

**Reconciling Conflicting Evidence on Low- and No-calorie
Sweeteners and Cardiometabolic Outcomes: An Umbrella
Review Using Naïve and Bias-Adjusted Methods**

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Manuscripts

1 **Reconciling Conflicting Evidence on Low- and No-calorie Sweeteners and Cardiometabolic**
2 **Outcomes: An Umbrella Review Using Naïve and Bias-Adjusted Methods**

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21
22 **Abbreviations used:** AMSTAR2=assessment of multiple systematic reviews;
23 ApoB=apolipoprotein B; BMI=body mass index; CHD=coronary heart disease; CI=confidence
24 interval; CVD=cardiovascular disease; DBP=diastolic blood pressure; GRADE=Grading of
25 Recommendations Assessment, Development, and Evaluation; HbA1c=glycosylated
26 hemoglobin; HDL=high-density lipoprotein; HR=hazard ratio; LDL-C=low-density lipoprotein
27 cholesterol; LNCS=low- and no-calorie sweeteners; MASLD=metabolic dysfunction-associated
28 steatotic liver disease; MD=mean difference; PICOTS=participants, intervention, comparator,
29 outcome, time, study design; PRISMA=Preferred Reporting Items for Systematic Reviews and
30 Meta-Analyses; RR=risk ratio; SBP=systolic blood pressure; SRMA=systematic review and
31 meta-analysis; T2D=type 2 diabetes; TG=triglycerides; WC=waist circumference; WHO=World
32 Health Organization.
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ABSTRACT

Inconsistency among evidence syntheses has led to opposing guidelines and public confusion regarding low- and no-calorie sweeteners (LNCS) in non-communicable diseases. To understand the role of different analytical approaches in assessing LNCS and cardiometabolic outcomes, we conducted an umbrella review of systematic reviews and meta-analyses. MEDLINE, EMBASE and Cochrane were searched for systematic reviews and meta-analyses of trials or cohorts that had at least two analytical approaches: naïve (LNCS versus all-comparators (trials) and prevalent (cohorts)) and bias-adjusted (LNCS versus intended or reference substitution (trials) and LNCS change or intended or reference substitution (cohorts)). GRADE assessed certainty of evidence. We included six trial- and five cohort-based analyses. In trials, LNCS reduced energy, body weight, and body fat in both analyses and BMI and systolic blood pressure had smaller HbA1c reductions than water in bias-adjusted only. In analyses of cohorts, LNCS was associated with higher obesity, diabetes, stroke, and cardiovascular and all-cause mortality in naïve analyses but lower body weight, waist circumference, obesity, CHD, and cardiovascular and all-cause mortality in bias-adjusted analyses. The certainty of evidence was generally moderate for trials and very low for cohorts. LNCS show benefits across analytical approaches in both analyses of trials. These results agree with bias-adjusted analyses of cohorts, in which LNCS are associated benefits across cardiometabolic outcomes, but not naïve analyses of cohorts. Systematic reviews and meta-analyses using bias-reduction methods support the use of LNCS as a sugars-reduction strategy.

Protocol registration: <https://doi.org/10.17605/OSF.IO/TSEQM>

Keywords: Non-nutritive sweeteners, low- and no-calorie sweeteners, Sweetening agents, sugar-sweetened beverages, randomized controlled trial, prospective cohort studies, cardiometabolic risk factors, cardiometabolic disease

58 **Take home message:** The evidence on low- and no-calorie sweeteners has often been cited to be
59 discordant between trials and cohorts. We show that when cohorts employ bias-adjusted
60 methodologies, the evidence consistently supports the use of sweeteners for sugar-reduction.
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INTRODUCTION

Dietary (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2025, U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2021) and clinical practice guidelines for obesity and diabetes (Twells et al., 2020, 2023, Sievenpiper et al., 2018, ElSayed et al., 2024) support low- and no-calorie sweeteners (LNCS) as a strategy to reduce caloric intake and manage weight (Twells et al., 2020, 2023, Sievenpiper et al., 2018, ElSayed et al., 2024, U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2025, U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2021). However, recent World Health Organization (WHO) guidance recommends against their use as a means of achieving weight control or reducing the risk of noncommunicable disease (World Health Organization, 2023). This guidance has raised concerns within the scientific community, as it conflicts with evidence of benefit from randomized controlled trials (henceforth referred to as trials) and robustly analyzed prospective cohort studies employing bias-adjusted methods (Khan et al., 2023, Hedrick et al., 2023). Trials consistently demonstrate benefit of using LNCS when displacing calories, with comparable effects to water on intermediate cardiometabolic outcomes (Toews et al., 2019, Laviada-Molina et al., 2020, Rios-Leyvraz et al., 2022, Rogers and Appleton, 2021, McGlynn et al., 2022). Similarly, prospective cohort studies using bias-adjusted methodologies - modeling changes in LNCS consumption and substitution for sugars to better-define LNCS exposures in the hopes of mitigating reverse causality and confounding - also reveal benefits for cardiometabolic outcomes, directly aligning with trial findings (Lee et al., 2022), indicating a potential role for LNCS in calorie reduction and weight management. The WHO guideline prioritized prospective cohort studies using 'naïve' i.e., prevalent exposure assessment, known to be susceptible to bias, while disregarding the more

85 informative cohort studies employing bias-adjusted methodologies like change and substitution
86 analyses. This approach not only undermines the established hierarchy of evidence (Brighton et
87 al., 2003) and best practices in nutritional research, but also conflicts with other WHO guidelines
88 that emphasize methodological alignment across study designs (Reynolds et al., 2022). It remains
89 unclear whether systematic reviews and meta-analyses (SRMAs) of LNCS adequately differentiate
90 findings based on naïve versus bias-adjusted methodological approaches.

91 In light of ongoing calls for rigorous LNCS research (Sievenpiper et al., 2017, Khan et al.,
92 2019, Khan et al., 2023, Mela et al., 2020, Malik, 2019, Hedrick et al., 2023, Higgins et al., 2024),
93 this umbrella review aims to synthesize existing SRMAs examining the association between LNCS
94 and cardiometabolic outcomes, encompassing disease risk, intermediate outcomes, and energy
95 intake. We will compare findings from reviews utilizing at least two distinct methodological
96 approaches - naïve and bias-adjusted - and assess the certainty of evidence using Grading of
97 Recommendations Assessment, Development, and Evaluation (GRADE).

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METHODS

100 *Design*

101 This umbrella review was conducted following the Cochrane Handbook for Systematic
102 Reviews of Interventions (Higgins et al., 2022) and reported according to the PRISMA guidelines
103 (Page et al., 2021). The study protocol was registered on the Open Science Framework (OSF,
104 <https://doi.org/10.17605/OSF.IO/TSEQM>)

105 *Search strategy*

106 **Supplementary table 1** shows the systematic search strategy, based on the PICOTS
107 framework (**Supplementary table 2**) (Moher et al., 2009). Medline, Embase and the Cochrane

108 Database of Systematic reviews were searched from inception through December 9th, 2024, using
109 search terms of exposure (e.g., “low- and no-calorie sweeteners” or “non-nutritive sweeteners”)
110 and study design (e.g., “systematic review” or “meta-analysis”). Manual searches using references
111 lists of included articles and other relevant reviews on the topic complemented the systematic
112 search.

113 *Study selection*

114 SRMAs of trials and prospective cohort studies were included if they report data on adults
115 (≥ 18 years) of all health backgrounds, if they had an intervention period of ≥ 7 days (for trials) or
116 a follow-up duration of ≥ 1 year (for cohort studies), and if they investigated the role of orally
117 consumed LNCS from any sources including beverages, table-top sweeteners and low- or no-
118 calorie foods on cardiometabolic disease and intermediate cardiometabolic disease outcomes
119 and/or energy intake (described below) employing both naïve and bias-adjusted analytical
120 approaches. These analytical approaches were defined as LNCS versus all comparators lumped
121 (naïve) and LNCS versus intended or reference substitution (bias-adjusted) for SRMAs of trials,
122 and prevalent LNCS analyses (naïve) or LNCS intended or reference substitution analyses with
123 additional adjustment for baseline adiposity, as was defined by each SRMA authors, (bias-
124 adjusted) for SRMAs of cohorts.

125 LNCS were defined as high-intensity sweeteners (e.g. aspartame, sucralose, stevia
126 glycosides, acesulfame-K, saccharin, etc.) whose caloric contribution was considered negligible
127 due to high sweetness intensity and minimal food quantities, excluding sugar alcohols (e.g., xylitol,
128 erythritol) which have lower sweetness intensity and non-negligible caloric contribution
129 (Magnuson et al., 2016). For each outcome, we prioritized the most recent SRMA to ensure up-to-
130 date synthesized evidence from both naïve and bias-adjusted methods.

131 *Data extraction and methodological quality assessment*

132 Two independent reviewers extracted data from each included SRMA. Extracted data for
133 all reviews included publication details, participant characteristics, and study setting. For trial-
134 based SRMAs, data extraction encompassed intervention details (LNCS type, dose, comparator
135 type, feeding control level [intended substitution or reference substitution]) and outcome
136 information (participant numbers, pooled effect estimates as mean differences [MDs] and standard
137 errors). For cohort SRMAs, extracted data included exposure information (LNCS type, dose in
138 highest/lowest quantiles, dose changes over time, substitution model type [intended substitution
139 or reference substitution]) and outcome data (case numbers, pooled effect estimates, and standard
140 errors). Review quality was independently assessed by two reviewers using the assessment of
141 multiple systematic reviews (AMSTAR 2) tool (Shea et al., 2017).

142 *Methodology types*

143 Outcomes were included only if SRMA data originated from at least two different
144 methodology types. Figure 1 depicts methodology types for trials and cohorts. For trials,
145 methodology types were: (1) naïve (lumping all comparators: sugars, water/placebo, usual diet),
146 bias-adjusted: (2) intended substitution (LNCS replacing sugars), or bias-adjusted: (3) reference
147 substitution (LNCS replacing water/placebo). Cohort methodology types were: (1) naïve analyses
148 (baseline or prevalent LNCS exposure only), bias-adjusted: (2) intended substitution analyses
149 (modeling LNCS substitution for sugars) with adjustment for baseline adiposity, bias-adjusted: (3)
150 reference substitution analyses (modeling LNCS substitution for water/placebo) with adjustment
151 for baseline adiposity, or bias-adjusted: (4) change analyses (modeling change in LNCS exposure
152 over time using repeated measures) with adjustment for baseline adiposity and in addition if they

153 employed any advanced g-methods to fully account for time-varying confounding (Mansournia et
154 al., 2017).

155 *Outcomes*

156 For trial SRMAs, outcomes included: changes in energy intake; adiposity measures (body
157 weight, BMI, body fat, waist circumference [WC]); glycemic control (fasting blood
158 glucose/insulin, 2-hour postprandial glucose, plasma glucose area under the curve, glycosylated
159 hemoglobin [HbA1c]); cardiovascular risk factors (systolic/diastolic blood pressure [SBP/DBP],
160 plasma lipids: low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol
161 [HDL-C], non-HDL-C, triglycerides [TG], apolipoprotein B [apoB]); and metabolic dysfunction-
162 associated steatotic liver disease (MASLD) markers (intrahepatocellular lipid, alanine
163 transaminase, aspartate aminotransferase, fatty liver index). For cohort SRMAs, outcomes
164 included: changes in energy intake, body weight, WC, and body fat (continuous); incidence
165 overweight/obesity; metabolic syndrome; type 2 diabetes (T2D); MASLD; cardiovascular disease
166 (CVD) and cardiovascular mortality (coronary heart disease [CHD], stroke, total cardiovascular
167 disease [CVD]); and all-cause mortality. For cohort studies, effect measures were extracted as
168 reported in the original SRMA studies (hazard ratios, relative risks, and odds ratios) and treated as
169 comparable estimates of association for reporting purposes, given that outcomes were relatively
170 rare and follow-up periods were comparable across studies and were referred to collectively as
171 measures of disease risk or incidence in our interpretation. We did not assess cancer as an outcome.

172 *Evidence synthesis & certainty of the evidence*

173 Extracted SRMA evidence including pooled estimates was report in tables and as
174 superplots. GRADE certainty assessments were reported as provided by original SRMAs. If no
175 GRADE assessment was available, at least two independent reviewers (SA-C, MEK and/or TAK)

176 evaluated the certainty of evidence for each SRMA using established GRADE criteria
177 (Schünemann et al., 2013) and published information. GRADE assesses evidence certainty as high,
178 moderate, low, or very low. RCT meta-analyses are initially considered high-quality evidence;
179 prospective cohort study meta-analyses are initially low-quality. Evidence downgrade was based
180 upon study limitations (risk of bias weight), inconsistency (substantial unexplained heterogeneity,
181 $I^2 \geq 50\%$ and $P < 0.10$), indirectness (factors limiting generalizability), imprecision (wide 95%
182 confidence intervals [CIs] crossing a minimally important difference), and publication bias (small-
183 study effects). Evidence upgrade was based upon large association magnitude (RR or $HR \leq 0.5$ or
184 ≥ 2), dose-response gradient, and attenuation by plausible confounding. Reviewer discrepancies
185 were resolved by consensus or arbitration by the senior author (JLS). For the interpretation of
186 the magnitude, we used the minimally important differences (MID) to assess the
187 importance of magnitude of our point estimate using the effect size categories according
188 to the new GRADE guidance (Santesso et al., 2020). Then, we used the MIDs to assess
189 the importance of the magnitude of our point estimates using the effect size categories
190 according to the GRADE guidance (Schünemann et al., 2013, Santesso et al., 2020,
191 Balshem et al., 2011) as follows: a large effect ($\geq 5 \times$ MID); moderate effect ($\geq 2 \times$ MID);
192 small important effect ($\geq 1 \times$ MID); and trivial/unimportant effect (< 1 MID).

193 *Role of the funder*

194 This work was supported by the Applied Physiology, Nutrition and Metabolism (APNM) Award
195 for Nutrition Translation. The award was established by the Canadian Nutrition Society (CNS),
196 in conjunction with the editorial staff of the journal of APNM – part of Canadian Science
197 Publishing - to recognize excellence in the translation of nutritional knowledge. The funders had
198 no role in the study design, collection, analysis, interpretation of the data, nor in writing of the

199 report. All authors had full access to the data and take full responsibility for the integrity and
200 accuracy of the data and analyses.

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RESULTS

203 *Search results*

204 **Figure 2** illustrates the study selection process. From 7,175 reports, we excluded 7,116
205 based on title or abstract. Full-text review of 59 reports yielded 33 meeting eligibility criteria.
206 Applying the criteria of latest review and at least two methodology types per outcome, we included
207 six trial-based SRMAs (N=24,497 across outcomes and methodology types). These reviews
208 reported on the following outcomes, assessed using reference substitution, intended substitution,
209 and lumping of all comparators methodologies: energy intake (Rios-Leyvraz et al., 2022,
210 Rostampour et al., 2024), body weight (Li s, 2023, Movahedian et al., 2024) BMI (Rios-Leyvraz
211 et al., 2022, Movahedian et al., 2024), body fat (McGlynn et al., 2022, Movahedian et al., 2024),
212 WC (McGlynn et al., 2022, Movahedian et al., 2024), HbA1c (McGlynn et al., 2022, Rios-Leyvraz
213 et al., 2022), fasting plasma glucose (McGlynn et al., 2022, Rios-Leyvraz et al., 2022), fasting
214 plasma insulin (McGlynn et al., 2022, Rios-Leyvraz et al., 2022), SBP (McGlynn et al., 2022,
215 Rios-Leyvraz et al., 2022), DBP (McGlynn et al., 2022, Rios-Leyvraz et al., 2022), LDL-C, HDL-
216 C cholesterol (McGlynn et al., 2022, Movahedian et al., 2023), and TG (McGlynn et al., 2022,
217 Movahedian et al., 2023). Although we searched for data on other outcomes (post-prandial
218 glucose, non-HDL-C, apoB, intrahepatocellular lipid, alanine transaminase, aspartate
219 aminotransferase, fatty liver index), we did not identify at least two analyses using the specified
220 methodologies for inclusion.

221 We also included five SRMAs of cohort studies (N=2,759,806 across outcomes and
222 methodology types). These meta-analyses reported on the associations between replacing free

223 sugars with LNCS and the following outcomes, using prevalent, change, intended substitution, and
224 reference substitution analyses: body weight (Rios-Leyvraz et al., 2022, Lee et al., 2022), WC
225 (Rios-Leyvraz et al., 2022, Lee et al., 2022), obesity incidence (Rios-Leyvraz et al., 2022, Lee et
226 al., 2022), T2D incidence (Lee et al., 2022, Li et al., 2023), CHD incidence (Lee et al., 2022,
227 Queiroz et al., 2024), stroke incidence (Lee et al., 2022, Queiroz et al., 2024), cardiovascular
228 mortality (Queiroz et al., 2024, Chen et al., 2024) and all-cause mortality (Queiroz et al., 2024,
229 Chen et al., 2024). Although we searched for data on other outcomes (BMI, body fat, energy intake,
230 metabolic syndrome, CVD and MASLD incidence, CHD and stroke mortality), we did not identify
231 at least two analyses using the specified methodologies for inclusion. **Supplemental Table 3** lists
232 the excluded systematic reviews and meta-analyses, with reasons for exclusion being either the
233 availability of more recent reviews or an insufficient number of methodology types for a given
234 outcome.

235 *Systematic review and meta-analyses characteristics*

236 **Tables 1 and 2** detail the characteristics of included trial-based and cohort-based SRMAs,
237 respectively. In SRMAs of trials, only one SRMA specifically included studies assessing the effect
238 of low- and no-calorie sweetened beverages (McGlynn et al., 2022), while the other five included
239 studies assessing the effect of LNCS in any food form. In SRMAs of cohorts, only one SRMA
240 specifically included studies assessing the effect of low- and no-calorie sweeteners in any food
241 form (Rios-Leyvraz et al., 2022), while the other four included studies assessing the effect of low-
242 and no-calorie sweetened beverages only. Mean LNCS dose reporting was limited in trial SRMAs
243 and LNCS type or dose was nearly absent in cohort SRMAs. Cohort SRMAs using bias-adjusted
244 analyses included only studies adjusted for initial adiposity, whereas those using naïve analyses
245 included a mixture of studies with or without adjustment for adiposity. **Supplementary tables 4a**

246 **and 4b** detail the AMSTAR2 ratings for SRMAs of trials and cohorts, respectively. AMSTAR2-
247 assessed risk of bias varied from low to high for trial SRMAs (majority high confidence) and low
248 to moderate for cohort SRMAs (majority moderate confidence).

249 *Outcomes*

250 **Figure 3** presents LNCS effects on outcomes from trial-based SRMAs, categorized by
251 methodology type. Naïve analyses (lumping all comparators; red font) showed LNCS use
252 associated with: a trivial energy intake reduction (-135.89 kcals [95% CI -205.39, -66.38 kcals],
253 $P<0.01$, $I^2=81\%$, $P_Q<0.01$, Low certainty); a small, important body weight reduction (-1.02 kg
254 [95% CI -1.57, -0.46 kg], $I^2=71\%$, $P_Q<0.0001$, Low certainty); and a trivial body fat reduction (-
255 1.09% [95% CI -1.90, -0.29%], $I^2=0\%$, $P_Q=0.803$, Low certainty). Bias-adjusted (blue font)
256 intended substitution analyses (LNCS for sugars) showed: a trivial energy intake reduction (-
257 175.26 kcals [95% CI -296.47, -54.06 kcals], $P<0.001$, $I^2=61\%$, $P_Q<0.001$, High certainty); a trivial
258 body weight reduction (-0.71 kg [95% CI -1.19, -0.24 kg], $I^2=78\%$, $P_Q<0.01$, Very low certainty);
259 a trivial BMI reduction (-0.21 kg/m² [95% CI -0.36, -0.06 kg/m²], $P<0.01$, $I^2=42\%$, $P_Q=0.040$, Low
260 certainty); and a trivial body fat reduction (-0.60% [95% CI -1.03, -0.18%], $I^2=0\%$, $P_Q=0.998$,
261 Moderate certainty). Bias-adjusted reference substitution analyses (LNCS for water/placebo)
262 showed: trivial increases in HbA1c that related to smaller reductions in HbA1c than water/placebo
263 (0.21% [95% CI 0.02, 0.40%], $P=0.034$, $I^2=93\%$, $P_Q<0.001$, Low certainty) and a small, important
264 reductions in SBP that related to larger reductions in SBP than water (-2.63 mmHg [95% CI -4.71,
265 -0.55 mmHg], $P=0.013$, $I^2=29\%$, $P_Q=0.275$, Low certainty). No differences were observed for
266 other outcomes.

267 **Figures 4 and 5** present associations of LNCS with outcomes from cohort-based SRMAs,
268 categorized by methodology type (Figure 4 shows MDs; Figure 5 shows ratios). Naïve prevalent

269 analyses (red font) showed LNCS use positively associated with: obesity risk (large magnitude,
270 HR 1.76 [95% CI 1.25, 2.49], $P<0.01$, $I^2=0\%$, $P_Q=0.620$, Low certainty); T2D risk (large
271 magnitude, RR 1.32 [95% CI 1.11, 2.56], $I^2=93\%$, $P_Q<0.001$, High certainty); stroke risk (at 1
272 dose/day) (moderate magnitude, HR 1.16 [95% CI 1.01, 1.32], $P=0.036$, $I^2=25\%$, $P_Q=0.260$, Very
273 low certainty); cardiovascular mortality risk (1 dose/day: large magnitude, HR 1.30 [95% CI 1.10,
274 1.53], $P=0.002$, $I^2=63\%$, $P_Q=0.030$, Very low certainty; 2 doses/day: large magnitude, HR 1.30
275 [95% CI 1.07, 1.57], $P=0.007$, $I^2=72\%$, $P_Q=0.010$, Very low certainty); and all-cause mortality risk
276 (at 1 dose/day) (moderate magnitude, HR 1.14 [95% CI 1.03, 1.26], $P=0.009$, $I^2=79\%$, $P_Q<0.01$,
277 Very low certainty).

278 Bias-adjusted (blue font) intended substitution analyses (LNCS for SSB substitution)
279 showed associations with: lower body weight (trivial magnitude, -0.12 kg [95% CI -0.14 , -0.10
280 kg], $P<0.01$, $I^2=46\%$, $P_Q=0.160$, Moderate certainty); lower obesity risk (large magnitude, RR 0.88
281 [95% CI 0.88, 0.89], $P<0.01$, Low certainty); lower CHD risk (large magnitude, RR 0.89 [95% CI
282 0.81, 0.98], $P=0.020$, $I^2=28\%$, $P_Q=0.220$, Low certainty); lower cardiovascular mortality risk (large
283 magnitude, RR 0.94 [95% CI 0.90, 0.99], $I^2=0\%$, $P_Q=0.647$, Very low certainty); and lower all-
284 cause mortality risk (large magnitude, HR 0.96 [95% CI 0.94, 0.98], $I^2=0\%$, $P_Q=0.578$, Very low
285 certainty). Bias-adjusted (blue font) change analyses showed associations with: lower body weight
286 (trivial magnitude, -0.01 kg [95% CI -0.01 , 0.00 kg], $P=0.010$, $I^2=66\%$, $P_Q=0.020$, Low certainty)
287 and lower WC (small important magnitude, -1.15 cm [95% CI -2.34 , -0.05 cm], $P<0.05$, $I^2=2884$,
288 $P_Q<0.01$, Low certainty). No differences were observed for other outcomes.

289 *Certainty of the evidence*

290 GRADE evaluations were available from the authors of included SRMAs for most
291 outcomes and methodology types. Exceptions were: trial-based SRMAs for body weight, intended

292 substitution, reference substitution (water), and reference substitution (placebo) (Li s, 2023);
293 cohort-based SRMAs for T2D incidence, prevalent analyses (Li et al., 2023), CHD incidence,
294 prevalent analysis (Queiroz et al., 2024); stroke incidence, prevalent analysis (1 and 2 doses/day)
295 (Queiroz et al., 2024); cardiovascular mortality, substitution analysis (Chen et al., 2024) and
296 prevalent analysis (1 and 2 doses/day) (Queiroz et al., 2024); and all-cause mortality, substitution
297 analysis (Chen et al., 2024) and prevalent analysis (1 and 2 doses/day) (Queiroz et al., 2024).
298 **Supplementary Table 4** presents our GRADE evaluations, conducted using published
299 information where original GRADE assessments were lacking.

300 Across trial-based and cohort-based SRMAs, evidence certainty ranged from very low to
301 high, with downgrades primarily due to risk of bias, inconsistency, indirectness, imprecision,
302 and/or publication bias. Upgrades were applied for dose-response gradients (trials only) and
303 attenuation by confounders (cohorts only).

305 DISCUSSION

306 This umbrella review synthesizes evidence from SRMAs of the association between LNCS
307 and cardiometabolic health and energy intake, categorized by study methodology. Our findings
308 reveal a crucial methodological dichotomy: naïve analyses versus bias-adjusted methodologies. In
309 trial-based SRMAs, both naïve (lumping all comparators) and bias-adjusted intended substitution
310 analyses demonstrated LNCS-associated reductions in energy intake, body weight, and body fat.
311 Specifically, while naïve analyses showed benefits, these were largely driven by the intended
312 substitution context—LNCS replacing sugars and displacing calories. This underscores that
313 LNCS's benefit lies in calorie displacement, offering a useful alternative to added sugars,
314 especially in SSBs which are the largest sources of added sugars (Brisbois et al., 2014, Malik and

315 Hu, 2022). Conversely, bias-adjusted reference substitution analyses in trials without calorie
316 displacement (LNCS replacing water/placebo) showed similar results across outcomes with only
317 minimal differences, including a small important reductions in SBP that related to greater
318 reductions than water, and a trivial increase in HbA1c that related to smaller reductions than water.
319 In cohort-based SRMAs, naïve prevalent analyses, susceptible to bias, showed positive
320 associations between LNCS intake and risks of obesity, T2D, stroke, cardiovascular, and all-cause
321 mortality. However, bias-adjusted intended substitution and change analyses in cohorts, designed
322 to mitigate bias, presented a contrasting picture, aligning with trial findings by showing
323 associations between higher LNCS intake and reduced risks of obesity, lower body weight, CHD,
324 cardiovascular, and all-cause mortality.

325 Cohort studies, often analyzed using naïve prevalent methods, are inherently prone to bias
326 like reverse causality (Sievenpiper et al., 2017, Lee et al., 2020). Our review highlights this
327 vulnerability, contrasting prevalent analyses, which suggest harm, with robustly analyzed cohorts
328 employing bias-adjusted intended substitution and change analyses (Sievenpiper et al., 2017, Khan
329 et al., 2019, Khan et al., 2023, Mela et al., 2020, Malik, 2019, Hedrick et al., 2023, Higgins et al.,
330 2024). The former naïve analyses method underpinned WHO guidelines advising against LNCS
331 for weight control (World Health Organization, 2023) which failed to capture the benefit revealed
332 by bias-adjusted approach we report here. Intended substitution modeling, mirroring real-world
333 sugar replacement, aligns with trial findings, demonstrating LNCS's potential benefits when used
334 to displace sugars. Reference substitution modeling, in contrast, shows negligible effects, further
335 emphasizing the importance of calorie displacement in mediating LNCS benefits. Change
336 analyses, though less common, reinforce these beneficial findings, indicating associations between
337 changes in LNCS exposure and favorable adiposity outcomes, consistent with trial evidence.

338 *Findings in the context of the literature*

339 Our findings diverge from other umbrella reviews of relying solely on biased prevalent
340 analyses of cohort SRMAs, which report inconsistent evidence on LNCS (Diaz et al., 2023,
341 Andrade et al., 2021, Lohner et al., 2017, Abbasi, 2019, Kim et al., 2019, Green and Syn, 2019).
342 This perceived inconsistency has fueled controversy (Khan et al., 2023, Khan et al., 2024, Hedrick
343 et al., 2023). The recent WHO guideline, prioritizing biased/naïve cohort evidence, amplified this
344 controversy. This, coupled with events like International Agency for Research on Cancer (IARC)'s
345 aspartame classification (Organization, 2023b, Organization, 2023a), has negatively influenced
346 public perception of LNCS. Our review demonstrates that the apparent trial-cohort disagreement
347 disappears when considering bias-reducing methodologies in cohorts. Trial-based SRMA results,
348 already largely consistent, become even more translatable to real-world scenarios when the
349 comparator context is considered.

350 *Strengths and limitations*

351 Our analysis has several strengths. First, we included recent SRMAs, with publication dates
352 from 2022 to 2025. Second, we are the first to incorporate the WHO-commissioned SRMA that
353 informed recent WHO guidelines on LNCS (Rios-Leyvraz et al., 2022, World Health
354 Organization, 2023). Third, we effectively disentangled effects/associations by methodology type,
355 enhancing result translatability. Fourth, we rigorously assessed overall evidence certainty using
356 GRADE. Fifth, dose-response gradients were evident in trial-based SRMAs (naïve analysis,
357 fasting insulin, as assessed by (Rios-Leyvraz et al., 2022)) and in cohort-based SRMAs (bias-
358 adjusted analysis of body weight, as assessed by (Lee et al., 2022), WC, as assessed by (Lee et al.,
359 2022), obesity, as assessed by (Lee et al., 2022), naïve analysis of T2D incidence (positive
360 relationship)). Sixth, attenuation by key confounders was observed in cohort analyses (bias-

361 adjusted analysis of CHD incidence, as assessed by (Lee et al., 2022) and naïve analysis of type 2
362 diabetes incidence, where results were attenuated by most of the included cohorts adjusting for
363 BMI (as observed in supplementary table 18)).

364 Limitations include: first, study limitations (risk of bias) in 9 of 41 trial-based analyses
365 (energy intake, BMI, fasting glucose, SBP/DBP) due to some studies with high risk of bias.
366 Second, inconsistency in 23 of 41 trial-based analyses (all outcomes) and 11 of 17 cohort-based
367 analyses (body weight, WC, T2D/CHD incidence, cardiovascular/all-cause mortality) due to
368 substantial unexplained heterogeneity. Third, indirectness in 11 of 41 trial-based analyses
369 (adiposity, lipids) and 16 of 17 cohort-based analyses (all outcomes) due to generalizability
370 limitations. Fourth, imprecision in 21 of 41 trial-based analyses (energy intake, adiposity, insulin,
371 HbA1c, SBP/DBP, lipids) and 14 of 17 cohort-based analyses (WC, obesity, T2D, CHD/stroke
372 incidence, all-cause mortality) due to wide 95% CIs. Finally, publication bias was detected in 1 of
373 41 trial-based analyses (naïve analysis of body fat, as assessed by (Movahedian et al., 2024)).
374 Balancing strengths and limitations, GRADE certainty for LNCS effects/associations on
375 cardiometabolic outcomes ranged from Very low to High.

376 *Implications*

377 Recent WHO guidelines, prioritizing cohort evidence from naïve analyses, made a
378 conditional recommendation against use of LNCS for weight control and disease risk reduction,
379 citing perceived lack of long-term benefit and potential harms (World Health Organization, 2023).
380 However, WHO cohort analyses relied on naïve but biased prevalent analyses, which, as we show,
381 do indicate increased cardiometabolic disease risk. Robust analytical methods (Sievenpiper et al.,
382 2017, Khan et al., 2019, Khan et al., 2023, Mela et al., 2020, Malik, 2019, Hedrick et al., 2023,
383 Higgins et al., 2024) that are bias-adjusted reconcile trial and cohort evidence, suggesting a need

384 to reconsider the WHO's conditional recommendation. These reconciled findings, warrant
385 dissemination to scientists and the public. LNCS, when used as sugar/calorie replacements, show
386 potential benefits, particularly for adiposity management and possibly for long-term T2D/CVD
387 risk improvement. Clinical practice, public health and nutrition guidelines would benefit from
388 considering and reporting on the context of LNCS use—substitution versus addition—and the
389 methodological limitations of naïve analyses. Future primary research and SRMAs employing
390 bias-adjusted methodologies are needed, especially for under-examined outcomes like
391 postprandial glucose, non-HDL-C, ApoB, MASLD outcomes, metabolic syndrome, and MASLD
392 incidence, to further refine our understanding of LNCS and cardiometabolic health. Additionally,
393 as there was limited reporting of LNCS doses across SRMAs, future primary research studies,
394 especially of cohorts, reporting should prioritize reporting the type of LNCS used in their
395 population as well as estimated average intake doses.

396 Finally, while bias-adjusted change and substitution analyses represent a significant
397 methodological improvement over naïve prevalent analyses by mitigating reverse causality, the
398 certainty of evidence could be strengthened through advanced causal inference techniques. The
399 bias-adjusted cohort analyses do not use g-methods, which are specifically designed to handle
400 time-varying confounders affected by past exposures (Mansournia et al., 2017). Future research
401 using these more sophisticated methods could provide more robust estimates by better accounting
402 for the dynamic relationship between LNCS consumption and cardiometabolic outcome over time.

403 **CONCLUSIONS**

404 Evidence from trial-based SRMAs consistently supports LNCS use, especially in
405 substitution for sugars, to reduce energy intake and improve adiposity outcomes, without harm to
406 other intermediate cardiometabolic outcomes. Cohort-based SRMA evidence appears inconsistent

407 when considering only naïve prevalent associations, as is often the case. However, bias-adjusted
408 intended substitution and change analyses reconcile trial-cohort discrepancies. Intended
409 substitution modeling, utilizing bias-reducing methods, demonstrates LNCS's potential to lower
410 adiposity, obesity incidence, cardiovascular and all-cause mortality, with no harm observed in
411 reference substitution modeling. Based on our findings, and when considering bias-adjusted
412 evidence, LNCS are a useful strategy to reduce calories from sugars and should be promoted to
413 reduce adiposity and disease risk, when used as substitutes for caloric sweeteners.

414

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488

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646 **Table 1: Characteristics of the included SRMAs of trials**

Outcome and Methodology type	Study (year)	N ^{Studies} (direct)	N ^{Studies} (indirect) ¹	N ^{Participants} (direct)	N ^{Participants} (indirect)	Model	GRADE ²	Eligibility criteria/PICOTS Framework							
								Participants	Interventions	Control/comparators	Time	Mean LNCS dose (mg)	Mean LNCSB dose (mL)	LNCS type	SRMA funding
Energy intake (kcal)															
Lumping of all comparators	Rios-Leyvraz and Montez 2022	25	NA	2208	NA	Random	Low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A
Intended substitution	Rostampour et al. 2024	18	NA	944	NA	Random	High	Adults	Direct and indirect consumption of NNS pure or in the context of foods and beverages.	Comparison with sugar sweetener beverage, and placebo or control group	≥ 4 weeks	480 - 4000	1000 - 1750	Mixed	A
Reference substitution	Rostampour et al. 2024	11	NA	655	NA	Random	Low	Adults	Direct and indirect consumption of NNS pure or in the context of foods and beverages.	Comparison with water	≥ 4 weeks	48 - 284	178 - 454	Mixed	A
Body weight (kg)															
Lumping of all comparators	Movahedian et al. 2024	17	NA	1451	NA	Random	Low	Adults with varying health statuses	Artificial and herbal sugar substitute sweeteners	Water, sucrose, or other high-calorie sweeteners	≥ 1 week	658	NR	Mixed	None
Intended substitution	Li et al. 2023	6	NA	401	NA	Random	Very low	Adults with BMI ≥ 24 kg/m ²	Any type of NNS, either alone or in combination with another NNS	Sucrose	≥ 4 weeks	NR	NR	Mixed	NR
Reference substitution (water)	Li et al. 2023	3	NA	560	NA	Random	Low	Adults with BMI ≥ 24 kg/m ²	Any type of NNS, either alone or in combination with another NNS	Water	≥ 4 weeks	NR	NR	Mixed	NR
Reference substitution (placebo)	Li et al. 2023	3	NA	318	NA	Random	Moderate	Adults with BMI ≥ 24 kg/m ²	Any type of NNS, either alone or in combination with another NNS	Placebo	≥ 4 weeks	NR	NR	Mixed	NR
BMI (kg/m²)															
Lumping of all comparators	Movahedian et al. 2024	11	NA	980	NA	Random	Low	Adults with varying health statuses	Artificial and herbal sugar substitute sweeteners	Water, sucrose, or other high-calorie sweeteners	≥ 1 week	531	NR	Mixed	None
Intended substitution	Rios-Leyvraz and Montez 2022	16	NA	1300	NA	Random	Low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A
Reference substitution (water)	Rios-Leyvraz and Montez 2022	6	NA	499	NA	Random	Low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A

Reference substitution (nothing)	Rios-Leyvraz and Montez 2022	4	NA	189	NA	Random	Low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A	
Body fat (%)																
Lumping of all comparators	Movahedian et al. 2024	7	NA	436	NA	Random	Low	Adults with varying health statuses	Artificial and herbal sugar substitute sweeteners	Water, sucrose, or other high-calorie sweeteners	≥ 1 week	583	NR	Mixed	None	
Intended substitution	McGlynn et al. 2022	7	14	210	559	Random	Moderate	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	1039	Mixed	A+I	
Reference substitution	McGlynn et al. 2022	4	14	124	559	Random	Moderate	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	618	Mixed	A+I	
Waist circumference (cm)																
Lumping of all comparators	Movahedian et al. 2024	5	NA	680	NA	Random	Very low	Adults with varying health statuses	Artificial and herbal sugar substitute sweeteners	Water, sucrose, or other high-calorie sweeteners	≥ 1 week	408	NR	Mixed	None	
Intended substitution	McGlynn et al. 2022	0	6	0	868	Random	Low	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	NR	Mixed	A+I	
Reference substitution	McGlynn et al. 2022	5	6	628	868	Random	Low	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	716	Mixed	A+I	

Fasting plasma glucose (mmol/L)															
Lumping of all comparators	Rios-Leyvraz and Montez 2022	16	NA	1494	NA	Random	Moderate	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A
Intended substitution	McGlynn et al. 2022	7	19	210	1183	Random	Moderate	Adults, no pregnant or breastfeeding women	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	1039	Mixed	A+I
Reference substitution	McGlynn et al. 2022	9	19	748	1183	Random	High	Adults, no pregnant or breastfeeding women	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	679	Mixed	A+I
Fasting plasma insulin (pmol/L)															
Lumping of all comparators	Rios-Leyvraz and Montez 2022	10	NA	759	NA	Random	Low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A
Intended substitution	McGlynn et al. 2022	7	16	210	512	Random	Low	Adults, no pregnant or breastfeeding women	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	1039	Mixed	A+I
Reference substitution	McGlynn et al. 2022	7	16	317	512	Random	Low	Adults, no pregnant or breastfeeding women	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	503	Mixed	A+I
HbA1c (%)															

Lumping of all comparators	Rios-Leyvraz and Montez 2022	6	NA	411	NA	Random	Moderate	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A	
Intended substitution	McGlynn et al. 2022	4	9	154	630	Random	Moderate	Adults, no pregnant or breastfeeding women		SSB	≥ 2 weeks	NR	1500	Mixed	A+I	
Reference substitution	McGlynn et al. 2022	4	9	236	630	Random	Low	Adults, no pregnant or breastfeeding women		Water	≥ 2 weeks	NR	250	Mixed	A+I	
Systolic blood pressure (mmHg)																
Lumping of all comparators	Rios-Leyvraz and Montez 2022	14	NA	1440	NA	Random	Moderate	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients. LNCSB. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A	
Intended substitution	McGlynn et al. 2022	3	10	56	706	Random	Moderate	Adults, no pregnant or breastfeeding women		SSB	≥ 2 weeks	NR	885	Mixed	A+I	
Reference substitution	McGlynn et al. 2022	4	10	425	706	Random	Low	Adults, no pregnant or breastfeeding women		Water	≥ 2 weeks	NR	944	Mixed	A+I	
Diastolic blood pressure (mmHg)																
Lumping of all comparators	Rios-Leyvraz and Montez 2022	13	NA	1137	NA	Random	Moderate	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural	No or lower doses of NSS consumption (i.e., any type of sugar, placebo, plain water or no intervention)	≥ 1 week	NR	NR	Mixed	A	

									caloric sweeteners) within the ADI						
Intended substitution	McGlynn et al. 2022	3	9	56	483	Random	Low	Adults, no pregnant or breastfeeding women	LNCSSB. Studies of LNCSSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	885	Mixed	A+I
Reference substitution	McGlynn et al. 2022	3	9	202	483	Random	Low	Adults, no pregnant or breastfeeding women	LNCSSB. Studies of LNCSSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	1022	Mixed	A+I
LDL-C (mmol/L)															
Lumping of all comparators	Movahedian et al. 2023	11	NA	760	NA	Random	Moderate	Adults	Artificial and herbal sugar substitute sweeteners	NR	≥ 1 week	642	480	Mixed	None
Intended substitution	McGlynn et al. 2022	6	16	183	894	Random	Moderate	Adults, no pregnant or breastfeeding women	LNCSSB. Studies of LNCSSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	952	Mixed	A+I
Reference substitution	McGlynn et al. 2022	7	16	486	894	Random	High	Adults, no pregnant or breastfeeding women	LNCSSB. Studies of LNCSSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	510	Mixed	A+I
HDL-C (mmol/L)															
Lumping of all comparators	Movahedian et al. 2023	15	NA	1080	NA	Random	High	Adults	Artificial and herbal sugar substitute sweeteners	NR	≥ 1 week	730	480	Mixed	None

Intended substitution	McGlynn et al. 2022	7	17	210	923	Random	Moderate	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	1038	Mixed	A+I
Reference substitution	McGlynn et al. 2022	7	17	488	923	Random	Low	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	511	Mixed	A+I
Triglycerides (mmol/L)															
Lumping of all comparators	Movahedian et al. 2023	16	NA	1154	NA	Random	Moderate	Adults	Artificial and herbal sugar substitute sweeteners	NR	≥ 1 week	770	480	Mixed	None
Intended substitution	McGlynn et al. 2022	7	17	210	923	Random	Moderate	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	SSB	≥ 2 weeks	NR	1039	Mixed	A+I
Reference substitution	McGlynn et al. 2022	7	17	488	923	Random	Very low	Adults, no pregnant or breastfeeding women	LNCBS. Studies of LNCSs added to foods, fortified beverages or nutrient-dense beverages (e.g., milk, juice, etc.) were excluded due to the presence of other macronutrients.	Water	≥ 2 weeks	NR	511	Mixed	A+I

647 ¹Indirect estimates only apply to network meta-analyses

648 ²If the authors of the SRMA evaluated the certainty of their evidence using GRADE, we report here their evaluation. If they did not evaluate the certainty of their evidence using
649 GRADE, we conducted our own GRADE assessment as shown in supplemental table 4.

650 A=agency funding; ADI=acceptable daily intake; BMI=body mass index; GRADE=Grading of Recommendations Assessment, Development, and Evaluation; HbA1c=glycosylated
651 hemoglobin; HDL-C=high-density lipoprotein cholesterol; I=industry funding; LDL-C=low-density lipoprotein cholesterol; LNCSB=low- and no-calorie sweetened beverages;
652 LNCS=low- and no-calorie sweeteners; NA=not applicable; NSS=non-sugar-sweeteners; NR=not reported; PICOTS=participants, intervention, comparator, outcome, time, study
653 design; SRMA= systematic reviews and meta-analysis; SSB=sugar-sweetened beverages
654

655 **Table 2: Characteristics of the included SRMAs of cohorts**

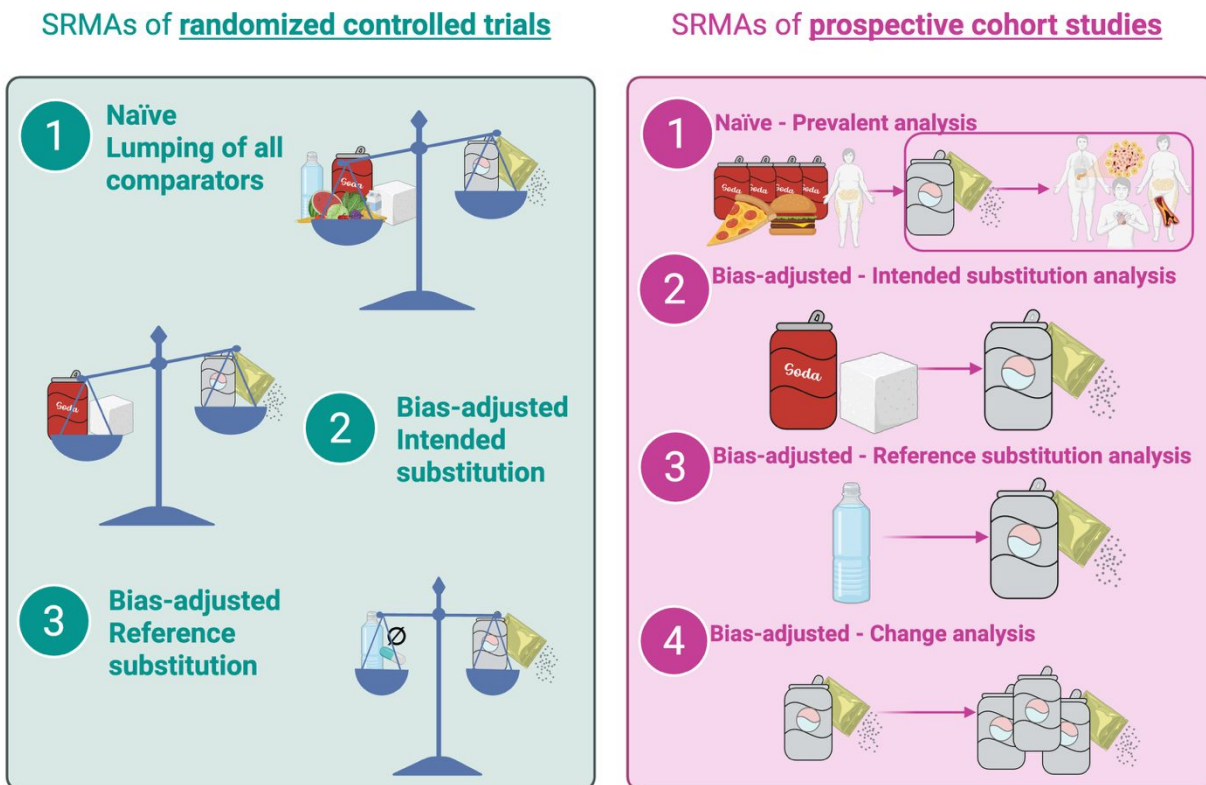
Outcome and Methodology type	Study (year)	N _{studies}	N _{participants}	Model	GRADE ¹	Eligibility criteria			Including studies that adjusted for initial adiposity	Mean LNCS dose (mg) in the lowest quantile	Mean LNCS dose (mg) in the highest quantile	Mean LNCSB dose (mL) in the lowest quantile	Mean LNCSB dose (mL) in the highest quantile	Increment change dose	LNCS type	SRMA funding
						Participants	Exposure	Time								
Body weight (kg)																
Prevalent analysis	Rios-Leyvraz and Montez 2022	4	118457	Random	Very low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI	≥ 1 year	Mixed	NR	NR	NR	NR	NA	Mixed	A
Intended substitution analysis	Lee et al. 2022	3	165579	Fixed	Moderate	Adults	LNCSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Reference substitution analysis	Lee et al. 2022	1	173	Fixed	Very low	Adults	LNCSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Change analysis	Lee et al. 2022	5	130020	Fixed	Low	Adults	LNCSB	≥ 1 year	Only	NR	NR	NR	NR	1 serving = 330mL	NR	A+I
Waist circumference (cm)																
Prevalent analysis	Rios-Leyvraz and Montez 2022	3	12886	Random	Very low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI	≥ 1 year	Mixed	NR	NR	NR	NR	NA	NR	A
Intended substitution analysis	Lee et al. 2022	1	173	Fixed	Very low	Adults	LNCSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Change analysis	Lee et al. 2022	1	9294	Fixed	Low	Adults	LNCSB	≥ 1 year	Only	NR	NR	NR	NR	1 serving = 330mL	NR	A+I
Obesity incidence																
Prevalent analysis	Rios-Leyvraz and Montez 2022	2	1668	Random	Low	Generally healthy adults	Any type of NSS (excluding sugar alcohols and natural caloric sweeteners) within the ADI	≥ 1 year	Mixed	NR	NR	NR	NR	NA	NR	A
Intended substitution analysis	Lee et al. 2022	1	15765	Fixed	Low	Adults	LNCSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Reference substitution analysis	Lee et al. 2022	1	15765	Fixed	Very low	Adults	LNCSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Type 2 diabetes incidence																
Prevalent analysis	Li et al. 2023	10	367447	Random	High	Adults	ASBs	≥ 2 years	Mixed	NR	NR	NR	NR	NA	NR	A

Intended substitution analysis	Lee et al. 2022	5	281855	Fixed	Very low	Adults	LNCBSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Reference substitution analysis	Lee et al. 2022	4	257202	Fixed	Very low	Adults	LNCBSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Change analysis	Lee et al. 2022	3	192352	Fixed	Very low	Adults	LNCBSB	≥ 1 year	Only	NR	NR	NR	NR	1 serving = 330mL	NR	A+I
CHD incidence																
Prevalent analysis (1 dose/d)	Queiroz et al. 2025	2	NR	Random	Very low	NR	ASBs, Diet soda, Diet Soft drinks, Non-sugar artificially sweetened beverages, low-calorie Soft drinks, Low-calorie artificially sweetened beverages.	NR	Mixed	NR	NR	<355mL per month	≥355mL per day	NA	NR	None
Intended substitution analysis	Lee et al. 2022	6	233676	Random	Low	Adults	LNCBSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Stroke incidence																
Prevalent analysis (1 dose/d)	Queiroz et al. 2025	4	NR	Random	Very low	NR	ASBs, Diet soda, Diet Soft drinks, Non-sugar artificially sweetened beverages, low-calorie Soft drinks, Low-calorie artificially sweetened beverages.	NR	Mixed	NR	NR	<355mL per month	≥355mL per day	NA	NR	None
Prevalent analysis (2 dose/d)	Queiroz et al. 2025	2	NR	Random	Very low	NR	ASBs, Diet soda, Diet Soft drinks, Non-sugar artificially sweetened beverages, low-calorie Soft drinks, Low-calorie artificially sweetened beverages.	NR	Mixed	NR	NR	<355mL per month	≥710mL per day	NA	NR	None
Intended substitution analysis	Lee et al. 2022	1	127456	Fixed	Very low	Adults	LNCBSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
Reference substitution analysis	Lee et al. 2022	1	127456	Fixed	Very low	Adults	LNCBSB	≥ 1 year	Only	NR	NR	NR	NR	NA	NR	A+I
CVD mortality																
Prevalent analysis (1 dose/d)	Queiroz et al. 2025	5	NR	Random	Very low	NR	ASBs, Diet soda, Diet Soft drinks, Non-sugar artificially sweetened beverages, low-calorie Soft drinks, Low-calorie artificially sweetened beverages.	NR	Mixed	NR	NR	<355mL per month	≥355mL per day	NA	NR	None
Prevalent analysis (2 dose/d)	Queiroz et al. 2025	4	NR	Random	Very low	NR	ASBs, Diet soda, Diet Soft drinks, Non-sugar artificially sweetened beverages, low-calorie Soft drinks, Low-calorie artificially sweetened beverages.	NR	Mixed	NR	NR	<355mL per month	≥710mL per day	NA	NR	None

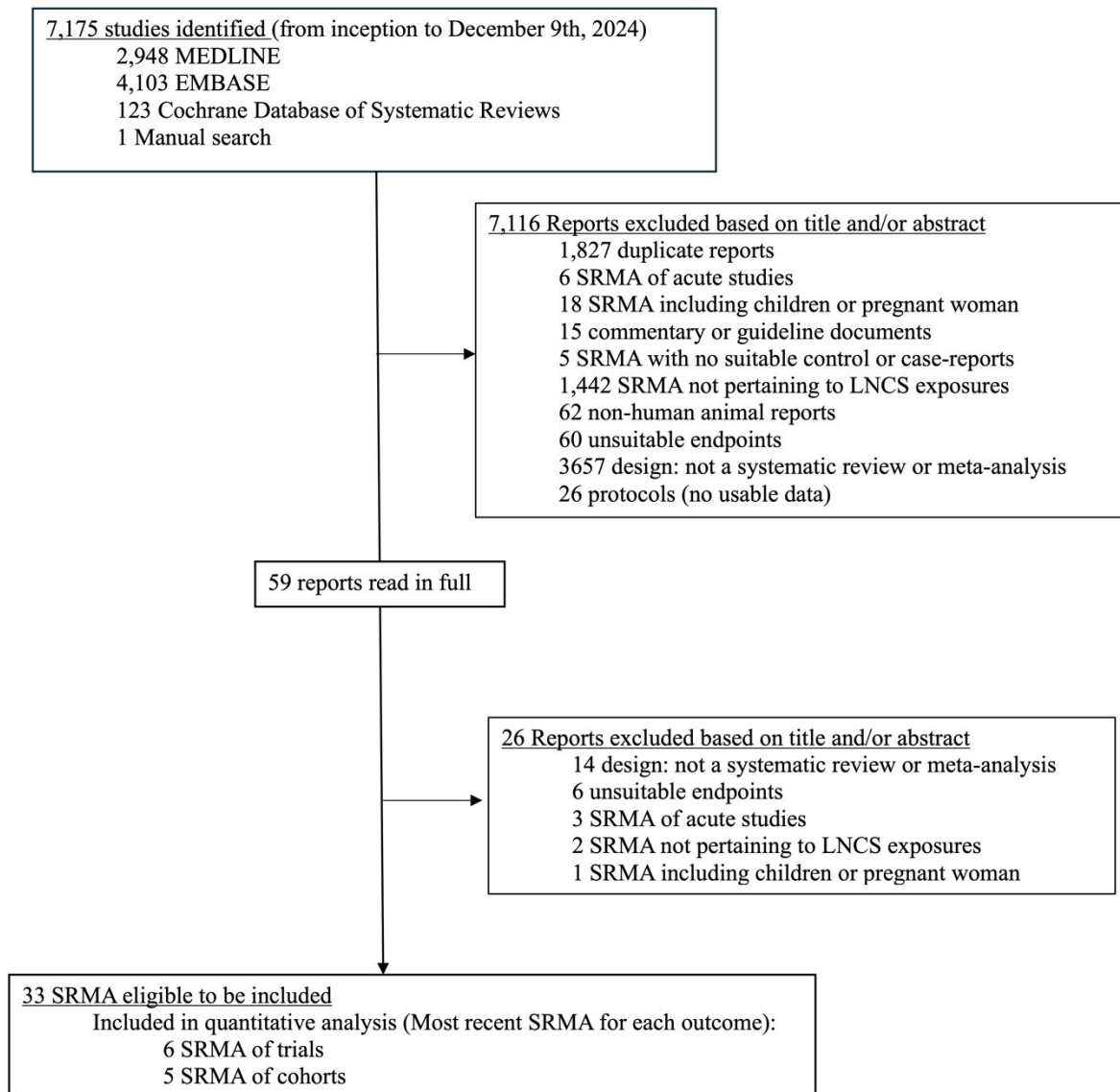
Intended substitution analysis	Chen et al. 2024	4	434110	Random	Low	Generally healthy adults	≥3 levels of ASB consumption	NR	Only	NR	NR	~0 355mL servings/d	≥2 355mL servings/d	NA	NR	A+I	
All-cause mortality																	
Prevalent analysis (1 dose/d)	Queiroz et al. 2025	6	NR	Random	Very low	NR	ASBs, Diet soda, Diet Soft drinks, Non-sugar artificially sweetened beverages, low-calorie Soft drinks, Low-calorie artificially sweetened beverages.	NR	Mixed	NR	NR	<355mL per month	≥355mL per day	NA	NR	None	
Prevalent analysis (2 dose/d)	Queiroz et al. 2025	5	NR	Random	Very low	NR	ASBs, Diet soda, Diet Soft drinks, Non-sugar artificially sweetened beverages, low-calorie Soft drinks, Low-calorie artificially sweetened beverages.	NR	Mixed	NR	NR	<355mL per month	≥710mL per day	NA	NR	None	
Intended substitution analysis	Chen et al. 2024	4	268472	Random	Very low	Generally healthy adults	≥3 levels of ASB consumption	NR	Only	NR	NR	~0 355mL servings/d	≥2 355mL servings/d	NA	NR	A+I	

656 ¹If the authors of the SRMA evaluated the certainty of their evidence using GRADE, we report here their evaluation. If they did not evaluate the certainty of their evidence using
 657 GRADE, we conducted our own GRADE assessment as shown in supplemental table 4.
 658 A=agency funding; ADI=acceptable daily intake; ASB=artificially-sweetened beverage; CHD=coronary heart disease; CVD=cardiovascular disease; GRADE=Grading of
 659 Recommendations Assessment, Development, and Evaluation; I=industry funding; LNCS=low- and no-calorie sweeteners; LNCSB= low- and no-calorie sweetened beverages;
 660 NA=not applicable; NR=not reported; NSS=non-sugar-sweeteners; SRMA= systematic reviews and meta-analysis.
 661

662 **Figure 1: Visual depiction of the different methodology types for SRMAs of trials and**
 663 **cohorts investigating LNCS**



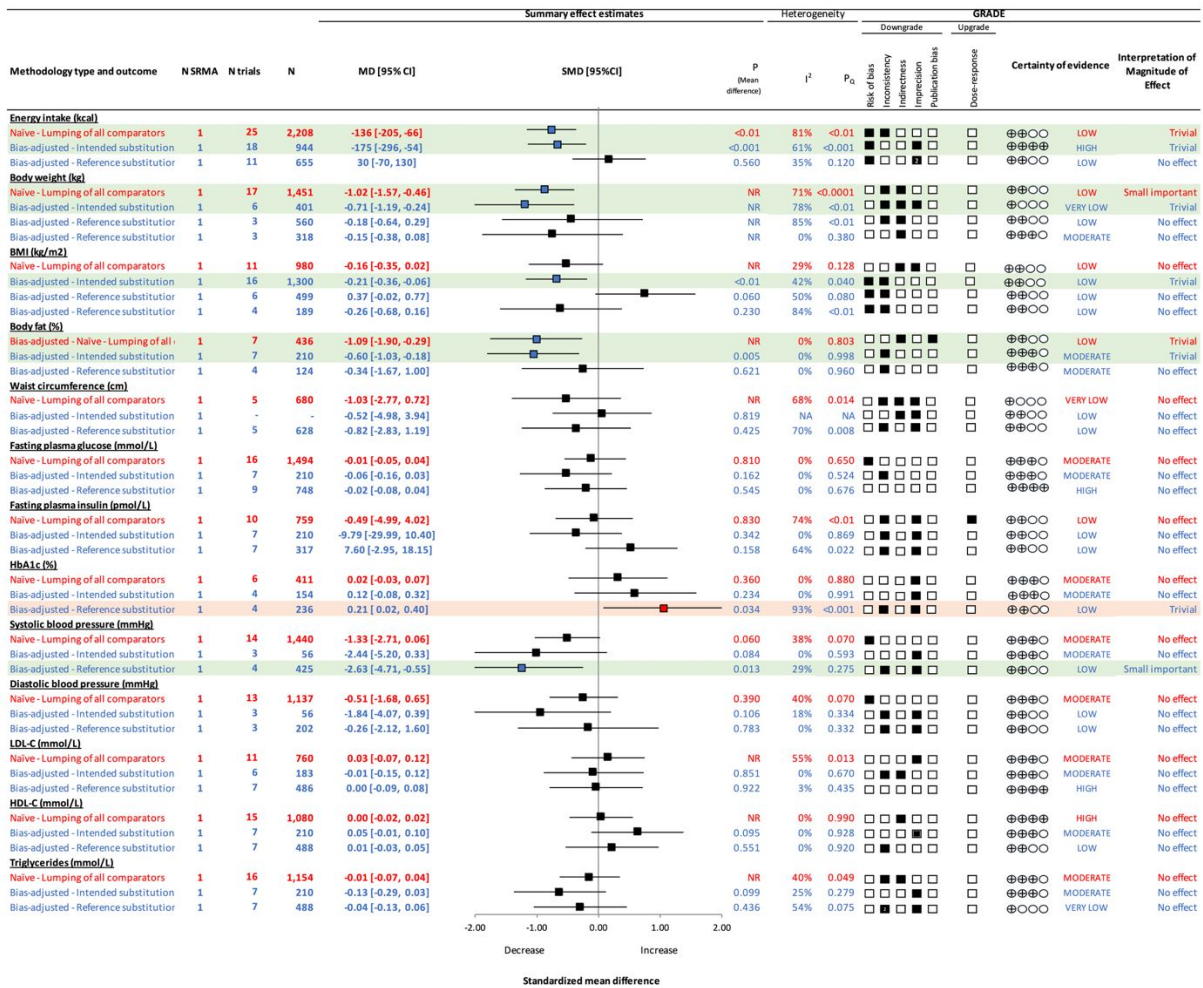
664
 665 For trials, methodology types were: (1) naïve (lumping all comparators: sugars, water/placebo, usual diet), bias-adjusted: (2)
 666 intended substitution (LNCS replacing sugars), or bias-adjusted: (3) reference substitution (LNCS replacing water, placebo or no
 667 intervention). Cohort methodology types were: (1) naïve analyses (baseline or prevalent LNCS exposure only), bias-adjusted: (2)
 668 intended substitution analyses (modeling LNCS substitution for sugars) with adjustment for baseline adiposity, bias-adjusted: (3)
 669 reference substitution analyses (modeling LNCS substitution for water/placebo) with adjustment for baseline adiposity, or bias-
 670 adjusted: (4) change analyses (modeling change in LNCS exposure over time) with adjustment for baseline adiposity.;
 671 SRMA=systematic review and meta-analysis.

672 **Figure 2: PRISMA flow**

673
 674
 675
 676

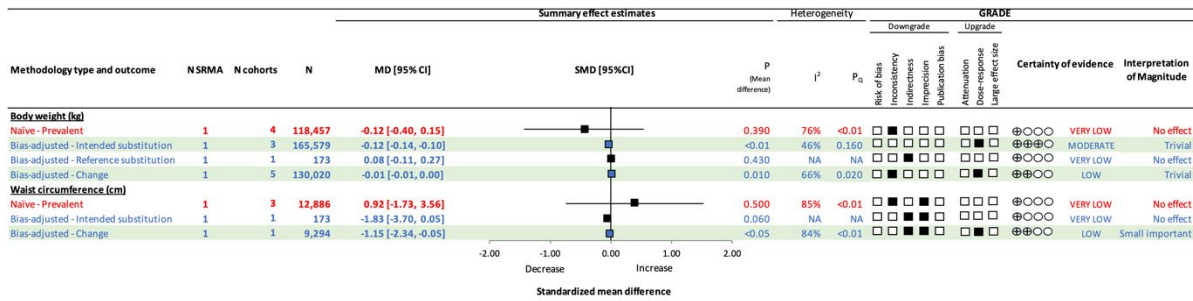
Flow of the literature for the effect/association of LNCS and energy intake, intermediate cardiometabolic outcomes, cardiometabolic disease and mortality risk. SRMA=systematic reviews and meta-analyses; LNCS=low- and no-calorie sweeteners.

677 **Figure 3: Summary plot for the effect of LNCS on included outcomes by methodology type**
 678 **in SRMAs of trials.**



679 Plotted data are standardized mean differences (95% CIs) and corresponding mean differences (95% CIs) are reported in the column
 680 to the left of the plot. Where the authors of each original SRMAs conducted a GRADE evaluation to evaluate the certainty and
 681 strength of the evidence, this evaluation was taken and summarized as shown in supplemental table 4. In red font are the naïve (prevalent) analyses, in blue
 682 font we conducted our own GRADE assessment as shown in supplemental table 4. In red font are the naïve (prevalent) analyses, in blue
 683 font are the bias-adjusted (intended and reference substitutions and change analyses) analyses. Significant results are highlighted
 684 in orange (for harm) and green (for benefit).
 685 BMI=body mass index; GRADE=Grading of Recommendations, Assessment, Development, and Evaluation; HbA1c=glycosylated
 686 hemoglobin; HDL-C= high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; MD=mean difference;
 687 NA=not applicable; NR=not reported; SMD=standardized mean differences; SRMA= systematic reviews and meta-analysis
 688
 689

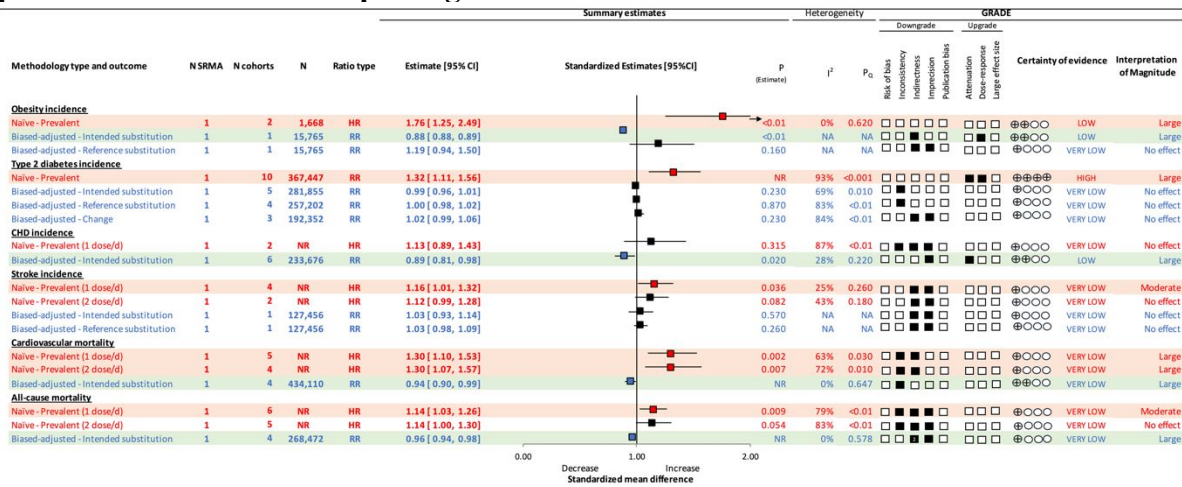
690 **Figure 4: Summary plot for the effect of LNCS on each included outcomes by methodology**
 691 **type in SRMAs of cohorts reporting mean differences.**



692 Plotted data are standardized mean differences (95% CIs) and corresponding mean differences (95% CIs) are reported in the column
 693 to the left of the plot. Where the authors of each original SRMA conducted a GRADE evaluation to evaluate the certainty and
 694 strength of the evidence, this evaluation was taken and summarized for each outcome. If no GRADE evaluation was conducted,
 695 we conducted our own GRADE assessment as shown in supplemental table 4. In red font are the naïve (prevalent) analyses, in blue
 696 font are the bias-adjusted (intended and reference substitutions and change analyses) analyses. Significant results are highlighted
 697 in orange (for harm) and green (for benefit).

698 BMI=body mass index; GRADE=Grading of Recommendations, Assessment, Development, and Evaluation; MD=mean
 699 difference; NA=not applicable; NR=not reported; SMD=standardized mean differences; SRMA= systematic reviews and meta-
 700 analysis
 701

702 **Figure 5: Summary plot for the effect of LNCS on each included outcomes by methodology**
 703 **type in SRMAs of cohorts reporting ratios.**



704 Plotted data are standardized estimates (95% CIs) and corresponding estimates (95% CIs) are reported in the column to the left of
 705 the plot. Where the authors of each original SRMA conducted a GRADE evaluation to evaluate the certainty and strength of the
 706 evidence, this evaluation was taken and summarized for each outcome. If no GRADE evaluation was conducted, we conducted our
 707 own GRADE assessment as shown in supplemental table 4. In red font are the naïve (prevalent) analyses, in blue font are the bias-
 708 adjusted (intended and reference substitutions and change analyses) analyses. Significant results are highlighted in orange (for
 709 harm) and green (for benefit).

710 CHD=coronary heart disease; GRADE=Grading of Recommendations, Assessment, Development, and Evaluation; MD=mean
 711 difference; NA=not applicable; NR=not reported; SMD=standardized mean differences; SRMA= systematic reviews and meta-
 712 analysis
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