# Acute glycemic and insulinemic effects of low energy sweeteners: A systematic review and meta-analysis of randomized controlled trials<sup>1-2</sup>

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Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

## Abbreviations:

Ace K: Acesulfame potassium

BNR: Blinding not reported

CI: Confidence interval

CO: Cross-over study design

D: Double-blind

iAUC: Incremental area under the curve

LES: Low energy sweeteners

NR: Not reported

O: Open label

PPG: Postprandial glucose response

PPI: Postprandial insulin response

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

S: Single-blind

SD: Standard deviations

SE: Standard Error

RoB: Risk of bias

T1D: Type-1 diabetes mellitus

T2D: Type-2 diabetes mellitus

## 1 Abstract

**Background:** It has been suggested that low energy sweeteners (LES) may be 2 3 associated with an increased risk of metabolic diseases, possibly due to stimulation of glucose-responsive mechanisms. 4 **Objective:** We conducted a systematic review and meta-analysis of human intervention 5 6 studies examining the acute effect of LES intake on postprandial glucose (PPG) and 7 insulin (PPI) responses, in order to comprehensively and objectively quantify these 8 relationships. 9 Methods: We systematically searched Medline, OVID FSTA and SCOPUS databases 10 until January 2020. Randomized controlled trials comparing acute postprandial effects on PPG and/or PPI after exposure to LES; either alone, with a meal or other nutrient-11 12 containing preloads to the same intervention without LES were eligible for inclusion. 13 PPG and PPI responses were calculated as mean incremental area under the curve 14 divided by time. Meta-analyses were performed using random effects models with inverse variance weighing. 15 Results: Twenty-six papers (34 PPG trials and 29 PPI trials) were included. There were 16 17 no differences in the effect of LES on PPG and PPI responses compared to control 18 interventions. Pooled effects of LES intake on the mean change difference in PPG and 19 PPI were -0.02 mmol/l [95% CI -0.09, 0.05] and -2.39 pmol/l [95% CI -11.83, 7.05] respectively. The results did not appreciably differ by the type or dose of LES 20 21 consumed, co-intervention type or fasting glucose and insulin levels. Among patients 22 with type 2 diabetes, the mean change difference indicated a smaller PPG response after exposure to LES vs. control (-0.3 mmol/l [95% CI -0.53, -0.07]). 23

24	Conclusions: Ingestion of LES, administered alone or in combination with a nutrient-
25	containing preload, has no acute effects on the mean change in postprandial glycemic or
26	insulinemic responses compared to a control intervention. Apart from a small beneficial
27	effect on PPG (-0.3 mmol/l) in studies enrolling patients with type 2 diabetes, the effects
28	did not differ by type or dose of LES, or fasting glucose or insulin levels.
29	Keywords: Non-caloric sweeteners; Non-nutritive sweeteners; Artificial sweeteners;

30 Postprandial; Glucose; Insulin; Diabetes

## 32 Introduction

Low-energy sweeteners (LES) are often used to replace sugars in food and beverage 33 34 formulations because they can provide sweet taste with little or no energy contribution or cariogenicity. As such, a range of different LES are common in the global food 35 supply (1), and frequently used by manufacturers providing lower calorie or sugar 36 37 alternatives to various food and beverage products. In the United Sates National Health and Nutrition Examination Survey 2007–2012, about 50% of respondents reported 38 39 consuming LES-containing products over a 2-day period (2). 40 Despite extensive safety evaluations of these compounds by regulatory bodies (3-5), there is an ongoing debate regarding potential detrimental health effects of LES intake 41 (6, 7). Concerns have been expressed, mainly based on selected animal and human 42 43 observational studies, that LES consumption may increase risks of metabolic disease, especially obesity and type 2 diabetes (8-11). It has been suggested that this might arise 44 45 in part as a result of LES stimulation of gut or systemic mechanisms responsive to sweet 46 stimuli and glucose (5, 11, 12). However, while LES stimulation of such systems has mainly been demonstrated in vitro and with animal models, it is uncertain whether these 47 48 effects are physiologically relevant in humans (13, 14). Furthermore, a substantial body of human intervention data suggests that overall, LES intake has no significant acute or 49 chronic effects on measures of glucose homeostasis (10, 15-18). 50 A key question underpinning the putative link between LES and metabolism is the 51 52 presence and magnitude of an effect of LES, ingested as part of a non-caloric or caloric

53 (nutrient-containing) preload, on glycemic responses. To date there has been no

reported quantitative meta-analysis of the effects of LES intake on two-hour (120 min)

55 postprandial glucose (PPG) and insulin (PPI) responses, which is a standard way of testing for and expressing the systemic glycemic and insulinemic exposures induced by 56 57 meals. Dietary patterns giving higher post-meal glycemic excursions are associated with increased risk of type 2 diabetes (19, 20), whereas drugs lowering PPG have been 58 shown to reduce the risk of progression from pre-diabetes to diabetes (19, 21). Our 59 objective was therefore to perform an up-to-date systematic review with meta-analysis 60 of controlled human intervention studies investigating the acute effects of LES intake on 61 62 PPG and PPI responses.

63 Methods

The protocol for this systematic review and meta-analysis was registered in the international prospective register of systematic reviews (PROSPERO, registration number: CRD42018099608), and conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (22).

69 Search strategy

To qualify for inclusion, trials had to meet the pre-defined inclusion criteria outlinedin Table 1.

PubMed/Medline, OVID FSTA, and SCOPUS were searched (from the date of
inception until January 2020) to identify potentially relevant studies conducted in
human participants and published in English. Titles, abstracts and keywords were
searched for variations and combinations of the following terms: *Artificial sweetener(s)*, *non-nutritive sweetener(s), low calorie sweetener(s), low energy sweetener(s)*,

*sucralose, aspartame, stevia, steviol, saccharin(e), acesulfame, erythritol, diet(beverage OR drink OR soda), low calorie(beverage OR drink OR soda)), low-energy(beverage OR drink OR soda), glucose, insulin and glyc(a)emic* (full PubMed search syntax in the
Supplementary Methods). Bibliographies from obtained publications were also screened
for additional potentially relevant studies.

#### 82 Screening and selection of trials

A two-step screening and selection process was followed. During the first step, titles, abstracts and keywords of publications were screened separately by two of the authors (AG & DJM) to identify potentially eligible studies. During the second step, the full texts of these publications were examined to gauge eligibility based on the stated inclusion criteria. In cases of inter-reviewer disagreement, questions on study eligibility were resolved through consensus and consultation with the other co-authors (KMA & AR).

## 90 Data extraction and quantification

91 The following information was extracted from eligible publications by means of a predefined data extraction file: 1) publication details (author, year of publication, 92 93 country); 2) study design characteristics (crossover or parallel, blinding); 3) subject 94 characteristics (age, gender and health status); 4) intervention and control treatment characteristics (type and dosage of LES, presence and type of meal/nutrient-containing 95 preload, type of control); 5) postprandial glucose and insulin incremental area under the 96 97 curve (iAUC) and associated measures of variance; 6) risk of bias indicators. If no iAUC values were reported, postprandial data per measured timepoint were extracted 98 99 (either from tables and text or from figures by means of a web-based plot digitizing tool 100 (23)). Data were extracted by 2 independent reviewers (AG, DJM) and differences101 resolved by consensus.

#### 102 Data synthesis and statistical analysis

Where postprandial data at individual timepoints were extracted, the iAUC was 103 calculated by the trapezoidal method (24). The variances of these iAUCs were based on 104 105 the standard deviations (SD) of the respective individual timepoints and, calculated by 106 means of matrix algebra involving a covariance matrix with the assumed correlation structure being compound symmetry (25). For this purpose, the correlation between 107 108 timepoints was assumed to be 0.75 for glucose and 0.5 for insulin. These assumptions were based on PPG and PPI measurements at repeated timepoints in previous studies 109 conducted by our group (26-29). 110

111 Prior to meta-analysis, all glucose and insulin data were transformed into SI units

112 (mmol/l for glucose (= 0.0555 \*mg/dl) and pmol/l for insulin (=  $6 \text{*}\mu \text{U/ml}$ )). The

113 outcomes were expressed as mean postprandial changes by dividing the iAUCs by the

duration of the postprandial measurement period (120 min). When measures of

115 variance were not reported, they were imputed using variance data from the other

studies included in the meta-analysis (30).

For both glucose and insulin, the principal effect measure was the difference in the mean postprandial changes between LES and control interventions. Pairwise analyses were applied to all crossover trials as described by Elbourne et al (31). The weighted effect estimates and corresponding 95% confidence intervals (CI) were calculated using random effects models with inverse variance weighting (32) using the PROC MIXED procedure in SAS (SAS v9.4, SAS Institute, Cary, NC, USA).. Pooled effects calculated by means of fixed effects models served as sensitivity analyses. Several
trials included in the meta-analyses included two or more different comparisons (e.g.
different doses or types of LES) in the same subjects (33-41). To ensure that these trials
did not contribute a disproportionate weight to the meta-analyses due to double counting
of the same subjects, the weight of each comparison was divided by the total number of
included comparisons in the respective trial (42).

129 Influence analyses were conducted by systematically excluding one study at a time and re-analyzing the remaining data to determine whether a specific study was exerting 130 131 excessive influence on the overall outcomes. Where enough data were available, the potential effects of pre-defined covariates on the overall outcomes were assessed by 132 means of subgroup (minimum of 4 comparisons per subgroup) and weighted meta-133 134 regression analyses (minimum of 10 comparisons per covariate) (43, 44). The predefined covariates were: LES type, health status (healthy; having type 2 diabetes), co-135 136 exposure type (i.e. LES consumed in a fasted state; LES consumed with a meal or other nutrient-containing preload), baseline fasting glucose and insulin and LES dose. 137

## 138 Risk of bias assessment

139 Assessment of the risk of bias (RoB) in the included studies was done by means of

140 the Cochrane Collaboration's tool for assessing RoB (45). For this purpose, seven

141 different domains were considered (random sequence generation, allocation

142 concealment, blinding of participants and personnel, blinding of outcome assessment,

143 incomplete outcome data, selective reporting and other sources of bias). The

144 assessments were carried out independently by 2 authors (AG and DJM), and

145 differences resolved by consensus.

146	Publication bias was evaluated by means of visual inspection of funnel plots
147	(constructed by plotting inverse SE against the respective weighted mean difference in
148	glucose and insulin iAUC for each trial) and Egger's regression test (with P<0.1
149	indicating asymmetry) (46).
150	Heterogeneity was assessed by means of the Cochran's Q statistic (significant at
151	P<0.1) and quantified by the I <sup>2</sup> -statistic (with values of 25%, 50% and 75% considered
152	to be low-, moderate- and high-level heterogeneity respectively) (47). In the absence of
153	a enough studies with head-to-head comparisons of the PPG and PPI effects of the
154	different LES types included in the review, a post-hoc frequentist network meta-analysis
155	was conducted in order to study any potential heterogeneity (or informative lack
156	thereof) in this regard. Analyses were conducted using the netmeta package on the R
157	statistical software (48).

158

## 159 **Results**

160 Included trial characteristics

The systematic searches retrieved a total of 5,105 potentially relevant papers after removal of duplicates (**Figure 1**). After exclusion of those that did not meet the predefined inclusion criteria, 26 papers remained that were included in the quantitative synthesis (meta-analysis) (33-41, 49-65). The 26 included papers reported on 34 trials (experiments) with information on PPG responses (yielding 55 comparisons) and 29 trials with information on PPI responses (yielding 50 comparisons). The characteristics of these trials are summarized in **Table 2**. Additionally, 18 papers (66-83) that reported

168	glucose and/or insulin responses for time periods <120 minutes post-prandially were
169	included in the qualitative synthesis, and are summarized in Supplementary Table 1.
170	A total of 452 individual participants took part in the 55 comparisons for PPG, and
171	394 participants in 50 comparisons provided data for PPI. The number of participants
172	per comparison ranged from 6 to 31. Mean age ranged from 18 to 66 years. Forty-one
173	comparisons included healthy lean participants. The remaining 14 comparisons were
174	comprised of patients with diabetes ( $n = 9$ type 2 diabetes and $n = 1$ type 1 diabetes) and
175	participants with obesity but no other health condition $(n = 4)$ .
176	In all comparisons, participants started from a fasting baseline. In 12 comparisons,
177	LES was administered to participants in a non-caloric vehicle (capsules, water, "diet"
178	beverage or intragastric infusion). In the remaining comparisons, LES was
179	administered either in conjunction with a standardized carbohydrate-containing meal (n
180	= 23) or a 75g glucose load (n = 20). The types of LES administered were: sucralose
181	(13 comparisons), l-arabinose ( $n = 10$ ), aspartame ( $n = 9$ ), saccharin ( $n = 5$ ), erythritol
182	(n = 3), stevia/steviosides $(n = 3)$ , accsulfame potassium $(n = 4)$ and combinations of
183	sucralose and acesulfame potassium ( $n = 6$ ), and sucralose, acesulfame potassium and
184	aspartame ( $n = 1$ ). The types of control treatments administered were: water or other
185	unsweetened beverage (31 comparisons), iso-caloric (and iso-carbohydrate) meals or
186	beverages without LES ( $n = 21$ ), saline ( $n = 2$ ), and corn starch placebo capsules ( $n =$
187	1).

## 188 Effects of LES intake on PPG and PPI responses

In the primary meta-analyses using random effects models, there were no statisticallysignificant effects of LES intake on the mean change differences in PPG and PPI

responses (-0.02 mmol/l mean PPG [95% CI -0.09, 0.05] and -2.39 pmol/l mean PPI
[95%CI -11.83, 7.05] respectively) (Figure 2 and 3). In meta-analyses using fixed
effects models, the overall estimates of PPG and PPI mean change differences remained
similar (-0.01 mmol/l mean PPG [95% CI -0.04, 0.02] and -1.41 pmol/l mean PPI
[95%CI -4.12, 1.29] respectively).

#### 196 Meta-regression and subgroup analyses

197 Meta-regression analyses found no statistically significant influence of baseline fasting glucose and insulin or dose of LES used, on the mean change differences in PPG 198 199 and PPI responses to LES (Table 3). However, sub-group analyses of health status 200 (Table 4), indicated a statistically significant difference in the mean change difference 201 in PPG response to LES when comparing healthy participants and those with type 2 202 diabetes: thus, there was a small statistically significant reduction in mean PPG for LES 203 vs control in the type 2 diabetes subgroup (-0.3 mmol/l [95% CI -0.53, -0.07]) whereas no change was evident in the healthy subgroup (-0.01 mmol/l [95%CI -0.07, 0.06]). No 204 205 further influences on PPG or PPI mean change differences were evident when dividing studies by LES type or co-exposure type (LES consumed in a non-caloric vs a meal or 206 207 nutrient-containing preload).

## 208 Influence analyses, assessment of potential biases and heterogeneity

Influence analyses conducted by omitting any single study from the meta-analyses did not materially affect results for PPG or PPI (Supplementary Table 2). Overall, all studies had some risk of bias, most notably regarding blinding (most studies were single blind as participants could not be blinded due to the nature of the interventions), as well as unclear reporting of random sequence generation and allocation concealment (Supplementary Table 3). To evaluate potential effects of (lack of) blinding, a post-hoc
analysis including only the seven trials (16 comparisons)(34, 36, 38, 63, 64) reported as
being double-blind was conducted. The outcomes of both random and fixed effect
meta-analyses were similar to those of the main analyses (Supplementary Table 4).

Both PPG and PPI mean change differences showed low to moderate heterogeneity (P value for Q statistic <0.01;  $I^2 = 44.7\%$  and P <0.01,  $I^2 = 48.3\%$  respectively) between studies. Egger's linear regression test did not indicate the potential presence of publication bias (P value of intercept = 0.48 and 0.83 for PPG and PPI respectively). In addition, visual inspection of the funnel plots did not confirm an obvious presence of publication bias, with the PPG and PPI changes scattered relatively uniformly around the overall estimates (**Figure 4 A and B**).

The network meta-analyses produced similar results to the main analyses. For PPG 225 226 and PPI mean change differences, there were no direct evidence of an effect of the 227 different LES types versus each other or the control intervention. For each outcome, the posterior between-study SD was below 0, suggesting low heterogeneity and 228 (Supplementary material, Network meta-analysis section). For stevia, indirect evidence 229 suggested a smaller PPG response compared to control -0.79 mmol/l [95%CI -1.56; -230 231 0.02], sucralose -0.81 mmol/l [95%CI -1.59; -0.02], aspartame -0.82 mmol/l [95%CI -1.60; -0.04], erythritol -0.87 mmol/l [95%CI -1.65; -0.09] and the combination of 232 sucralose and aspartame -0.89 mmol/l [95%CI -1.73;-0.05]. 233

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235

## 236 **Discussion**

This meta-analysis quantifying evidence from 34 randomized controlled intervention 237 238 trials found that intake of LES had no statistically significant effects on the mean change differences in acute post-prandial glucose or insulin responses compared with a 239 control intervention. Our findings for LES in a non-caloric (e.g. water) vehicle are in 240 241 accordance with the outcome of a recent meta-analysis that found no acute effects on 242 PPG measured over a range of postprandial time periods (15), as well as another recent 243 systematic review of PPG responses to LES (84). This is now confirmed based on a standard 120 min postprandial period of analysis for glucose and for insulin as well. A 244 245 somewhat older network meta-analysis that compared the effects of different caloric and 246 non-caloric sweeteners on 120 min PPG responses, concluded that the data were 247 inconclusive (85); however, many relevant trials have been published since that analysis, which included only two of the 34 trials here. 248 249 LES are often consumed in conjunction with caloric nutrients i.e. protein, fat and 250 carbohydrates. As such, for the first time, our meta-analysis also included studies where LES were administered along with standardized mixed meals, carbohydrate-containing 251 beverages or a 75g glucose preload. In this regard, sub-group analyses found a similar 252 253 absence of effect of LES on the mean change differences in PPG and PPI when

consumed either with or without a carbohydrate or nutrient containing preload. This

suggests that nutrient and/or food matrix interactions probably do not play a role in

256 determining potential effects of LES intake on acute glycemic responses.

257 The outcomes of the 18 studies in which glucose and/or insulin responses were
258 measured for time periods <120 minutes postprandially, are mostly consistent with the</p>

results of our meta-analyses. Most studies reported no effects (67, 69-78, 83) or very
small changes (70, 74, 76) in PPG and PPI responses after LES ingestion.

261 The findings of the few included trials of immediate cephalic phase responses were 262 inconsistent, with four of these (66, 68, 79, 82) reporting no effects on glucose or insulin, and two (80, 81) reporting increased cephalic phase PPI responses but no effects 263 on PPG. This is noteworthy since, although effects of sweetness itself have been 264 265 suggested (86, 87), it would seem that sweet taste stimuli alone are not sufficient to 266 elicit meaningful acute glycemic responses. A recent systematic review of studies 267 utilizing pre-ingestive sweet taste stimulation designs, also suggested that oral sweet taste activation from LES has limited effects on human glucose homeostasis (84). 268

Meta-analyses of data from some observational studies suggest an association
between LES intake and an increased risk of developing metabolic diseases, particularly

type 2 diabetes (8, 9). However, difficulties in the accurate assessment of LES exposure

and problems with reverse causality and confounding factors raise concerns regarding

the reliability and interpretation of associations from observational studies (88-90).

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275 intervention studies suggest no effects of LES intake on postprandial glucose responses.

Conversely, our meta-analysis and other reviews (15, 84), show that data from human

We note, however, that among patients with type 2 diabetes, the mean change difference indicated a smaller PPG response after exposure to LES *vs.* control. Similar effects were also noted in the meta-analysis of Nichol et al. (15). This might suggest a potential direct glucose-lowering benefit of LES intake for these individuals. However, effect sizes are small and were found from only 9 comparisons, all of which were judged to be of high risk of performance bias and included only 86 individuals. Moreover, it is uncertain whether the 0.3 mmol/l reduction in PPG response is truly replicable or would be of any
long-term clinical relevance in diabetes management. A number of longer-term trials of
LES show no significant effects on glycemic control in this population (16). We have
no obvious explanation or hypothesis for any differential response in the short term,
although this could be related to the poorer glycemic control in people with diabetes.

287 Several limitations of this meta-analysis should be noted. Firstly, we did not have an 288 a priori hypothesis that different types of LES would differ in their effects on the mean 289 change in PPG or PPI responses. We therefore assumed that it was appropriate to pool 290 the effects of different LES types in the same meta-analysis. Concerns have however been raised that different LES types might differ in the physiological effects (91). As 291 such, a network meta-analysis might therefore have been a more appropriate approach. 292 293 Network meta-analysis allows for the pooling of outcomes derived from direct and indirect evidence across multiple different treatments while preserving the benefits of 294 295 randomized comparisons within each trial. We did conduct a post hoc network meta-296 analysis to study any potential informative (lack of) heterogeneity in this regard. The outcomes were in line with our main analyses, suggesting no direct evidence of a 297 298 difference in PPG or PPI effects for the different LES types versus each other or a 299 control treatment. The outcome of this analysis should be interpreted with caution however, since it was conducted after the studies, data and outcomes of the main 300 301 analyses were known.

Secondly, most of the included studies had relatively small sample sizes, potentially obscuring possible intervention effects due to a lack of statistical power. However, small study biases are generally associated with the erroneous overestimation of effect size and statistical significance (92, 93). Thirdly, as a result of the sweet tasting nature 306 of the interventions, only a small number of the included studies that had specific design 307 considerations (i.e. administration via capsules/gastric infusion or concomitantly with 308 glucose/sucrose) were double-blinded. It is possible that detection bias has occurred in studies where the participants and, in some cases, the investigators were not blinded as 309 to the treatments. However, a post-hoc analysis including only the studies reported as 310 311 being double-blind had outcomes similar to those of the main analyses. This suggests that potential performance bias was likely not an issue in this case. Regarding the 312 313 subgroup and post-hoc analyses, another potential limitation is that many aspects of the studies covary. For example, all of the double-blind studies were conducted in healthy 314 subjects whereas all of the studies in subjects with type 2 diabetes were not blinded 315 316 (potentially high risk of performance bias), and all of the sucralose and l-arabinose studies are relatively recent whereas most of the aspartame and saccharin studies are 317 318 older. As such, the outcomes of the sub-group analyses should be interpreted with caution. Lastly, most of the studies included in this meta-analysis investigated the 319 effects of a single LES administered alone. No differences were found based on LES 320 321 type, but many current food and beverage products contain combinations of two or more types of LES. We only had enough data to perform a sub-group analysis on one 322 323 potential combination (acesulfame potassium + sucralose). Our conclusions in this 324 regard can, therefore, not be extrapolated to other combinations of LES. There is, however, currently no evidence or reasonable explanatory hypothesis as to why the 325 intake of a combination of LES would have different effects on glucose homeostasis 326 327 compared with a single LES alone.

In conclusion, this review provides an up-to-date overview of controlled humanintervention studies on the effects of LES consumption on acute postprandial glycemic

330 and insulinemic responses. Our analyses indicate that under acute conditions, whether administered alone or in combination with a nutrient-containing load, LES do not exert 331 332 an independent effect on the mean change in postprandial blood glucose or insulin responses compared to a control intervention. Some small reductions in PPI, based on 333 limited studies, were found in studies enrolling patients with type 2 diabetes, but overall 334 the null results do not seem to differ appreciably by the type of LES consumed, dose of 335 LES, or fasting glucose or insulin levels. A post-hoc network meta-analysis suggested 336 337 no direct evidence of a difference in PPG or PPI effects for the different LES types versus each other or a control treatment. In light of concerns that different LES types 338 may differ in their physiological effects, future work adopting an *a priori* network meta-339 340 analysis approach is recommended.

## 341 Author contributions

The authors' responsibilities were as follows—DJM and AG: conceived and designed the study, conducted the literature review, and drafted the manuscript; AG: conducted the statistical analysis; and KMA and AR: amended and approved the protocol, provided critical revision and important intellectual content. All of the authors made significant contributions to this manuscript. All authors read and approved the final manuscript.

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## Tables

Table 1. Trial selection criteria.

Inclusion	Exclusion
Participants/population	
Human children (3-10 years of age), adolescents (10-18 years of	
age) and adults ( $\geq 18$ years of age);	
Healthy participants and those with impaired glucose homeostasis	Hospitalized/critically ill patients
(i.e. prediabetes, diabetes type 1 or 2, impaired glucose tolerance	
and overweight or obese individuals)	
Intervention	
Acute exposure to LES; either alone, in water, as diet beverage or	Co-intervention with insulin or drugs affecting glucose
intragastric infusion, or with a meal or other nutrient-containing	homeostasis
preloads	
Comparators	
The same intervention without inclusion of LES	
Outcomes	
Acute postprandial blood glucose response (defined as incremental	Trials measuring postprandial blood glucose or insulin responses
Area Under the Curve) after exposure to LES or Control	for < 120 min (for quantitative meta-analysis only)
Acute postprandial insulin response (defined as incremental Area	
Under the Curve) after exposure to LES or Control	

First author, year	Study	Ν	Mean Age	Health	LES type	LES dose Control		Meal test	Meal carbohydrate	Outcome
[country]	design		(years)	status		(mg)			content (g)	
Ahmad, 2018 (49)	CO, S	20	24.1	Healthy	Stevia	3000	Isocaloric	Mixed meal	50	PPG
[Pakistan]							meal			
Azari, 2017 (50)	CO, S	10	33.5	Healthy	Saccharin	18	Water	75g glucose	75	PPG, PPI
[US]										
Brown, 2009 (51)	CO, BNR	22	18.5	Healthy	Sucralose + Acesulfame K	45.6; 25.9	Carbonated	75g glucose	75	PPI
[US]							water			
Brown, 2012 (52)	CO, BNR	25	18.8	Healthy	Sucralose + Acesulfame K	45.6; 25.9	Carbonated	75g glucose	75	PPG
[US]		9	18.2	T1D			water			
		10	17.9	T2D						
Burns, 1991 (33)	CO, BNR	8	26.1	Healthy	Aspartame	500	Unsweetened	100g sucrose	100	PPG, PPI
[US]							beverage	None	0	
Cooper, 1988 (53)	CO, BNR	17	62.2	T2D	Saccharin	93*	Isocaloric	Mixed meal	47	PPG, PPI
[Australia]							meal			
Ford, 2011 (54)	CO, S	8	22-27	Healthy	Sucralose	41.5	Water	None	0	PPG, PPI
[UK]										

**Table 2.** Characteristics of studies included in the meta-analysis

First author, year	Study	Ν	Mean Age	Health	LES type	LES dose	Control	Meal test	Meal carbohydrate	Outcome	
[country]	design		(years)	status		(mg)			content (g)		
Gregersen, 2004 (55)	CO, BNR	12	65.8	T2D	Stevioside	1000	Corn starch	Mixed meal	55	PPG, PPI	
[Denmark]											
Halschou-Jensen, 2015	CO, D	17	22.5	Healthy	L-Arabinose	2900	Isocaloric	Mixed meal	68	PPG, PPI	
(34)						5900	meal				
[Denmark]						2500			72		
						4900					
		6	23.3	Healthy	L-Arabinose	10200	Isocaloric	Solid mixed	72		
							meal	meal			
								Semi-solid			
								mixed meal			
						15000		Liquid	75		
								mixed meal			
Helou, 2019 (64)	CO, D	15	20.1	Healthy	Acesulfame K	3500	Isocaloric	Mixed meal	116	PPG, PPI	
[Lebanon]		15	21.7	Obese		3500	meal				

First author, year	Study	Ν	Mean Age	Health	LES type	LES dose	Control	Meal test	Meal carbohydrate	Outcome
[country]	design (years) status		(mg)			content (g)				
Horwitz 1988, (35)	CO, 0	12	28	Healthy	Aspartame	400 Unsweeter		Fasted	0	PPG, PPI
[US]					Saccharin	135	beverage			
		10	57	T2D	Aspartame	400				
					Saccharin	135				
Krog-Mikkelsen, 2011	CO, D	15	25	Healthy	L-Arabinose	1000	Isocaloric	75g sucrose	75	PPG, PPI
(36)						2000	beverage			
[Denmark]						3000				
Ma, 2009 (37)	CO, S	7	24	Healthy	Sucralose	800	Saline	Fasted	0	PPG, PPI
[Australia]						80				
Nichol, 2020 (65)	CO, BNR	10	27	Healthy	Sucralose	48	Water	75g glucose	75	PPG, PPI
[US]		11	29.5	Obese						
Overduin, 2016 (56)	CO, S	10	33.4	Healthy	Erythritol	8000	Isocaloric	Mixed meal	NR	PPG, PPI
[UK]		10	33.6	Obese			meal			
Parimalavalli, 2011	CO, BNR	6	NR	T2D	Stevia	2000	Isocaloric	Mixed meal	50	PPG
(57)							meal			
[India]										

First author, year	, year Study N Mean Age Health LES type		LES type	LES dose	Control	Meal test	Meal carbohydrate	Outcome		
[country]	design		(years)	status		(mg)			content (g)	
Pepino, 2013 (58)	CO, BNR	17	35.1	Obese	Sucralose	48	Water	75g glucose	75	PPG, PPI
[US]										
Prat-Larquemin, 2000	CO, BNR	24	23.2	Healthy	Aspartame	270	Isocaloric	Mixed meal	90	PPG, PPI
(59)							meal			
[France]										
Slama, 1984 (60)	CO, BNR	12	51-57	T2D	Saccharin	40	Isocaloric	Mixed meal	70	PPG, PPI
[France]							meal			
Solomi, 2019 (61)	CO, BNR	10	27.2	Healthy	Aspartame + Acesulfame K	55.9; 38.5†	Water	25g glucose	25	PPG
[UK]					(Diet Coke)					
Steinert, 2011 (38)	CO, D	12	23.3	Healthy	Acesulfame K	220	Water	Fasted	0	PPG, PPI
[Switzerland]					Aspartame	169				
					Sucralose	62				

First author, year	Study	N	Mean Age	Health	LES type	LES dose	Control	Meal test	Meal carbohydrate	Outcome
[country]	design		(years)	status		(mg)			content (g)	
Sylvetsky, 2016 (39)	CO, BNR	30	29.7	Healthy	Sucralose	68	Water	75g glucose	75	PPG, PPI
[US]						170				
						205				
		31	27.4	Healthy	Sucralose + Acesulfame K	68; 41	Carbonated	75g glucose	75	PPG, PPI
					(Diet Rite Cola)		water			
					Sucralose + Acesulfame K	18; 18; 57				
					+ Aspartame (Diet					
					Mountain Dew)					
					Sucralose + Acesulfame K	68; 41				
Temizkan, 2015 (40)	CO, S	8	45	Healthy	Aspartame	72	Water	75g glucose	75	PPG, PPI
[Turkey]					Sucralose	24				
		8	51.5	T2D	Aspartame	72				
		Ŭ	0110	120	Sucralosa	24				
					Sucraiose	24				

First author, year	Study	Ν	Mean Age	Health	LES type	LES dose	Control	Meal test	Meal carbohydrate	Outcome		
[country]	design		(years)	status		(mg)			content (g)			
Wolf-Novak, 1990 (62)	CO, BNR	7	27	Healthy	Aspartame	200	Isocaloric	Beverage	60	PPG, PPI		
[US]							beverage					
Wölnerhanssen, 2016	CO, D	20	25.9	Healthy	Erythritol	75000	Water	Fasted	0	PPG, PPI		
(63)												
[Switzerland]												
Wu, 2016 (41)	CO, S	10	33.6	Healthy	Acesulfame K	200	Water	75g glucose	75	PPG, PPI		
[Australia]					Sucralose + Acesulfame K	46; 26						
					Sucralose	52						

\*dose not given but reported as equivalent sweetness to 28g sucrose; dose calculated considering a sweetness equivalence of 300:1

†dose not reported; estimated according to content of Aspartame + Acesulfame K in commercially sold diet cola

BNR: Blinding not reported; CO: Cross-over study design; D: Double-blind; PPG: Postprandial glucose; PPI: Postprandial insulin; LES: Low energy sweetener; NR: Not reported; O: Open-label; S: Single-blind; T1D: Type-1 diabetes mellitus; T2D: Type-2 diabetes mellitus

Covariates	Mean cha	nge differen	ce in PPG	Mean change difference in PPI			
	β	SE	Р	β	SE	Р	
Baseline fasting glucose (per 1 mmol/l increase)	-0.059	0.04	0.15	2.17	2.87	0.45	
Baseline fasting insulin (per 1 pmol/l increase)	-0.001	0.001	0.32	-0.04	0.11	0.75	
Sucralose dose (per 10 mg increase)	0.004	0.003	0.22	0.08	0.19	0.66	
L-Arabinose dose (per 1000 mg increase)	0.001	0.024	0.96	0.96	3.93	0.81	

**Table 3.** Impact of continuous covariates on PPG and PPI responses to LES

PPG: Postprandial glucose; PPI: Postprandial insulin

			Mean	change diffe	rence in l	PPG					Mean	n change diff	erence in	n PPI		
	No. of	Effect		P within	$I^2$	Chi <sup>2</sup>	df	P between	No. of	Effect		P within	$I^2$	Chi <sup>2</sup>	df	P between
Subgroup	studies	(mmol/l)	95% CI	subgroup				subgroups	studies	(pmol/l)	95% CI	subgroup				subgroups
LES type						7.11	6	0.31						2.57	6	0.86
Sucralose	13	0.05	-0.07, 0.18	0.40	33.45				13	-3.58	-21.06; 13.90	0.69	12.99			
L-Arabinose	10	-0.03	-0.22, 0.16	0.77	34.91				10	-6.90	-32.63; 18.83	0.60	45.41			
Aspartame	9	0.05	-0.09, 0.20	0.46	0				9	1.82	-13.27; 16.92	0.81	49.51			
Sucralose + Ace K	6	0.12	-0.14, 0.38	0.36	0				4	25.32	-24.28; 74.92	0.32	0			
Saccharin	5	-0.04	-0.20, 0.13	0.66	0				5	-0.29	-17.03; 16.44	0.97	0			
Ace K	4	-0.12	-0.29, 0.05	0.16	0				4	2.74	-21.07; 26.54	0.82	0			
Co-exposure						0.48	1	0.48						0.09	1	0.77
Without nutrient	12	0.02	-0.11, 0.15	0.76	44.8				12	-0.57	-15.85, 14.71	0.94	0			
With nutrient preload	43	-0.03	-0.11, 0.04	0.40	41.46				38	-3.48	-15.38, 8.42	0.57	56.31			
Health status						5.56	1	0.02*						0.45	1	0.5
Healthy	41	-0.01	-0.07, 0.06	0.80	36.31				39	-2.86	-12.01, 6.30	0.54	56.31			
Type 2 diabetes	9	-0.30	-0.53, -0.07	0.01*	32.69				7	4.87	-15.63, 25.37	0.64	18.67			

Table 4. Mean change difference in PPG and PPI after LES intake within different subgro	oups.
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Ace K: Acesulfame potassium; Df: degrees of freedom; PPG: Postprandial glucose; PPI: Postprandial insulin

## **Figure legends**

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of the study selection procedure.

**Figure 2. Forest plot showing mean change difference in PPG after LES intake.** Horizontal lines represent 95% confidence intervals. The diamond represents the pooled estimate determined using a random effects model.

## Figure 3. Forest plot showing mean change difference in PPI after LES intake.

Horizontal lines represent 95% confidence intervals. The diamond represents the pooled estimate determined using a random effects model.

#### Figure 4. Funnel plot used to assess risk of publication bias for (A) PPG and (B) PPI.

Weights (1/SE<sup>2</sup>) are plotted against the changes in PPG (*A*) and PPI (*B*) from a total of 55 comparisons (452 individual participants) for PPG and 50 comparisons for PPI (394 individual participants) respectively. Both PPG and PPI effects showed moderate heterogeneity (P value for Q statistic <0.01;  $I^2 = 59.5\%$  and P <0.01,  $I^2 = 61.2\%$  respectively) between studies.