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Perspective:

Standards for research and reporting on low-energy ("artificial") sweeteners

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Nonstandard abbreviations:

ADME	Absorption, distribution, metabolism, and excretion
LES	Low energy sweeteners
RCT	Randomized controlled (intervention) trial

Abstract

Widely differing views exist amongst experts, policy makers and the general public, regarding the potential risks and benefits of reduced- or low-energy sweeteners (LES) in the diet. These views are informed and influenced by different types of research in LES, with differing hypotheses, designs, interpretation and communication. Given the high level of interest in LES, and the public health relevance of the research evidence base, it is important that all aspects of the research process are framed and reported in an appropriate and balanced manner. In this perspective, we identify and give examples of a number of issues relating to research and reviews on LES, which may contribute toward apparent inconsistencies in the content and understanding of the totality of evidence. We conclude with a set of recommendations for authors, reviewers and journal editors, as general guidance to improve and better standardize the quality of LES research design, interpretation, and reporting. These focus on clarity of underlying hypotheses, characterization of exposures, and the placement and weighting of new research within the wider context of related prior work.

Key words: Energy, sweetness, guidance, communication, recommendations

Research and review papers convey a range of differing conclusions about the potential impact of low-energy ('artificial' or nature-derived) sweeteners (LES) on public health, ranging from harmful to neutral to beneficial. Some commentators have highlighted concerns that use of LES may raise risks for obesity and metabolic disorders (1-4), while others are equally clear in expressing likely benefits of LES with regard to many of these same outcomes (5, 6). This has not been resolved by recent systematic reviews with meta-analyses (7-9), which generated differing conclusions.

There is consistent international guidance to industry and the public to reduce sugars intakes (10), and LES are a major alternative to sugars in many products, making this is an important public health issue to resolve. Furthermore, given that LES and LES-containing products receive a high level of attention from media and consumers, there is additional responsibility for experts to frame and communicate their views and research data in an appropriate context. As such, high standards for research designs and the representation and weighting of evidence are needed to ensure a balanced interpretation, context and reporting in research and reviews on LES.

A recent expert stakeholder panel proposed a number of research priorities for LES and health outcomes (11). While that panel did not specifically address issues relating to the execution of research and reporting on LES, others have highlighted issues in experimental design and interpretation that can magnify apparent inconsistencies in the evidence base (12-14). In this commentary, we highlight specific practices which can be considered as part of guidance to improve the design, reporting and interpretation of research on LES. We illustrate the issues with examples, and conclude with some recommended practices for authors, reviewers and journal editors.

Be clear about the hypothesis: What question is being tested?

From a public health perspective there is need for an evidence base of research that decisively addresses the benefits and risks of LES, i.e. generating reliable data and analyses on how the use of LES, as a replacement for sugars or on their own, influence metabolic health. Research on LES and non-communicable disease risks fit broadly under 3 underlying *a priori* hypotheses, reflecting questions about exposure to energy reduction, sweetness or LES-specific (metabolic or safety) effects. The design of studies, particularly in terms of the exposure and relevant comparators, should follow from and correspond to the underlying hypothesis and primary research question being posed.

1) Energy reduction: Testing effects of low-/non-caloric vs caloric sweeteners

   Where the research question tests exposure to LES as a generic low- or non-caloric source of sweetness, the hypothesis as stated is usually independent of the specific sweetener(s) considered. The appropriate comparator will be the same test product (usually food or beverage) vehicle(s) or dietary regimen with caloric sweeteners, tested against LES with a significantly lower energy content per unit consumed, and similar in sweetness and other sensory attributes.

2) Sweetness: Testing effects of sweetness (sweet stimuli) exposure *per se*

   Where the research question tests exposure to LES as a 'pure' (non-caloric) sweet stimulus, the hypothesis as stated is usually independent of the specific sweetener(s) considered. The main exception to this would where the hypothesis is based on to interactions of a specific LES structure and sweet taste receptor(s). The appropriate comparison is to exposure to the same or similar delivery vehicle(s) or dietary regimen, at the same energy and nutrient density, with and without LES.

   Depending on the hypothesis, the research may test oral exposure to LES as sweet stimulus, or LES as chemical stimuli for receptors sensitive to 'sweet' tastants in the gut or internal tissues. For oral exposure, the most common comparison would be LES-

sweetened beverages vs water, but this has also been tested with solid foods (15). To isolate the post-oral gastrointestinal or systemic exposures, LES in capsules would typically be used, or perhaps nasoenteric intubation (16) .

In order to interpret whether any putative effects are a response to LES specifically vs sweet stimuli in general, these studies should optimally include an additional comparison of sweet vs non-sweet caloric stimuli, such as glucose (sweet) vs pure short-chain maltodextrin (non-sweet, rapidly hydrolyzed glucose polymer).

3) Sweetener-specific: Testing specific post-ingestive (metabolic, physiological, toxicologic) effects of a specific LES or group of LES

These types of research questions are clearly based around one or more specific LES, with the underlying hypothesis relating to unique physiological effects that may arise from their particular characteristics. In this case the appropriate comparison is to the same test product (food, beverage, capsule) vehicle(s) or dietary regimen with no LES or, to sharpen the interpretation, preferably a different LES lacking the characteristic(s) of interest. Because sweeteners differ markedly in their absorption, distribution, metabolism and excretion (ADME), they can also differ in the potential presence of the intact material or metabolites in different body sites (17, 18). This point is often overlooked, yet may be highly relevant for the interpretation and extrapolation of experimental and population data, and is considered further in the next section.

Differences in the (stated or unstated) hypotheses, lack of clarity or mixing of hypotheses can have important consequences. Examples of this can be seen in the assessment of effects of LES intervention trials on outcomes relating to energy balance in 3 recent systematic reviews with meta-analyses. Rogers et al (8) separately analyzed and reported comparisons of LES vs sugars, LES vs water, and LES vs placebo capsules. In contrast, Azad et al. (7) and Toews et al. (9) did not make this distinction between comparators. For energy intake and weight change, a benefit of LES is more plausible when compared to a caloric than a non-

caloric alternative (19), so the decision of whether to make this distinction can significantly impact the combined effect sizes and conclusions (as can be seen in those reviews). There may be valid arguments for either approach in meta-analysis; however, the primary research studies invariably differentiate these comparisons in their hypotheses and designs.

A further consideration is whether the underlying hypotheses are or should be sweetener-specific. This has implications for study selection and the interpretation (extrapolation) of results. For example, the protocol and objectives for the systematic review of Toews (9) are framed in a way that is independent of the specific sweetener, although the review only included studies where the sweetener was specified. This criterion largely excludes studies where free-living subjects consume a mix of commercial LES-containing 'diet' products, generating much smaller evidence base than other contemporary systematic reviews for similar outcomes (7, 8, 20).

Control and specify exposures where relevant

There are research hypotheses and designs where the nature of the exposure and specific LES may be important. By definition, LES all share the characteristics of being sweet and low in energy when used in place of sugars. For hypotheses based on exposure to energy or sweetness, effects are usually assumed to be related to variation in the energy content or taste attributes of the test materials (19, 21). In the absence of other hypotheses, it is generally reasonable to presume that similar results would be seen using other LES to achieve the same calorie reduction or taste profile. Nevertheless, specific LES may differ with regard to their stimulation of different "sweet taste" receptors, digestion or uptake in the gut, and appearance and pharmacokinetics in different body pools, which results in differing potential for interactions with specific gut or systemic receptors and systemic or gut (including microbiota) metabolism (14, 17, 18, 22, 23). For example, protein or peptide sweeteners are rapidly digested and absorbed as their constituent amino acids, so will not enter the colon. Sucralose is usually reported to be almost completely excreted intact in feces (70-90%), although this has recently been questioned (24). Steviol glycosides on the

other hand are actively metabolized by the colonic microflora, bacterial cleavage of the glycoside component allowing absorption of steviol which is systemically available after hepatic glucuronidation and renally excreted.

There is currently considerable interest in the possible effects of LES on the gut microbiota composition, which has been reported for saccharin, sucralose and steviol glycosides in humans (25). The plausibility of these observations is directly linked to the molecular and thus ADME properties of the specific LES, and cannot be generalized. Moreover, as the functional capacity of the microbiota may be more relevant than purely taxonomic accounts of composition, the extrapolation from these observations to health implications must also take account of the nature and properties of the specific LES exposures. The majority of these studies have also been in rodents, which have been valuable in generating new hypotheses, especially where these are not amenable to direct testing in humans (e.g., 26). However, important differences in specific animal research models and test conditions contribute to many inconsistencies in this literature (12, 27), and direct relevance to human nutrition and metabolism cannot simply be assumed. Approaches in animal studies such as very excessive dose loading may be appropriate for some safety and toxicological research but can have distorting consequences for nutrition-related outcomes. A further issue for interpretation and replication is that many studies have fed animals commercial ‘tabletop’ LES preparations which are of unknown, impure or variable composition, where the sweetener comprises perhaps only a small percentage of the total content (28-30). Notably, the non-LES filler material or bulking agents in these compositions may also include fermentable carbohydrates.

Exposures in studies may be short- or long-term, and hypotheses should also logically relate to this. Despite *in vitro* evidence of variation in stimulation of oral, gut and systemic receptors by LES, a large body of short-term physiological studies in humans find no consistent generic or LES-specific effect on acute postprandial responses (31-33). However, there is more limited evidence testing potential variation in chronic LES-specific exposure effects on glycemic or gut hormone responses. Here it would be crucial that hypotheses relate to the

153 metabolic fates of specific LES, which might differentially affect physiology in the long-term, a
154 different and possibly more important question than what single-dose acute studies can
155 address. Measurable differences in physiological responses to different LES, mediated by
156 mechanisms independent of their actions at sweetness receptors, may be almost inevitable
157 given their extreme chemical diversity. It is important to confirm whether these differences
158 produce consistent and meaningful variation in health-related outcomes (34).
159 Depending on the hypotheses, human research studies may also need to take account (e.g.
160 by selection or pre-planned statistical analyses) of participant characteristics, particularly
161 whether they are habitually high or low consumers of LES. It is likely that these groups also
162 differ in regard to other habitual dietary and other lifestyle behaviors or personal
163 characteristics (e.g. microbiome), which may significantly influence responses to
164 interventions or their interpretation (14, 18). Establishing the nature of prior LES exposures of
165 populations may also have important implications for the interpretation of cross-sectional and
166 prospective observational studies measuring birth or long-term health outcomes. It seems
167 essential (and yet is rare) that researchers consider which particular LES were available to
168 the cohort at the time and place of data collection or index events (such as conception,
169 pregnancy), so the plausibility of causal interpretations can be placed in the context of the
170 relevant ADME properties and prior physiological or safety studies. For example, as noted
171 above, LES differ substantially in their uptake and access to systemic circulation or tissues.
172 Lastly, a limitation noted in a recent systematic review of the relationship between sweet
173 taste exposure and subsequent liking and preference for sweet stimuli was that few studies
174 had made any quantitative assessments of the perceived sweetness of test materials or diets
175 (21). Instead, the content or even just the presence of sugars or sweeteners in foods or diets
176 was often used as a proxy indicator of exposures to sweetness. While matching of test
177 materials should be relatively easy in laboratory-based trials, the characterization of
178 exposures to sweetness is more challenging where the subjects or cohort are consuming a
179 range of commercially available foods. Recent efforts to generate “sensory-diet” databases
180 (35, 36) are an important development, as they can provide a basis for objectively quantifying

and comparing exposures to sensory attributes of foods and diets in large populations. For both behavioral and physiological research focused on the effects of orosensory exposure to sweetness in foods or beverages, it seems essential that some effort is made to verify the actual exposures.

Considering all these potential sources of variability in research materials or exposures, design and outcomes, it is vital that the hypotheses, design and interpretation of research are consistent with the specific LES source(s), the doses and means of delivery, and the putative mechanisms or sites of action, which may primarily be oral, gastrointestinal or systemic. Effects of specific sweeteners may be independent of sweetness, even where this is the main attribute of LES that underpins the reason to design and undertake the study. Depending on the hypothesis, the range of potential “off-target” effects may make it inappropriate to aggregate LES studies together and test for a class effect.

Place new research in the context of the totality of evidence

New or different types of research will have differing contributions to the overall totality of evidence, and should be viewed within this context (37). The impartial and balanced representation and dissemination of the evidence base can however be undermined by selective citation and citation distortion (citation bias and amplification) in biomedical research (38, 39). These practices include systematically ignoring data conflicting with prior beliefs, conveying hypothesis as fact, and preferential reference to statistically significant vs “neutral” outcomes (or vice-versa).

Reporting of research on LES is not immune to these issues. An extreme example is the pattern of citations to Suez et al (29), who proposed that consumption of intense sweeteners may alter the intestinal microbiota leading to adverse effects on glucose tolerance. As of November 2019 that publication had been cited over 1000 times, usually to highlight this as a potential or even confirmed risk of LES (2, 4, 40). In contrast, a 2013 systematic review of controlled human trials of LES effects on markers of glycemic control (41), with a differing conclusion, had been cited only 5 times. Similarly, reviews of the LES-microbiota-glycemia

hypothesis (e.g., 42) may also make little or no reference at all to the primary research papers and reviews of controlled human trials that have specifically tested sustained exposure to LES on glycemic control (32, 43, 44), nor the regulatory and safety reviews where these outcomes have been considered in depth for specific sweeteners (18, 45-49). The choice of this example is not to question the results of Suez et al (29) or whether LES affect microbiota or glycemic control. It is simply to illustrate where new research with provocative results needs to be placed in the context of the totality of prior evidence. In this case, the record of citations indicates a pattern of giving disproportionate weight to hypothesized adverse effects, relative to a large body of empirical evidence to the contrary (18). In other cases, hypothesized effects of LES are simply assumed, with seemingly no apparent need to consider the evidence at all. For example, a common argument against the use of LES as an approach to reduce sugars intakes, rests on the view that sweetness exposure 'drives' sweetness preferences. This idea is plausible and commonly expressed, and even appears in relatively high-level policy documents (50). Yet there seems to be little objective support for this view, and possibly even more evidence favoring the alternative that sweetness exposure satisfies (rather than drives) preferences (21).

The persistent failure to present and consider research in the context of the totality of prior evidence risks uncritically (re-)generating and sustaining hypotheses without adequately acknowledging where these have previously been robustly tested and perhaps rejected (see 51). From recent headlines, commentaries and narrative reviews, non-experts might be forgiven for being unaware that LES had been the subject of a substantial number of RCTs, and systematic reviews and meta-analyses of these. As a general principle, it is poor practice for professional papers to cite selected *in vitro*, animal and observational studies as the primary evidence for putative effects of LES, without balanced reference to the large corpus of human trials and safety assessments where the same markers and outcomes have been considered (45-49). When the totality of information is considered a very different picture may emerge. For example, animal data are often used to underpin the view that LES may lead to disordered appetite and weight gain. However, in our systematic review of human

and animal studies of LES and body weight (8), we identified 90 relevant animal studies of which only a small minority (mostly from one research group) reported increased body weight. The corresponding human RCT data also showed beneficial effects on energy intake and body weight. The impact of selective citation is reflected in the view of some members of a recent expert stakeholder panel, that additional LES intervention trials for weight control outcomes were mainly needed "…due to public perception and some vocal opposition" (11).

Acknowledge the limitations of observational and animal data

Even papers critical of LES acknowledge there are many discrepancies between the adverse health impacts hypothesized by some animal and human observational studies, in contrast to more often neutral or beneficial effects usually seen in human intervention trials (52). Differences in the weighting given to evidence from these different research designs contributes toward differing views of the perceived risks and benefits of LES. While there are limitations to the suitability and interpretation of RCT data for certain research questions (37), there is a need for particular caution in selective use and extrapolation from observational and animal data. This can be illustrated by the interpretation of research on the relationship between water intake and body weight, as an analogy to research on LES.

Water is a zero-energy food and beverage ingredient, widely recommended as a preferred beverage choice in the context of obesity, despite inconsistent evidence around its influence on weight management (53). Systematic reviews and meta-analyses of observational data on water consumption in relation to weight management have reported limited evidence of benefits, and even significant *adverse* associations of water consumption with body weight outcomes in children and adolescents (54, 55). Other analyses have found that water consumption was positively associated with all-cause mortality (56). The plausibility of adverse effects of water consumption on weight control could be further supported by reference to a considerable volume of animal research. It has long been known that greater water intake is positively correlated with greater food intake in animals (57), and greater

weight gain with the addition of water to the diet has been reported in experiments with several species (58-63).

This example shows the ease with which selective, uncritical reference to observational and animal research could be used to underpin an apparently compelling but intentionally absurd narrative. In the case of water, adverse effects suggested by the cited observational and animal studies are readily dismissed, despite the absence of a robust body of contrary RCT data. For LES, similar adverse effects suggested by observational and animal data are given much more weight as a basis for causal inferences, even where there are substantial RCT data to the contrary. There may be very valid reasons for this, but animal studies may lack generalizability (27, 64), and the limitations of observational studies and risk of assuming causation from association are well-known (65, 66). In the observational studies of water and body weight, confounding and reverse causality are readily invoked and accepted as reasons to conclude the observed relationships are spurious (54, 56, 67). Similar concerns have repeatedly been raised regarding interpretation of epidemiological associations of body weight and metabolic health with LES (3, 13, 68). LES may be disproportionately used in place of sugar by individuals with a pre-existing history or elevated risk of weight gain or diabetes, and this caveat is often highlighted in the original papers (e.g., 69). Moreover, in the case of LES, the likelihood that epidemiological associations are specious is reinforced where the corresponding RCT data for related outcomes consistently indicate neutral or beneficial effects (8, 41). As a result, several authors have expressed doubt about the weight that should be placed on observational (and animal) studies in this area for outcomes where data from sustained RCTs are available (8, 13, 70, 71).

This ultimately comes down to ensuring the research approach has been appropriately designed to address a specific hypothesis, and that the limitations - including potential for confounding or post-hoc use of the same data to answer other research questions - are adequately acknowledged in drawing conclusions. All types of study designs have potential weaknesses, and all can contribute in different ways to the totality of evidence (37). Observational and animal research on LES can generate hypotheses and address questions

that cannot be directly tested in humans, such as longer-term disease outcomes and toxicology, and potential multi-generational effects (26, 27, 72, 73). Nevertheless, such data should be very cautiously interpreted, particularly where they conflict with results from robust RCTs.

A note on bias: white hats and black hats

Application of the guidance proposed here would improve the quality of communication and discourse on LES, independent of the views or interests of who is delivering the messages. All stakeholders may potentially be guilty of "white hat bias", the well-intentioned but biased "distortion of information in the service of what may be perceived to be righteous ends" (74). We as authors hold certain views based on our reading of the evidence and our own research (8, 19, 21), and also acknowledge potential conflicts of interest such as funding sources and collaborations. In research on LES, as in other areas of nutrition, the potential for industry-related ("black hat") bias has been widely discussed. Indeed, Mandrioli et al (75) recently concluded that reviews of LES and health were biased by sponsorship and financial conflicts of interest, although the risk of bias was mainly relevant to narrative rather than systematic reviews. However, commercial associations are not the only possible source of bias, and absence of such interests is no assurance of impartiality (39, 74, 76-79). The personal reputation, conference invitations, and travel and research support for "independent" researchers may also benefit from the particular views they take. A continued flow of provocative research results and atmosphere of uncertainty around LES undoubtedly also improves the chances for further funding of research on the topic.

These different biases can influence the design, interpretation, and reporting of research on LES, undermining an impartial and balanced scientific and public consideration of the possible benefits or risks of their use. This places even greater demand on authors and journal editors to ensure the faithful representation and appropriate weighing of evidence. With this in mind, we encourage others, and especially those with differing views, to offer

other examples from the current literature that would support further refinement of the recommendations that follow here.

Conclusions and recommendations

There are significant issues in how the evidence base on LES is generated, interpreted and communicated by the expert community, with implications for public health, industry and future research needs. We have discussed a number of these, with examples, to illustrate the need for a more consistent standard of practice in the conceptualization and reporting of both primary research and reviews of that research. These issues also emphasize areas for more careful and critical scrutiny of research publications by wider stakeholders, including research end-users.

Importantly, in relation to public health, LES are not a case where the “precautionary principle” necessarily applies. Where adverse effects of LES exposures are confirmed by evidence-based expert risk assessment, these rightly should be considered in regulatory and public health policies. However, there may also be value gained from the use of LES, for example as a tool for maintaining the acceptability of foods, beverages and diets reduced in sugar, facilitating progress towards widely-advised goals to reduce sugar intakes (80, 81). In short, there are risks to be considered not just from exposure to LES but also from prematurely advising the public to avoid them.

We believe it should be possible to formulate guidance to address the issues raised here, which can be widely embraced by individual researchers, and those involved with the funding, communication and use of research. Many of our concluding recommendations in Table 1 apply to nutrition research in general, but they have particular relevance to research with LES. We hope these can be broadly accepted by the expert community, and welcome further their consideration and development.

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Table 1. Recommendations for research and reporting on LES

- Research hypotheses should be explicit, and the underlying research question(s) reflected in the choice of exposures, comparators and analyses.
- The justification and interpretation of primary research studies and their representation in reviews should reflect the stated hypotheses, with particular regard to caloric vs non-caloric comparators, and potential for extrapolation to LES in general vs specific LES.
- Where outcomes are not attributable to energy reduction or perceived sweetness, interpretation relies on the chemical and ADME properties of specific LES.
- The selection and citation of existing research should fairly represent the balance and weight of different types of evidence, particularly where there are data from RCTs with relevant exposures and populations.
- Animal research and other studies generating evidence related to safety and toxicology should specifically refer to that literature.
- Reporting of evidence on health associations with LES from observational studies, including prospective cohort studies, should be clear that these are subject to residual confounding including reverse causality, and may have been designed to answer a different research question.
- Hypotheses generated by observational and animal data must be interpreted in relation to the specific exposures, plausible causal pathways, and results of any related human intervention trials.