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Low-calorie sweetener use and energy balance: Results from experimental studies in animals, and large-scale prospective studies in humans

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Abstract

For more than a decade, pioneering animal studies conducted by investigators at Purdue University have provided evidence to support a central thesis: that the uncoupling of sweet taste and caloric intake by low-calorie sweeteners (LCS) can disrupt an animal's ability to predict the metabolic consequences of sweet taste, and thereby impair the animal's ability to respond appropriately to sweet-tasting foods. These investigators' work has been replicated and extended internationally. There now exists a body of evidence, from a number of investigators, that animals chronically exposed to any of a range of LCSs – including saccharin, sucralose, acesulfame potassium, aspartame, or the combination of erythritol + aspartame – have exhibited one or more of the following conditions: increased food consumption, lower post-prandial thermogenesis, increased weight gain, greater percent body fat, decreased GLP-1 release during glucose tolerance testing, and significantly greater fasting glucose, glucose area under the curve during glucose tolerance testing, and hyperinsulinemia, compared with animals exposed to plain water or – in many cases – even to calorically-sweetened foods or liquids. Adverse impacts of LCS have appeared diminished in animals on dietary restriction, but were pronounced among males, animals genetically predisposed to obesity, and animals with diet-induced obesity. Impacts have been especially striking in animals on high-energy diets: diets high in fats and sugars, and diets which resemble a highly-processed 'Western' diet, including trans-fatty acids and monosodium glutamate.

These studies have offered both support for, and biologically plausible mechanisms to explain, the results from a series of large-scale, long-term prospective observational studies conducted in humans, in which longitudinal increases in weight, abdominal adiposity, and incidence of overweight and obesity have been observed among study participants who reported using diet sodas and other LCS-sweetened beverages daily or more often at baseline. Furthermore, frequent use of diet beverages has been associated prospectively with increased long-term risk and/or hazard of a number of cardiometabolic conditions usually considered to be among the sequelae of obesity: hypertension, metabolic syndrome, diabetes, depression, kidney dysfunction, heart attack, stroke, and even cardiovascular and total mortality. Reverse causality does not appear to explain fully the increased risk observed across all of these studies, the majority of which have included key potential confounders as covariates. These have included body mass index or waist circumference at baseline; total caloric intake and specific macronutrient intake; physical activity; smoking; demographic and other relevant risk factors; and/or family history of disease. Whether non-LCS ingredients in diet beverages might have independently increased the weight gain and/or cardiometabolic risk observed among frequent consumers of LCS-sweetened beverages deserves

further exploration. In the meantime, however, there is a striking congruence between results from animal research and a number of large-scale, long-term observational studies in humans, in finding significantly increased weight gain, adiposity, incidence of obesity, cardiometabolic risk, and even total mortality among individuals with chronic, daily exposure to low-calorie sweeteners – and these results are troubling.

Keywords

Low-calorie sweeteners; Non-nutritive sweeteners; Diet sodas; Diet beverages; Weight; Obesity

1. Introduction

Obesity prevalence in the U.S. increased dramatically during the 8-year period between the end of the National Health and Nutrition Examination Survey (NHANES) II, conducted from 1976 to 1980, and NHANES III, conducted from 1988 to 1994 [1]. Before 1980, saccharin, a coal-tar derivative, had been the main low-calorie sweetener (LCS) in the U.S. for a century [2] – consumed at relatively modest levels, and marketed, for much of that time, primarily to individuals with diabetes [3]. Between 1980 and 1988, however, LCS consumption in the U.S. increased dramatically following the introduction of aspartame, a methyl ester of aspartic acid and phenylalanine, as a tabletop sweetener in 1981 and as an ingredient in diet sodas, other beverages, and foods in 1983. Over the next three decades, obesity prevalence continued to rise – as did LCS intake, with the introduction of sucralose, a chlorinated hydrocarbon, in 1998, followed by neotame (2002); acesulfame potassium (AceK: 2003); and advantame (2014) [2], and the introduction of stevia and monk fruit extracts in 1994 [4] and 2010 [5], respectively. So: have the epidemics of obesity, metabolic syndrome, and diabetes which have swept our nation since 1980 occurred *despite* the use of LCS? Would they have been *even worse* had LCS not been so widely used? Or have our epidemics of obesity and its sequelae been *fueled* by the widespread use of these products, especially among highest-risk individuals?

2. Results from animal studies

Investigators at Purdue University have pioneered animal research into the relationship between LCS exposure and changes in a number of weight-related variables, including food consumption, weight change, and adiposity. Research by the team of Davidson [6–10], Swithers [11–21], and colleagues have provided evidence, for more than a decade, to support a central thesis: that the uncoupling of sweet taste and caloric consequences by LCS can disrupt an animal's ability to predict the metabolic consequences of sweet taste, and thereby impair the animal's ability to respond appropriately to sweet-tasting foods. In 2004 [6] they reported results for two groups of rats exposed for 10 days to sweetened liquids: in one group (the ‘non-predictive’ group), rats were fed two different liquids – one saccharin- and the other glucose-sweetened – so that sweet taste did not reliably predict caloric intake. In the other (‘predictive’) group, rats were also fed two different sweet liquids, but both were sweetened with sugar (either sucrose or glucose), so that – for this group – sweet taste always predicted caloric intake. After this dual-exposure period, both groups were fed a

small, calorically sweetened snack, and then allowed ad lib consumption of chow for an hour. Caloric intake from the snack was similar in both groups; post-snack chow intake in the 'non-predictive' group, however, was quadruple that in the 'predictive' group [6]. In subsequent experiments with adult Sprague-Dawley rats, conducted over 14 days, those animals for which the sweet taste of a yogurt diet was non-predictive of calories, consumed significantly more total calories, gained more body weight, and developed greater adiposity – measured as percent body fat by DEXA – compared with 'predictive' group members [13]. Several potential mechanisms which could have contributed to such changes included significantly higher post-ingestion thermogenesis, as measured by increases in core body temperature within the first 30 min after feeding in the predictive group, compared with the non-predictive group [13]; and, during this same period, higher point estimates for the activity departure from baseline in the predictive, vs. the non-predictive group – a difference which approached significance [13]. The investigators later extended the scope of their research to assess the impact of AceK exposure on adult male Sprague-Dawley rats, with similar results: rats given access to either AceK- or saccharin-sweetened yogurt for 7 of 14 days exhibited significantly greater weight gain than rats in a comparison group given glucose-sweetened yogurt throughout this period [14].

Other investigators extended this research to include additional LCS products, including Splenda, a widely-used sweetener featuring sucralose. Abou-Donia et al. [22] investigated the impact of Splenda on relative weight change in four groups of adult male Sprague-Dawley rats administered various concentrations of Splenda-sweetened water solutions – from 100 to 1000 mg/kg/day by oral gavage – for 12 weeks; the animals' weights were then monitored for an additional 12 weeks: the 'recovery' period. By the end of the first 12 weeks, rats exposed to the lowest Splenda dose, 100 mg/kg/day, had gained significantly more weight, relative to baseline, than rats in the plain-water control group [22]. Following discontinuation of Splenda, however, relative weights of rats in all Splenda-exposed groups continued to climb, and by the end of week 24, point estimates for weight gain in all Splenda-exposed rats were higher than those for water controls; these differences were significant for the 100 and 500 mg/kg/day groups [22]. Similarly, Feijó et al. [23] extended these investigations by studying weight gain in Wistar rats exposed to aspartame. In their study, rats exposed to either saccharin or aspartame experienced significantly increased weight gain, compared with rats exposed to sucrose – despite similar caloric intake among the groups. Polyák et al. also reported increased body weight gain in both male and female CBA/CA inbred mice treated with saccharin, and in males treated with cyclamate, following 25 weeks of LCS supplementation, even though – as in the previous study – no significant differences in food intake were found among the groups [24].

The impact of LCS in several of these studies varied with intrinsic characteristics of the animals, including sex [19,25] and genetic predisposition to obesity [19]; the animals' diet [7,19]; and whether the animals were already obese at the outset of the study [19,26]. In one representative study, substantial body-weight gains were observed in inbred obesity-prone females fed saccharin- vs. glucose-sweetened yogurt, but corresponding differences were not found in inbred obesity-resistant females [19]. Nonetheless, fat mass was significantly higher in all saccharin-exposed female rats, regardless of their inbred obesity phenotype [19]. LCS impact also varied with characteristics of the diet, including whether the animals

had been placed on high-energy diets, or were kept on regular low-fat chow, or on dietary restriction [7;19]. Swithers, Davidson, et al., for example, reported greater LCS-related weight gain in males than females [19], and observed that saccharin exposure caused substantial weight gain in female rats with diet-induced obesity, fed a high-fat, high-sugar diet – but not in females on regular, low-fat chow, or in diet-restricted animals [19].

Animal studies have also identified three important factors extrinsic to the animals themselves, which modulate the impact of LCS: metabolic and other physiologic characteristics of the specific LCS used in the study; the dosage administered; and the timing of the initiation of exposure. Saccharin, sucralose, and AceK, for example, remain almost completely unmetabolized, and thus are mainly excreted from the body in their original form. But aspartame is rapidly metabolized in the upper intestinal mucosa into its three main constituents – phenylalanine, aspartic acid, and methanol – and significant elevations of the amino acids, both in plasma and in the brain, have been detected for at least an hour following bolus ingestion in rodents [27,28]. These differences have likely contributed to the differential impacts reported for LCSs on weight change, glucose homeostasis, and the gut microbiota in various studies. The dosages of specific LCSs, and whether they have been administered in isolation or in combination with other compounds, have also varied dramatically, and have ranged from a 5% LCS + 95% glucose solution reported in one study [29], for example, to the maximum acceptable daily intake (ADI) of specific individual LCSs in another [24], to a 0.006% LCS solution reported in yet another study [30]. Although the initiation of LCS administration has generally begun in adulthood, some studies have initiated it prenatally or neonatally [25,31,32]. These multiple factors – combined with the overall variety of animal strains included in the experiments, their environments of origin, and resultant differences in their gut microbiota [33], even prior to the initiation of LCS – have undoubtedly contributed to the range of outcomes reported in these studies, and would be expected to influence variation in the outcomes of human studies, as well.

The findings of Collison et al. from 2013 [32] are of particular interest in highlighting the striking impact of extrinsic factors – in this case, other dietary components – on the relationship between LCS exposure and cardiometabolic changes. Four groups of male C57BL/6J offspring mice were exposed from conception (through maternal diet) on through age 20 weeks to different combinations of the following dietary supplements: trans-fatty acids (TFA) only, TFA + monosodium glutamate (MSG), TFA + aspartame (ASP), or the sum of all three: TFA + MSG + ASP. The results were intriguing: when obesity- and diabetes-prone mice received dietary supplementation with TFA + ASP, they exhibited no increase in adipose weight, compared with TFA alone, although they developed 26% more visceral adipose weight when they received supplementation with the combination of TFA + MSG. When, however, their diet was supplemented with all three – TFA + MSG + ASP – the results were dramatic: compared with TFA-only controls, these mice developed a 55% increase in visceral adipose mass, with 'striking alterations in gene transcription' in their adipose tissue, and striking increases in TNFα, fasting glucose, and HOMA IR – even in the absence of significantly increased weight gain [32]. The scale of these interactions was reminiscent of those reported by Lau et al. [34] among several common food additives – including aspartame – for other endpoints [34]. Such interactions underscore the dramatic

and unpredictable effect modification that can be triggered by non-LCS dietary components which are common throughout the U.S. food supply.

As these results from Collison underscore, deleterious impacts observed in animal studies were not limited to – and sometimes occurred in the absence of – increased weight gain. Mitsutomi et al. [26], examining the impact of exposure to a combination of non-nutritive sweeteners ('NNS': 99% erythritol and 1% aspartame) in mature C57BL/6 mice with diet-induced obesity, reported not only dramatically higher epididymal white tissue mass in the NNS-exposed group – despite comparable food intake – but also insulin levels that were double those in a plain-water control group [26]. Similarly, in 2012 Collison et al., studying C57BL/6J mouse offspring exposed to aspartame from conception through age 17 weeks, reported that aspartame exposure increased percent weight gain and reduced insulin sensitivity in males, and markedly increased visceral fat and fasting glucose in both sexes, even in the absence of increased weight gain in females [35]. In a second experiment also published in 2012, Collison et al. [25] reported significantly increased percent weight change in males – but not females – exposed to either ASP alone or the combination of ASP + MSG [25]. For both sexes, however, fasting glucose levels were 1.6-fold higher and insulin sensitivity was decreased in animals exposed to ASP alone, and fasting glucose levels were 2.25- and 2.3-fold higher for males and females, respectively, exposed to ASP + MSG, compared with controls [25]. Similarly, Swithers et al. reported that – in addition to experiencing significantly increased weight and adiposity – saccharin-exposed mice exhibited impaired glucose tolerance and impaired GLP-1 release during an oral glucose tolerance test [17]. These results were congruent with those of Mitsutomi et al. [26], who also found, among mice with diet-induced obesity exposed to NNS, significantly higher glucose area under the curve ($AUC_{glucose}$) during glucose tolerance testing, and higher insulin levels in NNS-exposed mice, vs. controls [26].

3. Lessons from animal studies, and their relevance to the design and interpretation of results from randomized clinical trials (RCTs)

As these and other animal studies have demonstrated, multiple factors clearly modulate the impacts of LCS on health outcomes. The presence of these factors – such as the amount of trans-fatty acids in the participants' diet – is sometimes either unknown or unmeasured in randomized clinical trials (RCTs); in other cases, relevant factors – such as female sex, or the requirement of dietary restriction – are in fact prescribed by the study design in a manner which would – whether intentionally or not – tend to minimize the occurrence of expected adverse effects in LCS-exposed participants. In either case, these factors can erode the comparability of RCT outcomes with those of animal studies and large-scale prospective observational studies. What is particularly striking about results from these animal studies is the key influence of the animal's diet in determining whether weight gain or other cardiometabolic consequences will follow LCS exposure. This raises the question – for those RCTs which have major dietary-change protocols embedded within them – whether their results can in fact be extrapolated to the general population. If indeed, for example, an RCT were to prescribe a healthful diet to participants, which minimized the intake of total calories, sugar, total fat, trans-fatty acids, and processed and restaurant food – which often

contains MSG as a flavor enhancer – as a backdrop for testing the impact of LCS on health, such an RCT might find no deleterious impact of LCS on weight and other cardiometabolic variables, specifically because the risk contributed by the typical Western diet itself – as might be widely used in the free-living U.S. population – had been minimized *by design* in such a study. If, in addition, increased physical activity – which can itself contribute to weight loss and increased insulin sensitivity [36,37] – were also included in an RCT protocol, these design elements might offer yet further protection against development of the kind of adverse outcomes repeatedly observed in animal studies, and in observational studies in humans, which have no such behavioral interventions. Thus, apparent discrepancies between results from some of these carefully structured RCTs, on the one hand, and both animal studies and long-term, large-scale observational studies, on the other, may derive – at least in part – from design elements of the RCT protocols themselves, which create a context for LCS use which is radically different from that among the majority of the community.

In order for the results of an RCT to be directly applicable to the general population, as a whole, it would ideally represent *proportionally*, as faithfully as possible, those population subgroups which animal studies and long-term observational studies in humans have identified as most likely to show adverse effects from LCS exposure: these include males and individuals genetically prone to obesity. Two-thirds of RCTs reviewed by Miller et al. [38], however, had included either disproportionately few [39–46] or no males [47–50], even though animal studies have shown greater weight gain following LCS exposure in males, compared with females [19,35]. Similarly, ethnic minorities – such as African Americans and Mexican Americans – and lower-income participants have typically been underrepresented in RCTs, even though obesity and diabetes prevalence is higher in these groups [51–53]. While ancillary interventions – including dietary restriction, significantly increased physical activity, and both group and personalized individual behavioral modification counseling/motivational sessions – are appropriate for an RCT evaluating the impact of *lifestyle change* on weight and cardiometabolic risk, they provide an unrealistic context for evaluating the expected impact of LCS exposure in the general adult U.S. population, which is predominantly sedentary, prone to consume high-energy diets – and 48% male [54].

4. Weight gain: the canary in the coal mine?

Why are we interested in weight change as an endpoint for LCS exposure, in the first place? Isn't it largely because weight gain may provide a warning of emerging – but not yet recognized – health risk? In this, it resembles the canary traditionally carried by miners into coal mines, to provide a warning of the impending danger of already-present but as yet undetected toxic gas. But what happens to the miner if the canary test fails?

As noted earlier, a number of animal studies reported increases not only in animals' weight and adiposity but also in cardiometabolic conditions that might have been presumed to be sequelae of the animals' obesity, including impaired glucose tolerance [17], decreased insulin sensitivity [26,35], and hyperinsulinemia [26]. In other animal studies, however, disruption of glucose homeostasis and – in some cases – even increased adiposity were observed *without* weight gain itself. Thus, Suez et al. observed increased glucose intolerance

– despite no reported increase in body weight – in mice exposed to either saccharin, sucralose, or aspartame [29]. Similarly, in 2013, Collison et al. found that TFA-fed male C57BL/6 mice, exposed to ASP + MSG from conception to age 20 weeks, displayed pre-diabetic changes in gene expression, and dramatically increased adipose weight, liver triglycerides, fasting glucose, and HOMA-IR which were significantly greater than changes observed in animals exposed only to TFA + MSG (without ASP), even though the TFA + ASP + MSG animals experienced no significant weight gain [32]. Palmnäs et al. [30] also reported paradoxical results: among diet-induced obese rats, aspartame exposure was associated with lower food consumption, final weight, body fat, and insulin – and yet higher fasting blood glucose, and $AUC_{glucose}$ in insulin tolerance testing. In these studies – in which weight gain did not occur in LCS-exposed animals – should its absence be construed as evidence of the healthfulness of these products'?

5. Congruent results from human observational studies

Several large-scale, long-term prospective observational studies in humans have yielded results comparable to the previously discussed animal studies. As long ago as 1986, Stellman and Garfinkel [55] reported results from an American Cancer Society cohort study: among 78,694 women followed for 1 year, saccharin users gained significantly more weight, within each quintile of relative weight at baseline; were more likely to gain 10 lb or more during the 1-year follow-up; and were more likely to gain ≥16% of their original weight during this time [55]. Weight differentials between users and non-users also tended to increase with baseline relative weight category. Colditz et al. [56], in 1990, reported a dose-response relationship between saccharin intake and subsequent weight gain among 31,940 participants in the Nurses' Health Study who were 35–54 years old in 1976 and followed for 8 yrs: weight gain was 54% higher among those with the highest saccharin intake, vs. none, from 1978 to 1980, and 34% higher in the highest saccharin-intake group vs. none from 1980 to 1984 [56]. In the San Antonio Heart Study (SAHS) [57], among 3465 participants originally examined during baseline exams from 1979 to 1988 who returned to follow-up 7 to 8 years later, we observed a dose-response relationship between the consumption of artificially sweetened (AS) beverages (ASB: sum of diet soda + AS coffee + AS tea) and weight gain over a 7- to 8-year follow-up. Participants in the fourth quartile of ASB intake – who consumed >22 ASB servings/week at baseline – experienced 78% greater gains in BMI than AS non-users, adjusted for baseline BMI and other relevant covariates. In addition, the risk of becoming either overweight or obese, among 1250 participants who had been normal-weight at baseline, was 93% higher among those in the fourth quartile of AS beverage intake, compared with non-users. Among 2571 participants who were either normal- or overweight (but not obese) at baseline, the risk of incident obesity was more than doubled among those with the highest ASB intake at baseline, compared with AS non-users. This difference occurred despite the fact that ASB users, overall – among participants for whom data from a 24-hour dietary recall were available (for the first of two SAHS cohorts) – had consumed significantly fewer kilocalories/day at baseline.

We extended these findings by examining the impact of diet soda consumption on change in abdominal adiposity among former SAHS participants, aged 65 years or older, who were subsequently enrolled in the San Antonio Longitudinal Study of Aging (SALSA) [58].

Following an extensive baseline examination (1992 to 1996), SALSA participants were invited to return to each of three separate follow-up examinations conducted over the next decade (mean follow-up: 9.4 years). Mean change in BMI of SALSA participants was minimal during this follow-up: as in other older individuals, their BMIs tended to plateau in their 70′s and decline thereafter [58], presumably due to age-related loss of lean body mass [59]. In this context, we observed a significant though modest positive trend between diet soda use and longitudinal BMI gain in SALSA. A dramatic, positive dose-response relationship emerged, however, between diet soda intake at the beginning of each follow-up interval and long-term change in waist circumference: those who consumed diet sodas daily or more often experienced subsequent waist circumference gains that were almost quadruple those of non-users; intermediate waist gains were observed in less-than-daily users, among whom waist gains were more than double those in non-users [58]. Increasing abdominal adiposity with aging has been associated with increased visceral fat [59], which in turn has been associated with increased inflammation [60], cardiometabolic risk [61,62], and risk of type 2 diabetes [60,63], depression [64,65], cognitive impairment [66], incidence of coronary heart disease [67,68], and mortality [69,70]. In fact, Després [71] has noted that weight loss itself does not constitute a sufficient goal for intervention, but rather that reducing waist circumference and increasing physical activity and fitness may be more relevant goals for health promotion. In this last connection, it is of interest that several RCTs and animal studies have reported point estimates for physical activity among LCS users that are – albeit non-significantly – lower than those of controls. And Peters et al., reporting 12-week follow-up results from a recent RCT, reported decreased weight among participants assigned to use, vs. avoid, LCS beverages, but no reduction in their waist circumferences [39].

Given the striking relationship between diet soda intake and long-term increases in abdominal adiposity in SALSA, it is not surprising that a number of other large-scale, long-term prospective observational studies have reported increased cardiometabolic risk among daily – or more frequent – consumers of diet sodas and/or other diet beverages. A detailing of these studies is not the main focus of this paper, but any full discussion of the impact of LCS on weight must acknowledge the repeatedly observed association, from a number of large-scale, prospective observational studies, between diet soda/beverage intake and subsequently increased risk of what might usually be considered sequelae of obesity. These reports include a 14% increase in incident hypertension in pooled analysis of data from the Nurses' Health Studies (NHS) I and II and the Health Professionals' Follow-up Study (HPFUS) [72]; 59% increased incidence of elevated waist circumference in the Multi-Ethnic Study of Atherosclerosis (MESA) [73]; 23 to 53% increased incidence of metabolic syndrome in MESA [73], the Atherosclerosis Risk in Communities study (ARIC) [74], the Framingham Heart Study [75], and the Coronary Artery Risk Development in Young Adults (CARDIA) study [76]; 38 to 68% increased incidence of diabetes in MESA [73] and the French *Etude Epidemiologique aupres des femmes de la Mutuelle Generale de l'Education Nationale*-European Prospective Investigation into Cancer and Nutrition (E3N-EPIC) study cohort [77]; 31% increased risk of depression in the NIH-AARP Diet and Health study [78]; doubling of the risk of kidney function decline in the NHS I and II [79]; 27% greater hazard of hemorrhagic stroke in pooled data from NHS and HPFUS [80]; 30 to 43% greater risk of

cardiovascular events, including heart attack and stroke, in the Northern Manhattan Study [81] and the Women's Health Initiative Observational Study (WHI-OS) [82]; and 50% increased hazard of cardiovascular mortality, and 30% increased hazard of overall mortality in the WHI-OS [82].

Thus, in both animal experiments and large-scale, long-term prospective observational studies in humans, daily exposure to high-intensity sweeteners has been associated – often in a dose-response manner – with increased cardiometabolic risk: increased weight gain, general and abdominal adiposity, incidence of overweight and obesity, and worsened glucose homeostasis. In observational studies in humans, daily and more-frequent users of diet sodas and diet beverages have also experienced significantly increased cardiometabolic risk and events, and mortality itself. Reverse causality does not appear to explain fully the increased risk consistently observed across these studies, the majority of which have included as covariates, in their analyses, key potential confounders, including baseline BMI, caloric intake, physical activity, and family history. Fagherazzi et al. [77], for example, in analyzing data for 66,118 French women in the E3N-EPIC study cohort, included a wide range of risk factors as covariates in their model – "years of education; smoking status; physical activity (metabolic equivalent task hours per week); hypertension; hypercholesterolemia; use of hormone replacement therapy; family history of diabetes; self-reported use of antidiabetic drugs; alcohol intake (g/d); omega-3 fatty acid intake; carbohydrate intake (g/d); coffee (mL/d), fruit and vegetables, and processed-meat consumption (g/d); and dietary pattern (Western or Mediterranean), total energy intake" and baseline BMI category: "<20, 20 to <25, 25–30, and ≥30" kg/m^2 – and still found a 68% increase in the 14-year risk of diabetes, among women consuming >603 mL/wk of ASBs, vs. non-users [77].

Several ironies have recurred in these animal and human studies: impact has often been *most severe* in animals and humans genetically disposed to obesity and diabetes, or concurrently on a Western-style diet, with high-fat and/or high-sugar content, or already overweight or obese. Thus, those most at risk often fared the *worst* by using LCS. In their 2013 paper, Swithers et al. sounded this cautionary note: 'These results suggest that the most negative consequences of consuming high-intensity sweeteners may occur in those most likely to use them for weight control, females consuming a "Westernized" diet and already prone to excess weight gain.' [19] Another irony is that cardiometabolic risk has sometimes escalated in LCS-exposed subjects in the *absence* of significantly increased weight gain.

The mechanisms through which LCS and LCS-sweetened beverages might increase the risk of cardiometabolic problems lie beyond the scope of this paper. It should be noted, however, that biologically plausible mechanisms have been identified through both animal and human studies, and include – among others – disruption of animals' ability to predict the caloric consequences of sweet taste [6,7,10]; decreased release of GLP1 in response to sweet-tasting food [17]; up- and down-regulation of gene transcription in adipose tissue [32]; disruption of neurometabolic function in the hippocampus [83]; altered reward processing of sweet taste in humans [84]; and adverse impacts on the gut microbiota [29,30,85]. This last topic – the potential impact of LCS on the gut microbiota – is of particular concern. Preliminary data [29] suggest that high intake of the LCS saccharin, at a level corresponding to the FDA's acceptable daily limit, may increase glucose intolerance in both humans and animals, and

that the impact may be through adverse influences on the gut microbiota [29]. Similarly, exposure to aspartame has been associated with elevated fasting glucose and diminished insulin-stimulated glucose disposal [30], as well as changes to the gut microbiota, related to increased production of propionate, a short-chain fatty acid which is a substrate for gluconeogenesis [30]. Because this topic is discussed elsewhere in this issue, it will not be addressed further here, except through one final question, below, regarding LCS vehicles.

6. A question for future research: what is the total impact of the most prominent LCS *vehicles* – diet sodas and other diet beverages – on cardiometabolic risk?

Up to this point, this discussion has been limited to the presentation of results from animal experiments, and from large-scale, prospective human studies, in which increases in weight gain, adiposity, and other cardiometabolic risk factors have been observed among subjects with daily LCS exposure. In each of the animal studies cited, the measured exposure of interest has been a specific *LCS*, or multiple LCSs. In observational studies in humans, by contrast, the measured exposures have been either *diet sodas*, specifically, or *LCS-sweetened beverages*, in general – which include diet sodas, and represent the most-frequently-used LCS vehicles in the U.S. [86–88]. LCSs represent only a tiny fraction, by weight, of diet beverages, however, so the question might be raised: do any other, non-LCS components of diet beverages *independently* increase weight gain and/or cardiometabolic risk? Diet sodas, for example, share a number of components with sugar-sweetened sodas: carbonated water; potassium citrate; potassium benzoate; caramel color – in colas – which contains advanced glycation end products [89,90]; caffeine, in caffeinated sodas; artificial colors and flavors; citric and/or phosphoric acid; and bisphenol A (BPA)[91,92], in canned sodas – to name a few. Some of these non-LCS ingredients of diet sodas have themselves been associated with increased health risks, including general and abdominal obesity [93,94], inflammation [95,96], decreased insulin sensitivity [97], diabetes [98,99] and its complications [96,100], hypertension [101], atherosclerosis [102,103], and other health problems [34,90,104–107]. An important additional public health question, then, given the popularity of diet sodas as vehicles for LCS consumption, might be: what independent impact might their *non-LCS* ingredients have on frequent users' weight gain and cardiometabolic risk? And might changes within the gut microbiota of frequent users – changes which might, for example, increase the efficiency of their nutrient harvesting [108–111] from ingested food, increase energy storage in their adipose tissue [108,112], or increase their intestinal permeability, with resultant leakage of lipopolysaccharides, and increased systemic inflammation [111] – mediate any part of the impact from these vehicles?

The gut microbiota represents a rich interior ecosystem: dynamic, teeming with life, yet also vulnerable. In the broadest sense, it could be likened, simplistically, to a rainforest. Could high-volume, daily intake of diet sodas affect the gut microbiota adversely – through either their LCS content itself [29,30,85], BPA content [91], advanced glycation endproducts, or other chemical components – in any way that is comparable to the impact that human-origin chemical exposures might have on an actual rainforest, by shifting the balance of microbiota subpopulations and their behaviors? Even subtle changes might influence cardiometabolic

risk itself. Further research on the potential *total* impact of prominent LCS vehicles seems warranted, in order to more adequately assess the full impact of these widely-used vehicles on both the gut microbiota and cardiometabolic risk, especially among frequent consumers.

What we know for now is this: results from both animal studies and a number of large-scale, long-term observational studies in humans have reported significantly increased weight gain, abdominal adiposity, and cardiometabolic risk among those frequently exposed to LCSs. Even after controlling for a wide range of key potential confounders, studies in humans have found significantly increased incidence of depression, metabolic syndrome, diabetes, cardiovascular events, cardiovascular mortality, and total mortality among frequent users of the most-widely-used LCS vehicles – diet sodas and other diet beverages – and these results are troubling.

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HIGHLIGHTS

- Prospective studies have reported greater long-term weight gain and cardiometabolic risk in low-calorie sweetener (LCS) users.
- Animal studies have also shown increased weight gain and adiposity, and impaired glucose homeostasis, in LCS-exposed animals
- Adverse impacts appear greater in males, obese animals, and those on high-energy diets resembling processed ‘Western’ diets.
- Animal studies have provided biologically plausible mechanisms for weight gain and metabolic derangement in LCS consumers.
- Reverse causality does not fully explain higher risks in humans, which remain after adjustment for key potential confounders.