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The impact of low and no-caloric sweeteners on glucose absorption, incretin secretion and glucose tolerance

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Abstract

The consumption of non-nutritive, low or no-calorie sweeteners (LCS) is increasing globally. Previously thought to be physiologically inert, there is a growing body of evidence that LCS not only provide a sweet taste but may also elicit metabolic effects in the gastrointestinal tract. This review provides a brief overview of the chemical and receptor-binding properties and effects on chemosensation of different LCS but focuses on the extent to which LCS stimulates glucose transport, incretin and insulin secretion, and effects on glucose tolerance. Aspartame and sucralose both bind to a similar region of the sweet receptor. For sucralose, the data are contradictory regarding effects on glucose tolerance in humans and may depend on the food or beverage matrix and the duration of administration, as suggested by longer-term rodent studies. For aspartame, there are fewer data. On the other hand, acesulfame-potassium (Ace-K) and saccharin have similar binding characteristics to each other but, while Ace-K may increase incretin secretion and glucose responses in humans, there are no data on saccharin except in rats, which show impaired glucose tolerance after chronic administration. Additional research, particularly of the effects of chronic consumption, is needed to provide concrete evidence for beneficial or detrimental effects of LCS on blood glucose regulation in humans.

Keywords: low-calorie sweetener, non-nutritive sweetener, glucose transport, incretin, glucagon-like peptide-1, glucose tolerance

Introduction

Over the past four decades, the use of low- or no-calorie sweeteners (LCS) in foods and beverages has increased worldwide (Yang 2010). This has led to increased interest in information on sugar substitutes as indicated by a Google Trends search between 2004 and 2016, with sugar substitutes being a popular “search term” in 2010 with a score of over 70 and trending incrementally to reach a maximum score of 100 in two subsequent years, 2013-2014. (Google Trends, 2016). LCS are also known as *artificial, non-nutritive, no-calorie* or *high-intensity* sweeteners and are used to create products in which sweetness is achieved with addition of few or no calories. Increasing demand for such products has resulted in an increase in the number of LCS approved and the number of products containing them has increased (Yang, 2010), furthering their consumption. In the United States from 2005 to 2009, of over 85,000 unique food and beverage products available, 6.6% contained LCS alone or in combination with caloric sweeteners. When actual purchases were analyzed, 15% of the total volume of foods and beverages bought by consumers contained LCS (Ng et al., 2012), likely contributing to the ongoing continued increase in prevalence of usage (Sylvetsky et al., 2012; Yang, 2010), including in children. In comparison to 1999-2000 cycle National Health and Nutrition Examination Survey (NHANES) data, by 2007-8 cycle a doubling in intake of LCS beverages by children ≥ 2 years in 2007-8 has also been noted, from a prevalence of 6.3% to 13.5% (Sylvetsky et al., 2012). In adults in the NHANES cohort, over the same period, prevalent consumers of LCS increased from 19.3% to 26.7% (Sylvetsky et al., 2012). From an anthropological perspective, it is argued that consumers of LCS now perceive them as health enhancing, thanks to the rise in popularity of dieting related to idealized body silhouettes and changes in marketing strategy by companies such as placing LCS in the drug aisle of supermarkets and department

stores (de la Pena, 2010). Experimentation of non-diabetic individuals with using LCS began in World War II because of sugar rationing (de la Pena, 2010). LCS use continued as women in particular began to exercise their right to autonomy with respect to how they fed their filies, fomented by various advertising campaigns (de la Pena, 2010). However, consuming LCS does not necessarily lead to consuming a healthier diet as indicated by data from the National Health and Nutrition Examination Survey (NHANES) cohort. Using 1999-2008 NHANES data, adult consumers of LCS had higher total Healthy Eating Index scores, although they also had worse subscores for saturated fat and sodium (Drewnowski and Rehm, 2014). Another study analyzed NHANES data 2003-2010 for dietary intakes and Neilson Homescan data 2000-2010 to evaluate purchases (Piernas et al., 2014). Two patterns emerged for both adult and child consumers of LCS. Those who avoided calorically-sweetened foods had lower overall energy and sugar intake than non-consumers (NC) but consumers of LCS plus calorically-sweetened beverages (LCS+CSB) had higher overall energy and sugar intake. A summary of the 24-h recall data (mean kcal/day) for adults and children illustrates this point: energy from all sources (NC=1901; LCS=1944; LCS+CSB=2262), from carbohydrates (NC=936; LCS=884; LCS+CSB=1102), from sugar (NC=416; LCS=338; LCS+CSB=507), from total fat (NC=643; LCS=706; LCS+CSB=793), from saturated fat (NC=216, LCS=233; LCS+CSB=264), and from protein (NC=305; LCS=328; LCS+CSB=336) (Piernas et al., 2014). These data suggest that some LCS consumers maintain an overall relatively healthy or “prudent” diet whereas others, who combine LCS and CSB consumption, have relatively less healthy diets. Aside from the timeline, the major difference in these studies of NHANES data (Drewnowski and Rehm, 2014; Piernas et al., 2014) is that the former analyzed single 24-hour dietary recalls whereas the latter measured food purchases, two 24-hour dietary recalls, and created dietary patterns by factor analysis.

Although consumption of foods containing LCS is increasing, consumer perceptions of flavor, safety and health impacts (Gardener, et. al., 2012) as well as changes in product formulation may impact the type of LCS consumed. Despite the growing economic drive to produce food products using LCS and to meet consumer demand for products with reduced calories but high palatability, large gaps remain in our knowledge of the effects of LCS on human physiology. Therefore, understanding the biochemical properties of LCS that lend themselves to providing a sweet taste and their ability to provoke other biological functions in humans is important. The purpose of this review is to identify the current knowledge regarding the mechanisms of action elicited by low calorie or non-nutritive sweeteners on taste receptors, which affects outcomes of (a) chemosensation in the gut; (b) gut physiology particularly with respect to incretin hormones (mainly glucagon-like peptide-1 (GLP-1)) and (c) potential for influencing glucose homeostasis and insulin secretion.

Overview of relationship between LCS and health

It is important to understand the potential direct and indirect effects of LCS on human physiology and metabolism. First, LCS consumption is associated with overall dietary patterns and may indirectly affect health outcomes. Second, more recent research evidence also indicates direct effects of LCS on human physiology and metabolism. A detailed discussion below will shed light on the possible mechanisms involved.

Indirect Effects: Recent studies have used NHANES or consumer purchasing data to examine relationships between dietary and LCS intakes in the United States. Piernas and colleagues (Piernas et al., 2014) found that consumers of LCS followed two distinct dietary patterns that included foods either equated to a prudent diet (which included home-cooking using whole plain

foods such as fruit, vegetables, grains, cooking fat and other foods) or from a pattern of ready-to-eat-meals/fast-foods (Piernas et al., 2014). NHANES data also revealed differences in dietary patterns associated with the type of beverage consumption depending on whether individuals were normal-weight or obese. In this study an overall increase in intake of foods with low nutrient density (typically high in fat, sugar, cholesterol and sodium) was associated with consumption of LCS beverages, and the pattern was more pronounced in obese respondents (An, 2016). Thus, LCS consumption may indirectly affect health due to poor nutritional quality of the diet, which may lead to micronutrient deficiency and risks for overweight and obesity.

However, there exists considerable controversy in the literature as to whether LCS exerts deleterious effects on human health and physiology. In a recent review article, Fowler (Fowler, 2016) argued that the current body of evidence supports the conclusion that use of LCS, in particular in beverages, are associated with negative health outcomes such as obesity, cardiovascular and metabolic diseases, and early mortality. Recent research has revealed that LCS consumers tend to have an increased risk of developing chronic disease even when data were adjusted for body mass index (BMI), total calorie intake and other potential confounders (Fowler, 2016). This author suggests the effects of various additives in the LCS beverages on weight gain and cardio-metabolic risks are unknown and deserve further investigation (Fowler, 2016). This association is consistent with studies in rodents showing that LCS consumption elicits weight gain (Swithers et al., 2009). Conversely, survey of people who maintained weight loss of at least 13.6 kg for 1 year found that 53% used LCS beverages for various purposes, including to reduce calories, and that about 40% felt such beverages were important in weight loss and maintenance (Catenacci et al., 2014). A recent systematic review and meta-analysis of randomized controlled studies (n=15 studies) concluded that provision of LCS to participants led

to small decreases in body mass and fat as well as waist circumference (Miller and Perez, 2014). However, evidence from prospective cohort studies (n=9) was not as clear, with LCS intake associated with a 0.03 kg/m² gain in BMI with follow-up ranging from 0.5-7.5 years (Miller and Perez, 2014). With respect to type 2 diabetes, meta-analysis of 10 prospective cohort studies identified a 25% increase in diabetes risk per one serving/day of beverage sweetened with LCS, indicating a positive association between increased risk for diabetes and consumption of beverage sweetened with LCS. This association was attenuated when adjusted for adiposity (Imamura et al., 2016), which is consistent with the notion of reverse causation. The authors also detected publication bias and residual confounding that could lead to a false positive result (Imamura et al., 2016). It is possible that randomized controlled trials (RCT) do not have long enough follow-up to capture weight gain, whereas in the prospective cohort studies it is difficult to be confident that all legitimate confounders have been taken into account.

Direct Effects: LCS may directly affect physiological systems by interacting with the same taste receptors as caloric sweeteners e.g. sucrose, which are found not only on the tongue but also on epithelial cells of the small intestine of rodents and other mammals (Margolskee et al., 2007, Mace et al., 2007, Jang et. al., 2007). For sweet taste, two proteins called T1R2 and T1R3 form a heterodimer, which is required for receptor activation. Although their existence in human intestine has been questioned, recent studies have positively identified T1R2 in human duodenum by immunohistochemistry and T1R3 by quantitative polymerase chain reaction; and like in rodents, subpopulations of T1R2 co-localized with either glucose-dependent insulinotropic polypeptide (GIP) or GLP-1-producing cells (Young et al., 2013; Young et al., 2009). Both GIP and GLP-1 are released from the gut in response to dietary carbohydrates and stimulate insulin release. Thus the presence of T1R2 and T1R3 in the gut indicates the presence

of a feedback mechanism involved in identification and absorption of simple carbohydrates/LCS in the gut as discussed in more detail below. LCS may also exert an effect on metabolism/physiology by influencing the gut microbiome (Suez et al., 2014) and the hedonic and regulatory centres of the brain such as the dopaminergic midbrain and certain cortical regions (Green and Murphy, 2012), and hippocampus, which is associated with memory (Cong et al., 2013). Thus, LCS may not simply be “inert” sweeteners but may directly or indirectly affect what people eat or how they respond to the nutrient absorption to impact health.

Chemical and structural properties of LCS

Properties of currently available LCS are summarized in Table 1, including the Acceptable Daily Intake recommended by the Canadian Diabetes Association (Canadian Diabetes Association, 2016). Note that different jurisdictions may limit the use of or not approve certain compounds. For a more in-depth discussion of the chemistry of these compounds, the reader is referred to the review by DuBois and Prakash (DuBois and Prakash, 2012).

LCS encompass a broad range of chemical forms, including cyclic sulfamates (acesulfame-potassium (ace-K) and cyclamate), dipeptides such as aspartame and other short peptides not yet in broad commercial application, organochlorines such as sucralose and complex glycosides including those now approved for commercial use from the *Stevia* family (Table 1). Other larger structures including peptides and oligosaccharides can also impart a sweet taste (DuBois and Prakash, 2012).

Natural sugars, sucrose and glucose, also react with binding sites on T1R2 and T1R3 but with different affinities, and variant conformational changes (Nie et al., 2005). Despite this, rodents lack ability to differentiate between the sweetness of sucrose, glucose and fructose

(Dotson and Spector, 2007), which implies a common signal transduction and neural signalling pathway. Natural sugars exhibit a linear concentration/response curve, interpreted as full agonism of the receptor (reviewed in Dubois, 2016). They do not bind to related receptor isoforms such as T1R1, which also forms a heterodimer with T1R3 to elicit umami, or with the receptors that detect bitter taste (Dubois, 2016). Paradoxically, attempts to identify effects of site-specific mutations on sucrose binding were unsuccessful, whereas much was learned regarding LCS as described below (Matsuda et al., 2012). This limits our ability to understand why most LCS are high-intensity sweeteners (that is, taste more sweet than sucrose at a given concentration as shown in Table 1), why LCS often have off-flavors (primarily bitterness), prolonged sensation of sweetness, reduced sweetness after multiple exposures (Dubois, 2016), or the impact of an LCS species binding to multiple sites on both T1R2 and T1R3.

While most LCS were initially considered to be “inert” from a physiological standpoint (Yang, 2010), the fact that they bind to the same taste receptor as mono- and disaccharides suggests that they may elicit other biological effects as well (Burke and Small, 2015). What is known of the receptor binding characteristics of several LCS is summarized in Table 1. In humans, ace-K, aspartame and sucralose bind to the amino terminal domain of T1R2 (Maillet et al., 2015; Masuda et al., 2012) in the T1R2+T1R3 heterodimer, similar to natural saccharides (Nie et al., 2005), whereas cyclamate and saccharin may bind to transmembrane domains (DuBois and Prakash, 2012; Jiang et al., 2005; Xu et al., 2004). Modeling studies suggest that steviol glycosides can bind to multiple sites on both T1R2 and T1R3 (Mayank and Jaitak, 2016). Divergence in binding sites, affinity and other properties (such as metabolism by gut microbes) suggests that biological effects may not be identical between the LCS compounds, which may affect outcomes of interest, such as their ability to stimulate glucose uptake and incretin secretion

in the gut. However, questions regarding the relevance of binding characteristics to taste properties have yet to be fully addressed (Dubois, 2016; Matsuda et al., 2012).

Chemosensation

Most of what is known about chemosensation has been learned by studying taste receptor signal transduction and neural pathways arising in the mouth. The T1R2/T1R3 heterodimer is coupled to a G-protein called gustducin, which upon ligand binding, activates a signalling pathway ultimately promoting calcium entry via a cation channel called transient receptor potential cation channel-5 (TRPM5), which elicits neurotransmitter release (reviewed in Brown and Rother 2012). Sensory afferent information is encoded in gustatory nerve fibers that project to the brain. Receptor binding properties of sweeteners can affect taste sensation. In the most extreme example, complete natural absence of T1R2 despite normal expression of T1R3 precludes heterodimer formation (Li et al., 2005) as happens in cats, which do not perceive sweet foods. Other interspecies differences in taste may also be due to genetic variance. For example, mice expressed a human transgene for T1R2 they perceived aspartame, which was absent in wild-type mice (Zhao et al., 2003). The amino terminal region of T1R2 has multiple binding sites, which partially overlap and have the potential for two sweeteners to potentiate sweet taste (Maillet et al., 2015). In addition, point mutations within the binding sites could affect taste perception. For example, in human T1R2 a E302A mutant reduced sensitivity to both aspartame and cyclamate by 100-fold, while E203G mutant maintained cyclamate sensitivity but impacted aspartame sensitivity significantly (Maillet et al., 2015). Such differences could have bearing on such practical matters as the pet food industry but more importantly for the subject of this review, on interpretation of experiments regarding taste sensation in rodents compared with humans, and

even within strains of mice, which exhibit specific sweetener preferences (Reed et al., 2004). In the gut, the basic cellular machinery (receptors, G proteins, signalling pathway molecules) for sweet perception is expressed in certain epithelial cells, as discussed further below.

Glucose transport or absorption

Potential functional effects of LCS on the gut have been studied in cell lines, rodents and humans. The majority of human studies examine acute effects of ingestion, whereas cell lines and rodent studies examine both acute and chronic outcomes. The main gut outcomes tested include glucose uptake and incretin secretion.

Cell lines have the high through-put capacity allowing many compounds to be tested in parallel but few studies have taken advantage of this. For analyzing incretin secretion or glucose uptake in response to T1R2/T1R3 binding, the most important cell lines are GLUTag cells, which are rodent-derived GLP-1-secreting (Drucker et al., 1992) and Hutu-80 cells, which also secrete GLP-1 but are derived from human duodenal cells (Schmidt et al., 1977). For studies of glucose transport alone, human-derived Caco-2 cells and other lines of intestinal origin may be relevant. All of these cells also express glucose transporters including SGLT1 and GLUT2, the most abundant sodium-dependent and –independent glucose transporters in the human small intestine (Zheng and Sarr, 2013). SGLT1 is expressed constitutively in apical membranes of intestinal epithelial cells whereas GLUT2 is expressed primarily on the basal membrane. However, in the presence of high glucose concentrations (> 30 mM), GLUT2 is translocated to the apical membrane and can account for up to 75% of glucose absorption under these conditions (Kellett et al., 2008).

Ace-K stimulates glucose uptake in Caco-2 and RIE-1 (rat-derived) cell lines (Zheng and

Sarr, 2013). Glucose uptake in this model was determined to be carrier-mediated because it could be inhibited with a downstream PKC inhibitor (Zheng and Sarr, 2013) and because ace-K had no impact on glucose transport (Zheng and Sarr, 2013) in a cell line lacking GLUT2 (IEC-6)(Zheng et al., 2012). A curvilinear dose response was illustrated with effects at physiological glucose concentrations (Zheng and Sarr, 2013). While these studies provide proof of principle, they are far removed from the complex physiological milieu in which other mediators such as GLP-2 are likely to be important. Studies of other LCS were not found.

Rat intestinal preparations *in vivo* were used to compare the impact of co-infusion of sweetener and glucose on glucose absorption (Mace et al., 2007). A glucose concentration that normally does not elicit GLUT2 translocation (and thus only basal glucose absorption) was used. In the presence of 1 mM sucralose or ace-K, however, glucose transport was doubled within minutes, an effect diminished by phloretin, a GLUT2 inhibitor; saccharin increased glucose transport by about 0.5-fold (Mace et al., 2007). Membrane insertion of GLUT2 was revealed by immunohistochemistry (Mace et al., 2007). These studies are more physiological; however they do not reveal whether the LCS exert direct or indirect effects on GLUT2 translocation and it should be noted that another study using rat jejunum found no effect of ace-K on glucose transport (Chaudhry et al., 2013). Longer-term exposure of tissues e.g. through dietary manipulation, could up-regulate expression of glucose transporter proteins, thereby increasing total transporting capacity. SGLT1 mRNA was increased after chronic feeding of ace-K, saccharin and sucralose but not aspartame in mice, correlating with enhanced absorption of glucose (Margolskee et al., 2007). Importantly, such effects were attenuated in mice lacking T1R3 or gustducin, the G-protein that transduces T1R2+T1R3 receptor binding (Margolskee et al., 2007). Piglets fed saccharin for 3 days had increased expression of SGLT1 mRNA

corresponding with increased glucose transport *ex vivo* but the exact mechanism is unclear because the antagonist of T1R3, lactisole, had no effect on saccharin-mediated effects (Moran et al., 2010). However, the relevance of these animal studies to human physiology is controversial because of experiments with contradictory results showing that sucralose did (Brown et al., 2009; Pepino et al., 2013) or did not (Ma et al., 2010) prime subsequent glucose absorption when consumed or infused into the duodenum of healthy humans. One difficulty in interpreting these results is that glucose concentration in the blood is the net of glucose transported minus the sum of glucose metabolized in the intestine and liver before it reaches peripheral blood in early stages, and uptake into peripheral tissues in later stages of the test, thus requiring an understanding of circulating insulin concentrations during the same time course. A strength of Ma et al. (Ma et al., 2010) is that similar results were seen with a non-metabolizable glucose analogue and that intraduodenal infusion avoided cephalic stimulation of insulin secretion.

Incretin Secretion

Glucose transport is possibly both a stimulant and a consequence of incretin secretion. Therefore, it is also important to understand the effects of LCS on incretin biology in physiological systems. Beginning with cell lines, studies have utilized both GLUTag (rat-derived) and NCI-H716 or Hutu-80 (human-derived) lines. All express T1R2+T1R3, gustducin, and proglucagon (Ohtsu et al., 2014), which is transcribed and processed primarily to GLP-1 and GLP-2 in the gut (Holst, 2009). There are consistent reports of LCS stimulating GLP-1 in cell lines, albeit using a limited number of compounds, mainly sucralose. Using Hutu-80 cells, the GLP-1 secretory responses to four LCS were quantified. All sweeteners (50 mM ace-K, sucralose, saccharin and 3 mM glycyrrhizin) had statistically significant GLP-1 responses when

assayed in the presence of low (3 mM) glucose. Furthermore, lactisole, a T1R3 antagonist blocked GLP-1 secretion. In NIH-716 cells, 5 mM sucralose elicited maximal GLP-1 secretion, which was blocked by either lactisole or siRNA against gustducin (Jang et al., 2007). Likewise, sucralose stimulated both GLP-1 and GIP secretion from GLUTag cells, which was blocked by gurmardin, an inhibitor of sweet taste receptor (Margolskee et al., 2007). Another rat-derived GLP-1 secreting cell line, STC-1 was incubated acutely with LCS diluted to similar sweetness equivalents to sucrose on a background of 5 mM glucose. All sweeteners tested (ace-K, aspartame, saccharin and sucralose) elicited secretion with potency ace-K>sucralose=sucrose=saccharin>aspartame (Geraedts et al., 2012).

Despite congruity of cell line results, studies of incretin secretion in animal models have yielded inconsistent results. For example, Fujita et al. (Fujita et al., 2009) compared the ability of ace-K, saccharin, sucralose and steviol glycoside to elicit GLP-1 secretion in rats but found that none did so when acutely gavaged into fasting animals. However, chronic feeding of saccharin did stimulate GLP-1 secretion in rats although in reduced amounts compared with glucose (Swithers et al., 2012). This suggests the mechanism of action *in vivo* might be indirect and require induction of specific genes within the intestinal epithelium.

Similar to rodents, acute administration of LCS alone including ace-K (Steinert et al., 2011), aspartame (Maersk et al., 2012; Steinert et al., 2011), or sucralose (Ma et al., 2009; Steinert et al., 2011) to humans generally has no effect on circulating GLP-1. Using duodenal biopsies of lean or obese subjects, Geraedts and colleagues (Geraedts et al., 2012) did not detect stimulation of GLP-1 by either sucrose or sucralose. In combination with a natural sugar (generally glucose or sucrose), reported effects of LCS on GLP-1 secretion are variable. When sucralose was given in advance of glucose to obese (Pepino et al., 2013) or type 2 diabetes

participants (Temizkan et al., 2015), GLP-1 secretion was not greater than water control; however, healthy participants did respond more robustly (Temizkan et al., 2015). Erythritol, which is a low-calorie bulk sweetener with low sweetness intensity, also stimulated GLP-1 after acute administration in combination with sucrose, similar to sucrose alone (Overduin et al., 2016). A combination of ace-K and sucralose (in a carbonated beverage) elicited GLP-1 secretion in healthy (Brown et al., 2009; Brown et al., 2012) but not diabetic humans (Brown et al., 2012). However, a more recent study from the same group found no difference in GLP-1 secretion when comparing preloads of seltzer water versus seltzer spiked with ace-K and sucralose; however, ace-K and sucralose in non-caffeinated cola evoked an increase in GLP-1 secretion and it was speculated that the cola flavouring potentiated the response (Sylvetsky et al., 2016b). The generally negative effects of acutely administered sweeteners by themselves have the potential to stimulate GLP-1 in combination or in the presence of natural sugars, which suggests a number of possibilities that have yet to be examined in detail: (1) chronic administration is needed to induce the cellular machinery necessary (as suggested by rodent studies); (2) although these LCS bind to receptors and elicit sweet taste responses from lingual sites, the binding characteristics are not sufficient to evoke incretin secretion except in the presence of complementary molecules (either other LCS or natural sugars). Involvement of distinct signaling pathways in taste sensation versus incretin secretion would be an important question because many food products use a combination of sweeteners, which would likely promote the latter. A recent study complicated interpretation even further, showing that LCS combinations (either sucralose+ace-K+aspartame or sucralose+ace-K) in caffeine-free carbonated beverages but not seltzer water or sucralose alone promoted GLP-1 responses to subsequent oral glucose (Sylvetsky et al., 2016b). In summary, the current research summarized

above suggests that ingredients in food or beverage matrices also have the potential to impact physiological responses to LCS.

Insulin secretion and/or effects on glucose tolerance

Irrespective of whether LCS promote incretin secretion, effects on insulin secretion and glucose tolerance are possible. In a variety of experimental settings, including LCS preloading prior to oral glucose tolerance tests (OGTT), or incorporation of LCS into meals or water, the majority of studies suggest no effect on glucose tolerance or insulin secretion whether the compounds are presented alone or as mixtures (Brown et al., 2009; Sakurai et al., 2012; Sylvetsky et al., 2016b; Temizkan et al., 2015); however, there are dissenting findings, even within the same study. For example, Temizkan et al. (Temizkan et al., 2015) identified no effects of aspartame whereas sucralose lowered glucose after a glucose tolerance test in healthy individuals. Ace-K increased the blood glucose during OGTT of healthy adults by about 5% whereas sucralose and aspartame were not different from glucose alone (Bryant et al., 2016). Conversely, sucralose worsened OGTT results despite increased insulin secretion in obese but insulin-sensitive people (Pepino et al., 2013). Although statistical significance was not reached, Sylvetsky et al. (2016b) noted an increase in insulin secretion that they believed could have biological significance. These human studies have been conducted under acute conditions and the study inclusion criteria generally do not mention habitual LCS consumption, except in the case of Pepino et al. (Pepino et al., 2013), who excluded people who consumed more than one can of diet beverage (or equivalent) per week. Another variation was to withdraw LCS for 48 hours prior to starting the experiment (Sylvetsky et al., 2016b); other studies did not specify any withdrawal period.

However, it is possible that adaptations could occur after long-term LCS usage, which

alter the results. For example, in a rat study, provision of saccharin on 3 of 7 days preceding a glucose tolerance protocol in which the animals drank the glucose led to worsened OGTT with no effect on insulin. In the same study, gavaging glucose in the glucose tolerance test yielded no difference of saccharin versus control diet, implying a cephalic contribution (Swithers et al., 2012). Chronic provision to rats of saccharin, sucralose or aspartame in drinking water elicited impaired glucose tolerance after 11 weeks, with no apparent effect on insulin secretion, although only fasted values were reported (Suez et al., 2014). Therefore, long-term intake may affect blood glucose outcomes of LCS consumption on glucose tolerance. In an RCT to compare the effects of water versus LCS-beverage intake on weight for 12 weeks, both treatments reduced fasting glucose to a similar extent, although the LCS-beverages elicited 1.8 kg greater weight loss (Peters et al., 2014). Other limitations of these human studies include small sample size and a variety of participants: healthy, obese and/or type 2 diabetic, which complicates interpretation of the findings. Sylvetsky and colleagues (Sylvetsky et al., 2016a) have recently provided a critical analysis of intervention studies and concluded that increased rigour in a number of additional parameters, such as LCS species, dose, route and duration of administration; choice of placebo condition including the food/beverage matrix; selection of participants to enhance generalizability or, conversely, to focus on specific populations such as children. In general, we observed that the inclusion/exclusion criteria for participants in studies was rarely specified in detail or that the inclusion criteria was very broad.

Conclusions and Future Directions

In light of the heterogeneity of results of LCS on outcomes in animals and humans, is there rationale for structural differences among the chemicals contributing to their effects? Ace-K and

saccharin appear to have similar binding characteristics to sweet taste receptors (Maillet et al., 2015); likewise aspartame and sucralose share convergent binding properties (Maillet et al., 2015; Masuda et al., 2012). In rodents, ace-K and saccharin do appear to share ability to stimulate glucose uptake (Mace et al., 2007; Margolskee et al., 2007) but for incretin secretion and any effects in humans there are too few data available for comparison. For aspartame and sucralose, there are insufficient data on glucose transport to compare. Regarding incretin secretion, the bulk of current available evidence suggests no effect of either compound in rodents or in humans (Fujita et al., 2009; Maersk et al., 2012; Pepino et al., 2013; Sakurai et al., 2012; Steinert et al., 2011), although the flavour of the matrix may influence results (Sylvetsky et al., 2016b). Results of preloading experiments with aspartame and sucralose on oral glucose tolerance and associated insulin secretion in humans are mixed, and possibly matrix-dependent, as noted above. Based on limited data, evidence suggests that structural interaction with the sweet taste receptor in the gut may indeed predict functional outcomes but further experiments are needed to confirm. Studies in rodents that examine changes in blood glucose and glucose transport concomitantly with incretin and insulin secretion patterns would help provide a more complete picture of how LCS may potentiate these parameters in the presence of natural sugars. In humans, trials with cross-over designs could be used for several purposes, such as comparing effects of several LCS or comparing one LCS in combination with different food matrices.

A relatively small body of evidence leaves plentiful gaps in our understanding of physiological responses to LCS. Cell line and animal experiments are useful for screening and identification of potential mechanisms, but lack of congruence with human studies limits applicability for more complex experiments. More studies on the basic biology of receptor binding and signal transduction, particularly in response to combinations of LCS, or LCS plus

natural sugars are required for both mouth and gut sweet sensing systems. Particularly in the gut, there is a paucity of data on afferent transmission of sweet sensing to other parts of the gastrointestinal tract or the brain. In humans, longer-term consumption trials are required to address the question of whether LCS exert detrimental or beneficial effects, since acute studies for the most part have not revealed conclusive answers. However, the design of experiments needs to be carefully considered in the context of participant characteristics and inclusion criteria as well as the properties of the LCS being studied. The analysis of such experiments should take into account confounders such as weight loss and long-term consumption of LCS. Furthermore, a better understanding of who consumes LCS, for what reasons, and with what impact on dietary patterns and diet quality is of interest from personal and population health perspectives.

In the meantime, new theories are emerging as to how LCS may affect glucose metabolism, weight gain and other outcomes. Predominant among these are potential impacts of LCS on (a) brain centers that regulate peripheral metabolism (Green and Murphy, 2012; Cong et al., 2013), (b) the gut microbiome (Suez et al., 2014; Nettleton et al., 2016) and associated gut functions, such as activity of alkaline phosphatase (Gul et al., 2017). Additionally, chemists and biochemists continue to develop new sweeteners as well as novel sweetness enhancers (DuBois and Prakash, 2012), whose biophysical properties are as yet poorly understood. Enhancers increase sweetness intensity of natural sugars (DuBois, 2016), which would allow food manufacturers to produce foods with less sugar and calories. However, given some of the surprises that have emerged in studies of LCS long after they were approved for human consumption, it will be important to study the biology of these novel compounds in rodent models and in humans in all of the areas discussed in this review related to glucoregulation as well as the new areas of research on the gut microbiome and the brain.

Conflict of Interest

The authors report no conflicts of interest associated with this manuscript.

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Table 1. Structural, chemical and biological properties of LCS

Compound (Acceptable Daily Intake) ¹	Binds to taste receptors	Sweetness compared to sucrose	Stimulates glucose uptake	Stimulates incretin secretion	Affects circulating insulin or glucose tolerance
Acesulfame- potassium (Ace-K) (15 mg/kg body weight)	EC ₅₀ =0.5 mM; T1R2 amino terminal domain similar to saccharin (Masuda et al., 2012)	11.6 (Dubois et al., 1991)	YES <i>Cell lines</i> – (Zheng and Sarr, 2013); <i>Rodents</i> - (Mace et al., 2007) & increases SGLT1 mRNA after chronic feeding (Margolskee et al., 2007) NO <i>Rodents</i> - rat jejunum (Chaudhry et al., 2013)	YES <i>Cell lines</i> - (Geraedts et al., 2012; Ohtsu et al., 2014); <i>Humans</i> – in combination with sucralose in healthy but not T2D humans – (Brown et al., 2009) NO <i>Rodents</i> - (Fujita et al., 2009); <i>Humans</i> - acute administration – (Steinert et al., 2011)	YES <i>Humans</i> – Increases glucose response slightly (Bryant et al., 2014)
Aspartame (40 mg/kg body weight)	EC ₅₀ =0.75 mM; T1R2 amino terminal extracellular domain (Maillet et al., 2015; Masuda et al., 2012)	16.0 (Dubois et al., 1991)	NO <i>Rodents</i> - SGTL1 mRNA not increased after chronic feeding (Margolskee et al., 2007)	YES <i>Cell lines</i> – (Geraedts et al., 2012) NO <i>Humans</i> - acute administration – (Maersk et al., 2012; Steinert et al., 2011)	YES <i>Rats</i> - chronic (11 wk) in drinking water impaired glucose tolerance, no effect on fasting insulin (Suez et al., 2014) NO <i>Humans</i> - In healthy & T2D, no effect on insulin or glucose in OGTT (Temizkan et al., 2015); no effect on glucose (Bryant et al., 2014)
Cyclamate (11 mg/kg body weight)	EC ₅₀ =2.6 mM; T1R3 trans-membrane domain – (Jiang et al., 2005; Xu et al.,	15.2 (Dubois et al., 1991)	No information found	No information found	No information found

	2004)				
Saccharin (5 mg/kg body weight)	EC ₅₀ =0.2 mM; T1R2 amino terminal domain similar to ace-K (Masuda et al., 2012)	10.1 (Dubois et al., 1991)	YES <i>Rodents & Pigs</i> - (Mace et al., 2007) & increases SGLT1 mRNA after chronic feeding (Margolskee et al., 2007); (Moran et al., 2010)	YES <i>Cell lines</i> - (Geraedts et al., 2012; Ohtsu et al., 2014); <i>Rodents</i> - reduced compared with glucose after chronic feeding (Swithers et al., 2012) NO <i>Rodents</i> – acute gavage (Fujita et al., 2009)	YES <i>Rats</i> – saccharin on 3/7 days, then OGTT (drinking, not gavaging) gave higher glucose, no insulin difference in OGTT (Swithers et al., 2012); chronic (11 wk) in drinking water impaired glucose tolerance, no effect on fasting insulin (Suez et al., 2014)
Sucralose (9 mg/kg body weight per day)	EC ₅₀ =0.1 mM; T1R2 amino terminal domain similar but not identical to aspartame (Masuda et al., 2012)	14.7 (Dubois et al., 1991)	YES <i>Rodents</i> - acutely increases transport & GLUT2 membrane insertion (Mace et al., 2007); increases transport & SGLT1 mRNA after chronic feeding (Margolskee et al., 2007) NO <i>Humans</i> – (Ma et al., 2009; Ma et al., 2010)	YES <i>Cell lines</i> – (Geraedts et al., 2012; Ohtsu et al., 2014); <i>Humans</i> - Yes - acute administration to healthy but not T2D (Temizkan et al., 2015); acute administration of sucralose+Ace-K as caffeine-free cola (Sylvetsky et al. 2016b) NO <i>Rodents</i> - acute gavage (Fujita et al., 2009); <i>Humans</i> - acute administration – (Pepino et al., 2013; Steinert et al., 2011); meal test incorporating mixed sweeteners (Ma et al., 2010; Sakurai et al., 2012); acute administration of sucralose+ace-K in seltzer water (Sylvetsky et al., 2016b)	YES <i>Rats</i> - chronic (11 wk) in drinking water impaired glucose tolerance, no effect on fasting insulin (Suez et al., 2014); <i>Humans</i> – Preload worsens OGTT despite increased insulin (Pepino et al., 2013); improves OGTT in healthy, no effect on insulin; no effect in T2D (Temizkan et al., 2015) NO <i>Humans</i> - Sucralose+ace-K preload (Brown et al., 2012); Combinations of sucralose with Ace-K ± aspartame in soda or water; also sucralose alone (Sylvetsky et al., 2016b); healthy men consuming meal with mixed sweeteners

					(Sakurai et al., 2012); healthy subjects (Bryant et al., 2014)
Erythritol (NA)	No information found	0.5-0.8 (Fujimori et al., 2012)	No information found	YES <i>Humans</i> - fed acutely (Overduin et al., 2016)	NO <i>Humans</i> – no acute effect on insulin (Overduin et al., 2016)
<i>Stevia</i> compounds (4 mg/kg body weight)	Diverse binding to T1R2 and T1R3 sites based on theoretical models (Mayank and Jaitak, 2016)	~10 (Dubois et al., 1991)	No information found	NO <i>Rodents</i> – acute gavage (Fujita et al., 2009)	No information found

Abbreviations: EC₅₀ – half maximal stimulatory concentration; GLUT2 – glucose transporter-2; OGTT – oral glucose tolerance test; SGLT1 – sodium-dependent glucose transporter-1; T2D – type 2 diabetes; T1R2, T1R3 – sweet taste receptor subunits

¹ From (Diabetes Canada, 2016).