Trends Endocrinol Metab* 2013;24:431-441.
25. Simon BR, Parlee SD, Learman BS, et al. Artificial sweeteners stimulate adipogenesis and suppress lipolysis independently of sweet taste receptors. *J Biol Chem* 2013;288:32475-32489.
26. Nakagawa Y, Nagasawa M, Yamada S, et al. Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS One* 2009;4:e5106. doi:10.1371/journal.pone.0005106
27. Suez J, Korem T, Zeevi D, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* 2014;514:181-186.
28. Swithers SE, Laboy AF, Clark K, Cooper S, Davidson TL. Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats. *Behav Brain Res* 2012;233:1-14.
29. Miller PE, Perez V. Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. *Am J Clin Nutr* 2014;100:765-777.
30. Peters JC, Wyatt HR, Foster GD, et al. The effects of water and non-nutritive sweetened beverages on weight loss during a 12-week weight loss treatment program. *Obesity (Silver Spring)* 2014;22:1415-1421.
31. Sylvetsky AC, Blau JE, Rother KI. Understanding the metabolic and health effects of low-calorie sweeteners: methodological considerations and implications for future research. *Rev Endocr Metab Disord* 2016;17:187-194.
32. de Ruyter JC, Olthof MR, Seidell JC, Katan MB. A trial of sugar-free or sugar-sweetened beverages and body weight in children. *N Engl J Med* 2012;367:1397-1406.
33. Maersk M, Belza A, Stodkilde-Jorgensen H, et al. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. *Am J Clin Nutr* 2012;95:283-289.
34. Tate DF, Turner-McGrievy G, Lyons E, et al. Replacing caloric beverages with water or diet beverages for weight loss in adults: main results of the Choose Healthy Options Consciously Everyday (CHOICE) randomized clinical trial. *Am J Clin Nutr* 2012;95:555-563.
35. Blackburn GL, Kanders BS, Lavin PT, Keller SD, Whatley J. The effect of aspartame as part of a multidisciplinary weight-control program on short- and long-term control of body weight. *Am J Clin Nutr* 1997;65:409-418.
36. Rogers PJ, Hogenkamp PS, de Graaf C, et al. Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. *Int J Obes (Lond)* 2016;40:381-394.
37. Sylvetsky AC, Rother KI. Nonnutritive sweeteners in weight management and chronic disease: a review. *Obesity (Silver Spring)* 2018;26:635-640.
38. Colagiuri S, Miller JJ, Edwards RA. Metabolic effects of adding sucrose and aspartame to the diet of subjects with noninsulin-dependent diabetes mellitus. *Am J Clin Nutr* 1989;50:474-478.
39. Brown RJ, Rother KI. Non-nutritive sweeteners and their role in the gastrointestinal tract. *J Clin Endocrinol Metab* 2012;97:2597-2605.
40. Simon BR, Learman BS, Parlee SD, et al. Sweet taste receptor deficient mice have decreased adiposity and increased bone mass. *PLoS One* 2014;9:e86454. doi:10.1371/journal.pone.0086454
41. Tucker RM, Tan SY. Do non-nutritive sweeteners influence acute glucose homeostasis in humans? A systematic review. *Physiol Behav* 2017;182:17-26.
42. Jang HJ, Kokrashvili Z, Theodorakis MJ, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci U S A* 2007;104:15069-15074.
43. Mace OJ, Affleck J, Patel N, Kellett GL. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol* 2007;582(Pt 1):379-392.
44. Kokrashvili Z, Mosinger B, Margolskee RF. Taste signaling elements expressed in gut enteroendocrine cells regulate nutrient-responsive secretion of gut hormones. *Am J Clin Nutr* 2009;90:822S-825S
45. Masubuchi Y, Nakagawa Y, Ma J, et al. A novel regulatory function of sweet taste-sensing receptor in adipogenic differentiation of 3T3-L1 cells. *PLoS One* 2013;8:e54500. doi:10.1371/journal.pone.0054500
46. Mosinger B, Redding KM, Parker MR, et al. Genetic loss or pharmacological blockade of testes-expressed taste genes causes male sterility. *Proc Natl Acad Sci U S A* 2013;110:12319-12324.
47. Eaton MS, Weinstein N, Newby JB, et al. Loss of the nutrient sensor TAS1R3 leads to reduced bone resorption. *J Physiol Biochem* 2018;74:3-8.
48. Pepino MY, Tiemann CD, Patterson BW, Wice BM, Klein S. Sucralose affects glycemic and hormonal responses to an oral glucose load. *Diabetes Care* 2013;36:2530-2535.
49. Sylvetsky AC, Brown RJ, Blau JE, Walter M, Rother KI. Hormonal responses to non-nutritive sweeteners in water and diet soda. *Nutr Metab (Lond)* 2016;13:71. doi:10.1186/s12986-016-0129-3
50. Margolskee RF, Dyer J, Kokrashvili Z, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na^+-glucose cotransporter 1. *Proc Natl Acad Sci U S A* 2007;104:15075-15080.

51. Sen S, Rouphael C, Houston S, Sylvetsky AC, Rother KI. Do sucralose and saccharin promote fat accumulation in cultured human adipose derived mesenchymal stem cells? *Diabetes* 2015;64:A560. Abstract 2199-P.
52. Abou-Donia MB, El-Masry EM, Abdel-Rahman AA, McLendon RE, Schiffman SS. Splenda alters gut microflora and increases intestinal p-glycoprotein and cytochrome p-450 in male rats. *J Toxicol Environ Health A* 2008;71:1415-1429.
53. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. Gut Microbiome response to sucralose and its potential role in inducing liver inflammation in mice. *Front Physiol* 2017;8:487. doi:10.3389/fphys.2017.00487
54. Bian X, Tu P, Chi L, Gao B, Ru H, Lu K. Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions. *Food Chem Toxicol* 2017;107(Pt B):530-539.
55. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *PLoS One* 2017;12:e0178426. doi:10.1371/journal.pone.0178426
56. Palmnas MS, Cowan TE, Bomhof MR, et al. Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. *PLoS One* 2014;9:e109841. doi:10.1371/journal.pone.0109841
57. Suez J, Korem T, Zilberman-Schapira G, Segal E, Elinav E. Non-caloric artificial sweeteners and the microbiome: findings and challenges. *Gut Microbes* 2015;6:149-155.
58. Daly K, Darby AC, Shirazi-Beechey SP. Low calorie sweeteners and gut microbiota. *Physiol Behav* 2016;164(Pt B):494-500.
59. Bright OMWD, White MS, Bleich SN, et al. Research priorities for studies linking intake of low calorie sweeteners and potentially related health outcomes. *Curr Dev Nutr* 2017;1:e000547. doi:10.3945/cdn.117.000547
60. Swithers SE, Davidson TL. A role for sweet taste: calorie predictive relations in energy regulation by rats. *Behav Neurosci* 2008;122:161-173.
61. Feijo Fde M, Ballard CR, Foletto KC, et al. Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels. *Appetite* 2013;60:203-207.
62. Boakes RA, Kendig MD, Martire SI, Rooney KB. Sweetening yoghurt with glucose, but not with saccharin, promotes weight gain and increased fat pad mass in rats. *Appetite* 2016;105:114-128.
63. Glendinning JI. Do low-calorie sweeteners promote weight gain in rodents? *Physiol Behav* 2016;164(Pt B):509-513.
64. Peters JC, Beck J. Low calorie sweetener (LCS) use and energy balance. *Physiol Behav* 2016;164(Pt B):524-528.