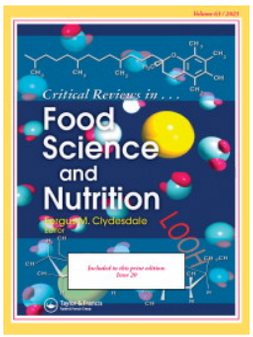

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Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Mina Movahedian, S. Amirhossein Golzan, Omid Asbaghi, Kousalya Prabahar & Azita Hekmatdoost (2023): Assessing the impact of non-nutritive sweeteners on anthropometric indices and leptin levels in adults: A GRADE-assessed systematic review, meta-analysis, and meta-regression of randomized clinical trials, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2023.2233615

To link to this article: https://doi.org/10.1080/10408398.2023.2233615

Published online: 13 Jul 2023.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2023.2233615

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REVIEW

Assessing the impact of non-nutritive sweeteners on anthropometric indices and leptin levels in adults: A GRADE-assessed systematic review, meta-analysis, and meta-regression of randomized clinical trials

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ABSTRACT

In today's world, non-nutritive sweeteners (NNSs) are recognized as substitutes for sugar or other high-calorie sweeteners, and their consumption is increasing dramatically. However, there is ongoing debate regarding the impact of NNSs on anthropometric indices. To fill this gap in knowledge, the current GRADE-assessed systematic review and meta-analysis of randomized controlled trials (RCTs) was conducted to evaluate the effects of artificial- and stevia-based sweeteners consumption on anthropometric indices and serum leptin level which is known as an appetite-regulating hormone. A comprehensive search was conducted on the Scopus, PubMed, and Embase databases up to November 2022 to identify randomized controlled trials (RCTs) investigating the effects of NNSs on anthropometric indices and serum leptin levels. Data extraction from qualified studies was performed independently by two researchers. A random- or fixed-effects model was used to estimate weighted mean differences (WMDs) and 95% confidence intervals (CIs) for anthropometric indices such as body weight (BW), body mass index (BMI), fat mass (FM), fat-free mass (FFM), waist circumference (WC) and serum leptin level. Heterogeneity between studies was assessed using Cochran's Q test and quantified using the I^2 statistic. From a pool of 3212 studies initially identified, 20 studies with a total sample size of 2158 subjects were included in the analysis. Results of the pooled analysis showed that NNSs consumption had a significant reducing effect on BW (WMD: −1.02, 95% CI: −1.57, −0.46 Kg), FM (WMD: −1.09, 95% CI: −1.90, −0.29), and FFM (WMD: −0.83, 95% CI: −1.42, −0.23), but did not have any significant effect on BMI (WMD: −0.16, 95% CI: −0.35, 0.02), WC (WMD: −1.03, 95% CI: −2.77, 0.72), or serum leptin level (WMD: −2.17, 95% CI: −4.98, 0.65). The findings of this study indicate that the consumption of artificial- and stevia-based sweeteners may lead to a reduction in body weight, fat mass, and free fat mass.

KEYWORDS

Body mass index (BMI); body weight (BW); fat free mass (FFM); fat mass (FM); leptin; non-nutritive sweeteners (NNSs); waist circumference (WC)

Introduction

The global prevalence of overweight and obesity is on the rise, and it is now considered an epidemic. Overweight and obesity are defined as abnormal or excessive accumulation of general or localized fat in the body and are considered with a body mass index (BMI) ≥25–<30 kg/m^2 and BMI ≥30 kg/m^2, respectively. These issues are recognized as two of the main public health care system concerns (Alami, Jafari, and Hosseini 2021). The prevalence of overweight or obesity increased between 1980 and 2013, impacting both adult men and women. (Alcaraz et al. 2021). In 2015, it was reported that the global prevalence of overweight and obesity was 1.9 billion and 609 million adults, respectively. (Chooi, Ding, and Magkos 2019). The multifactorial and chronic effects of overweight and obesity have economic consequences for both individuals and nations, with the most apparent direct healthcare costs being associated with the treatment of obesity-related diseases (Guh et al. 2009; Okunogbe et al. 2021). Obesity can lead to adiposity inflammation, which is pathogenically associated with chronic diseases such as cardiovascular disease and cancer (Guha et al. 2021). Moreover, obesity is commonly characterized by high levels of serum leptin, a hormone released from fat cells, which shows a strong direct relationship with body fat percentage. This peptide hormone plays a crucial role in regulating food intake, body mass, and reproductive function. Additionally, it is involved in fetal growth, pro-inflammatory immune responses, angiogenesis, and lipolysis (Obradovic et al. 2021).

The factors associated with overweight and obesity are unhealthy dietary patterns, a sedentary lifestyle,

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socioeconomic status, and urbanization (Alami, Jafari, and Hosseini 2021; Alcaraz et al. 2021). In more detail, some risk factors can contribute to energy imbalance such as dietary changes, larger portion sizes, and sedentarism (Buoncristiano et al. 2021). Furthermore, dietary surveys have shown that "empty calorie" foods, which are foods and beverages that contain added sugar but no other nutrients, are a major source of discretionary calories and contribute to energy imbalance (Alcaraz et al. 2021). It has been reported that increased intake of sugar-sweetened beverages (SSBs) is associated with a risk of overweight and obesity (Paraje and Gomes 2022; Qin et al. 2020), type 2 diabetes, hypertension, and all-cause mortality (Qin et al. 2020). Non-nutritive sweeteners (NNS) are often promoted as a healthier substitute for sugar-sweetened foods (Qin et al. 2020), due to the fact that they provide little to no calories (Tapanee et al. 2021). The Food and Drug Administration (FDA) has approved six NNS including saccharin, aspartame, acesulfame potassium, sucralose, neotame, and advantame, for use in foods and beverages (Movahedian et al. 2021). In addition, there are some natural and herbal sweeteners such as stevia which are recognized as substitutes for sugar-sweetened foods or NNS (Ajami et al. 2020).

During 2007–2008, NNS usage has been elevated from 18.7% to 24.1%, whereas the percentage of daily energy intake from added sugar decreased from 18.1% to 14.6% in these years (Tapanee et al. 2021). According to some clinical practice guidelines, NNS are suggested as an alternative of sugar-sweetened foods and beverages to reduce calorie and sugar intake and induce satiety (Gardner et al. 2012; Tapanee et al. 2021).

Some clinical trials declared that NNS or stevia consumption can help in reducing anthropometric indices such as BMI (Raben et al. 2002; Stamataki et al. 2020), body weight (BW) (Higgins and Mattes 2019; Peters et al. 2016; Raben et al. 2002; Stamataki et al. 2020), fat mass (FM) (Raben et al. 2002) and weight circumferences (WC) (Peters et al. 2016). While according to some other studies the associations between NNS or stevia with mentioned anthropometric indices are insignificant (Higgins, Considine, and Mattes 2018; Hsieh et al. 2003; Silva et al. 2006). Raben et al. study showed that an artificial sweetened diet can significantly reduce serum leptin levels (Raben et al. 2011), while other studies have found no significant association between certain artificial sweeteners and leptin (Higgins, Considine, and Mattes 2018; Romo-Romo et al. 2020).

Given the equivocal reported effects of NNS consumption on anthropometric indices and serum leptin levels, a comprehensive systematic review and meta-analysis of randomized clinical trials is needed to determine the main effects of NNS intake on these measures.

Methods

The present meta-analysis was conducted on all the RCTs examining the effects of NNSs or stevia-based sweeteners in comparison to control groups consuming water, sucrose, or other high-calorie sweeteners, on anthropometric indices and serum leptin levels in individuals with varying health statuses (such as diabetes, overweight, obesity, hypertension, hyperlipidemia, and healthy subjects). This study was conducted based on PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) protocol for reporting systematic reviews and meta-analyses (Page et al. 2021) and has been registered on the PROSPERO website (registration number: CRD42021250064).

Search strategy

A comprehensive literature search was performed in the online databases of PubMed, Scopus, and Embase, up to November 2022. The following key words were used in the search strategy ("non-nutritive sweetener" OR "rebaudioside B" OR "nonnutritive sweetener" OR "non-nutritive sweetener" OR "artificial sweetener" OR "natural sweetener" OR "low calorie sweetener" OR "low-calorie sweetener" OR "zero calorie sweetener" OR "zero-calorie sweetener" OR stevia OR advantame OR saccharin OR aspartame OR "Methyl aspartylphenylalanine" OR NutraSweet OR trichlorosucrose OR sucralose OR acetosulfame OR "acesulfame*"OR neotame OR "rebaudioside A") AND (Intervention OR "controlled trial" OR randomized OR random OR randomly OR placebo OR "clinical trial" OR Trial OR "randomized controlled trial" OR "randomized clinical trial").

The inclusion of publications was not restricted by the date and language of publication. In addition, the reference lists of relevant articles were reviewed to identify any potentially missing publications for inclusion in this study. All searched papers were included in the Endnote software for screening. Duplicate citations were then removed, and unpublished studies were omitted. Eligible studies with the following criteria were included: (1) randomized controlled clinical trials (RCTs), (2) studies involving adult individuals (age > 18 y), (3) studies with using different forms of artificial and herbal sugar substitute sweeteners, including saccharin, aspartame, acesulfame potassium, sucralose, neotame, advantame, rebaudioside A, D-Allulose, and stevia, (4) RCTs with interventions lasting for a minimum of one week, and (5) controlled trials that reported mean changes and their corresponding standard deviations (SDs) of anthropometric indices and serum leptin levels across the studies for 2 groups or provided sufficient information for calculating those effect sizes. In the case of multiple published articles on a specific dataset, the most comprehensive study was selected for inclusion in this study. Clinical trials that had an additional intervention group were treated as two separate studies. The following exclusion criteria were applied: studies with an experimental, cohort, cross-sectional, or case-control design, review articles, and ecological studies. Trials without a placebo or control group, those that were not randomized, and/or were conducted on children and adolescents were also excluded.

Data extraction

In the first pass, all the articles found by searching the mentioned keywords were screened by two researchers. Then, in

the second pass, the data extraction process for each eligible RCT was independently performed by these researchers. Additionally, any discrepancies in the selection of eligible trials or data extraction were discussed, resolved, and confirmed by a third investigator. The information extracted from each eligible RCT included the first author's name, publication year, country, individuals' demographic information (mean age, gender, and BMI), study design, the sample size for both control and intervention groups, type of sugar substitute intervention, the dosage of sweetener, type, and dosage of a placebo, duration of intervention, and mean changes with their SDs of anthropometric indices (BMI, body weight, fat mass, FFM, and waist circumference), and serum leptin levels across the trial for all groups. The most commonly used unit was adopted for analysis when anthropometric index data were reported in various units.

Quality assessment

The cochrane quality assessment tool was used to assess the risk of bias for each study in the current meta-analysis (JPT Higgins 2011). The mentioned tool consists of seven domains: (I) allocation concealment, (II) attrition bias, (III) random sequence generation, (IV) performance bias, (V) reporting bias, (VI) detection bias, and (VII) other sources of bias. If methodological flaws that could potentially impact the results of a study were identified, a "high risk" score was assigned to the corresponding domain. For domains with no defects, a "low risk" score was assigned, while an "unclear risk" score was assigned when there was insufficient information to determine the impact for that particular domain. If a study failed to consider factors such as dietary intake during the intervention, physical activity, and adjustment of baseline values in statistical analyses, it was classified as a high risk of bias in the "other sources of bias" domain. The overall risk of bias for an RCT study was determined based on the following criteria: Weak: if "low risk" was assigned to less than two domains, or (2) Good; if "low risk" was assigned to two or more domains (JPT Higgins 2011).

Statistical analysis

Mean changes and their SDs of anthropometric indices for both groups were used to obtain the overall effect sizes. If mean changes were not reported, the alterations of anthropometric indices and serum leptin level during the intervention were calculated. Also, standard errors (SEs), 95% confidence intervals (CIs), and interquartile ranges (IQRs) were converted to SDs according to the technique of Hozo, Djulbegovic, and Hozo (2005). To obtain the overall effect sizes, a random-effects model that takes between-study variations into account was applied. Moreover, heterogeneity was checked using I^2 statistics and the Cochran Q test. I^2 value > 50% or $p < .05$ for the Q-test was considered as significant between-study heterogeneity (Sadeghi et al. 2019; Zahedi et al. 2018). Subgroup analyses were conducted to identify potential sources of heterogeneity. Predefined variables such as intervention type (steviol glycoside *vs.* stevioside *vs.* aspartame *vs.* rebaudioside A *vs.* advantame *vs.* unknown NNS or combined type), intervention source (artificial or natural), intervention duration (≥24 or <24 wk), intervention dosage (≥710 or <710 mg/d) for different sweeteners, and baseline BMI (kg/m^2) categories (normal: 18.5–24.9, overweight: 25–29.9, or obese: >30) were used for subgroup analyses. The sensitivity analysis was conducted to detect whether the overall effect size was dependent on a particular study. The presence of publication bias was assessed using formal tests of Begg and Egger as well as visual inspection of funnel plots. The current meta-analysis was performed using version 14 of STATA, and a *p-value* < .05 was considered statistically significant.

Certainty assessment

The overall certainty of evidence across the trials was evaluated according to the guidelines of the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) working group (gradeworkinggroup.org), and classified into four categories based on the corresponding evaluation criteria: high, moderate, low, and very low (Guyatt et al. 2008).

Results

A total of 3212 publications were initially identified through our primary search, of which 1243 were found to be duplicates and were removed. Following the screening of the remaining 1969 records based on title and abstract, 1178 irrelevant articles were excluded. Additionally, 518 animal studies, 150 review papers, and 29 conference papers were removed. The full text of the remaining 94 articles was evaluated, of which 59 were excluded due to being irrelevant or lacking sufficient data. Seven articles could not be located, and two articles were excluded due to being written in a non-English language (Dávila et al. 2017). In addition, one study excluded because NNS was used in the placebo group (Han et al. 2018), and also one study was excluded due to a lack of reported data at the end of the study (Koyuncu and Balci 2014). Two eligible articles were found to be published on the same dataset (Raben et al. 2002; Sørensen et al. 2005), and therefore, data was extracted from only one of them (Raben et al. 2002). There was also another duplicate dataset for two articles (Peters et al. 2014, 2016), of which the most comprehensive one was included (Peters et al. 2016) in the current meta-analysis. Additionally, the study conducted by Maersk et al. was excluded from the meta-analysis due to reported data on anthropometric indices in relative changes, which could not be converted to absolute changes (Maersk et al. 2012). Furthermore, the study conducted by Ferri et al. was omitted from the meta-analysis due to the non-constancy of intervention doses during the study (Ferri et al. 2006).

Finally, a total of twenty eligible publications were included in the current systematic review and meta-analysis (Al-Dujaili et al. 2017; Barriocanal et al. 2008; Bonnet et al. 2018; Bueno-Hernández et al. 2020; Campos et al. 2015;

Chan et al. 2000; Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Hsieh et al. 2003; Leon et al. 1989; Madjd et al. 2017; Maki et al. 2008; Peters et al. 2016; Raben et al. 2011; Raben et al. 2002; Romo-Romo et al. 2020; Silva et al. 2006; Stamataki et al. 2020; Stern et al. 1976; Tate et al. 2012). The study selection flow diagram is shown in Figure 1.

Study characteristics

The characteristics of the included RCTs in the current systematic review and meta-analysis are shown in Table 1. These RCTs were published between 1976 and 2020 and originated from various regions, including North America (Bueno-Hernández et al. 2020; Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Leon et al. 1989; Maki et al. 2008; Peters et al. 2016; Romo-Romo et al. 2020; Stern et al. 1976; Tate et al. 2012), South America (Barriocanal et al. 2008; Silva et al. 2006), Europe (Al-Dujaili et al. 2017; Bonnet et al. 2018; Campos et al. 2015; Raben et al. 2011; Raben et al. 2002; Stamataki et al. 2020), and Asia (Chan et al. 2000; Hsieh et al. 2003; Madjd et al. 2017). Only one study was exclusively conducted on female individuals (Madjd et al. 2017), and others were on both genders. The sample sizes of the RCTs included ranged from 16 to 303 participants, resulting in a total sample size of 2158 subjects. The mean age of participants was between 20 and 70 years old. The dosage of sugar alternative sweetener varied between 48 and 1800 mg/d,and the duration of the intervention varied from 1 to 104 wk across selected RCTs. With the exception of two studies that used a crossover design (Al-Dujaili et al. 2017; Bonnet et al. 2018), all other studies employed a parallel design. Concerning the type of sweetener, eight studies prescribed Steviol glycoside, Stevioside or rebaudioside A for intervention group (Al-Dujaili et al. 2017; Barriocanal et al. 2008; Chan et al. 2000; Higgins and Mattes 2019; Hsieh et al. 2003; Maki et al. 2008; Silva et al. 2006; Stamataki et al. 2020), four studies administered Aspartame (Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Leon et al. 1989; Stern et al. 1976), three studies used sucralose for the intervention group (Bueno-Hernández et al. 2020; Higgins and Mattes 2019; Romo-Romo et al. 2020), with one of them additionally utilizing maltodextrine and dextrose along with sucralose (Romo-Romo et al. 2020). Only one study utilized saccharine during the intervention (Higgins and Mattes 2019). In addition, three other studies performed the intervention using a combination of different types of NNS; two

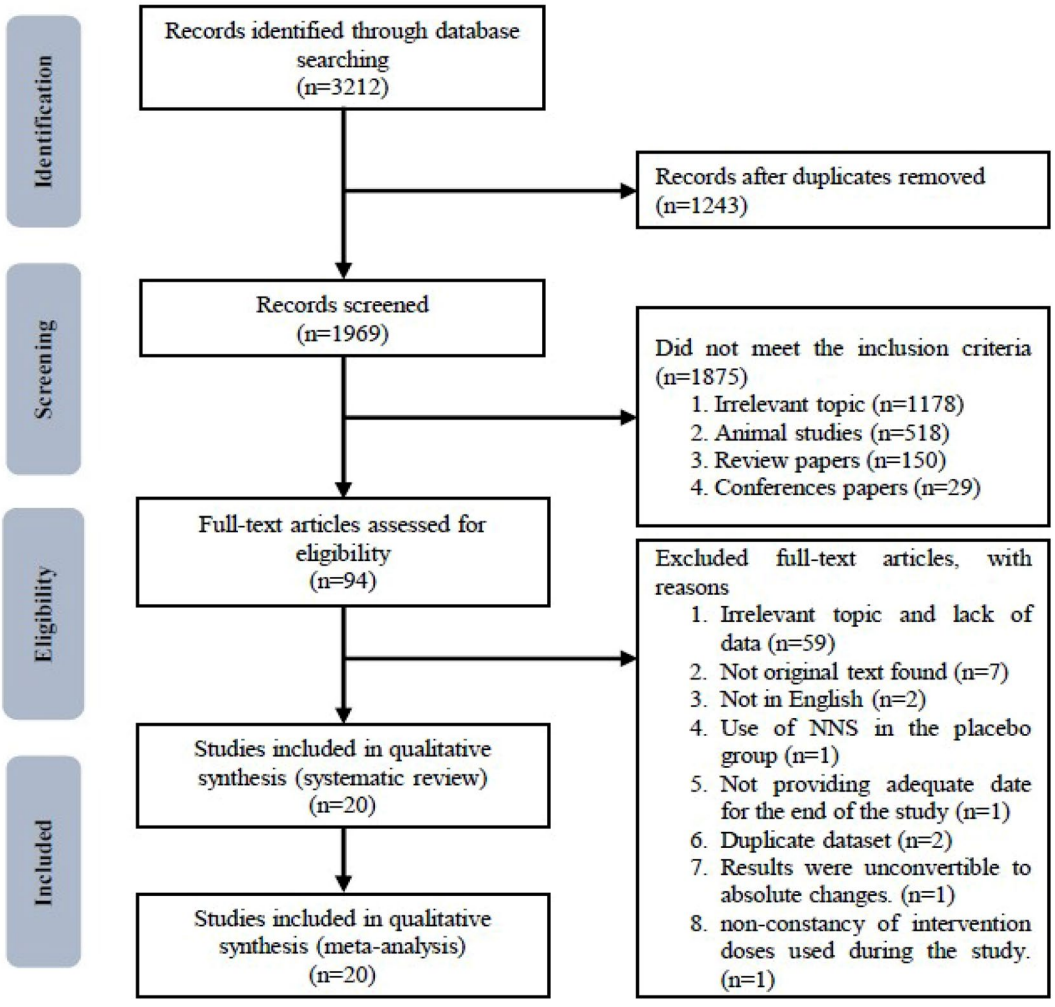

Figure 1. Flow diagram of study selection.

Table 1. Characteristic of included studies in meta-analysis.

Studies	Country	Study design	Participant	Sample size and sex	Sample size		Trial duration (Week)	Means age		Means BMI		Intervention		
					IG	CG		IG	CG	IG	CG	Intervention type	Intervention dose	Control group
Stern et al. (1976)	USA	Pa/Ra/Db/Pc	T2DM	M/F	33	29	13	21–70	21–70	NR	NR	Aspartame (capsule)	1800 mg/d	NR
Leon et al. (1989)	USA	Pa/Ra/Db/Pc	Healthy, paid volunteers	M/F (F:57, M:51)	53	55	24	31.4 ± 9.46	29.5 ± 10.38	NR	NR	Aspartame (capsule)	900 Mg/d	Placebo (300 mg microcrystalline & 0.9 mg silicon dioxide)
Chan et al. (2000)	China	Pa/Ra/Db/Pc	Hypertensive subjects	M/F (F:50, M:50)	56	44	52	54.1 ± 3.8	53.7 ± 4.1	23.2 ± 2.5	23.6 ± 2.8	Stevioside (capsule)	750 mg/d	NR
Raben et al. (2002)	Denmark	Pa/Ra	Overweight	M/F (F:35, M:6)	20	21	10	37.1 ± 9.83	33.3 ± 9.16	27.6 ± 2.23	28.0 ± 2.29	Artificial sweeteners (beverage & food) (54% Aspartame, 22% Acesulfame K, 23% Cyclamate & 1% Saccharin)	0.48, 0.57, or 0.67 G/D on Levels 1, 2, and 3, respectively (Level 1, 60–75 kg; Level 2, 75–90 kg; and Level 3, > 90 kg)	Sucrose
Hsieh et al. (2003)	China	Pa/Ra/Db/Pc	Stage 1 hypertension	M/F (F:84, M:84)	82	86	104	51 ± 6	52 ± 8	22.9 ± 2.6	23.8 ± 2.6	Stevioside (capsule)	1500 mg/d	Placebo
Silva et al. (2006)	Brazil	Pa/Ra/Db/Pc	Newly dignosed & untreated hyperlipidemia	M/F	23	20	13	20–70	20–70	25.78 ± 4.74	27.9 ± 4.02	Stevioside (capsule)	200 mg/d	Talcum
Barriocanal et al. (2008) **(A)**	Paraguay	Pa/Ra/Db/Pc	T1dm	M/F (Nr)	8	8	13	20–60	20–60	23.2	22.4	Steviol glycosides (capsule)	750 mg/d	NR
Barriocanal et al. (2008) **(B)**	Paraguay	Pa/Ra/Db/Pc	T2dm	M/F (Nr)	15	15	13	40–70	40–70	28.7	30.1	Steviol glycosides (capsule)	750 mg/d	NR
Barriocanal et al. (2008) **(C)**	Paraguay	Pa/Ra/Db/Pc	Healthy	M/F (Nr)	13	17	13	20–60	20–60	22.9	24.4	Steviol glycosides (capsule)	750 mg/d	NR
Maki et al. (2008)	USA	Pa/Ra/Db/Pc	T2dm	M/F (F:60, M:62)	60	62	16	59.1 ± 9.29	61.5 ± 8.66	33.7 ± 4.64	33.6 ± 4.72	Rebaudioside A) (capsule)	1000 mg/d	Placebo (Microcrystalline cellulose)
Raben et al. (2011)	Denmark	Pa/Ra	Overweight, healthy, not dieting, and for women not pregnant or lactating.	M/F (F:19, M:4)	11	12	10	35.5 ± 11.93	35.3 ± 9.69	27.6 ± 2.65	28.7 ± 2.42	Artificial sweeteners (beverage & food) (54% Aspartame, 22% Acesulfame K, 23% Cyclamate & 1% Saccharin)	Nr	Sucrose
Tate et al. (2012)	USA	Pa/Ra/Sb	Overweight and obese	M/F (F:178, M:35)	105	108	24	41.20 ± 11.2	43.2 ± 10.6	36.1 ± 6.2	35.8 + 5.2	Diet bevrege	Varying	Water
Campos et al. (2015)	Switzerland	Pa/Ra	Healthy subjects with BMI greater than 25 kg/m^2	M/F (F:13, M: 14)	14	13	12	Nr	Nr	31.5 ± 4.48	30.6 ± 5.40	Artificially Sweetened beverages	Nr	Sugar sweetened beverages (SSB)
Peters et al. (2016) **(A)**	USA	Pa/Ra	Weight-stable people with overweight and obesity	M/F (F:255, M:53)	154	149	52	48.3 ± 10.4	47.3 ± 10.6	33.92 ± 4.25	33.30 ± 3.98	Nns (beverage)	710 ml/d	Water
Peters et al. (2016) **(B)**	USA	Pa/Ra	Weight-stable people with overweight and obesity	M/F (F:255, M:53)	115	107	52	48.3 ± 10.4	47.3 ± 10.6	33.92 ± 4.25	33.30 ± 3.98	Nns (beverage)	710 ml/d	Water
Al-Dujaili et al. (2017)	Scotland	Cr/Ra/Pc	Healthy, non-smokers, not diabetics and not on medications that lower BP.	M/F (F:8, M:8)	8	8	1	27.75 ± 13.75	27.75 ± 13.75	26.33 ± 5.26	26.33 ± 5.26	Stevia (powder)	600 mg/d	Sucrose
Madjd et al. (2017)	Iran	Pa/Ra/Sb	T2dm	F (F:81)	40	41	24	35.45 ± 7.45	34.15 ± 6.99	33.19 ± 2.25	32.86 ± 1.67	Nns (diet beverage)	250 ml once daily after lunch (main meal) 5 times a week	Water
Bonnet et al. (2018)	France	Cr/Ra/Db/Pc	Normal and overweight	M/F (F:28, M:22)	25	25	12	31.1 ± 10.3	31.1 ± 10.3	24.7 ± 3.2	24.7 ± 3.2	Aspartame + Acesulfame K (129 mg Aspartame and 13 mg Acesulfame K)	660 ml/d	Carbonated beverages

(*Continued*)

Table 1. Continued.

Studies	Country	Study design	Participant	Sample size and sex	Sample size		Trial duration (Week)	Means age		Means BMI		Intervention		
					IG	CG		IG	CG	IG	CG	Intervention type	Intervention dose	Control group
Higgins, Considine, and Mattes (2018) **(A)**	USA	Pa/Ra/Pc	Healthy	M/F (F:32, M:30)	31	15	12	22.8 ± 1.0	24.2 ± 1.3	22.3 ± 0.3	21.7 ± 0.3	Aspartame (capsule)	350 mg/d	Dextrose + PABA
Higgins, Considine, and Mattes (2018) **(B)**	USA	Pa/Ra/Pc	Healthy	M/F (F:36, M:26)	31	16	12	21.8 ± 0.6	24.2 ± 1.3	22.1 ± 0.3	21.7 ± 0.3	Aspartame (capsule)	1050 mg/d	Dextrose + PABA
Higgins and Mattes (2019) (A)	USA	Pa/Ra/Sb/Pc	Overweight/obese adult	M/F (F:38, M:30)	29	10	12	25.8 ± 6.9	28.2 ± 9.5	28.8 ± 4.4	30.4 ± 4.1	Saccharin (beverage)	730 mg/d	Sucrose
Higgins and Mattes (2019) (B)	USA	Pa/Ra/Sb/Pc	Overweight/obese adult	M/F (F:36, M:33)	30	10	12	29.5 ± 12.0	28.2 ± 9.5	30.3 ± 5.3	30.4 ± 4.1	Aspartame (beverage)	580 mg/d	Sucrose
Higgins and Mattes (2019) (C)	USA	Pa/Ra/Sb/Pc	Overweight/obese adult	M/F (F:49, M:18)	28	10	12	27.1 ± 9.6	28.2 ± 9.5	29.9 ± 3.8	30.4 ± 4.1	Rebaudioside A (beverage)	660 mg/d	Sucrose
Higgins and Mattes (2019) (D)	USA	Pa/Ra/Sb/Pc	Overweight/obese adult	M/F (F:37, M:30)	28	9	12	25.9 ± 9.0	28.2 ± 9.5	28.7 ± 4.0	30.4 ± 4.1	Sucralose	160 mg/d	Sucrose
Romo-Romo et al. (2020)	Mexico	Pa/Ra	Healthy	M/F	30	8	2	23 ± 2.96	23 ± 2.22	21.5 ± 1.7	21.8 ± 1.7	Sucralose (Sachet- 12 mg Sucralose, 30 mg Maltodextrin and 958 mg Dextrose)	0.123 ± 0.0129 g/d (Women)/0.1577 ± 0.0129 g/d (Men)	Control (followed the same procedures without receiving any intervention)
Bueno-Hernández et al. (2020) **(A)**	Mexico	Pa/Ra/Db/Pc	Healthy	M/F (F:40, M:24)	30	17	10	22.9 ± 3.5	22 ± 3.2	24.1 ± 2.9	24.2 ± 3.8	Sucralose	48 mg/d	Water
Bueno-Hernández et al. (2020) **(B)**	Mexico	Pa/Ra/Db/Pc	Healthy	M/F (F:38, M:27)	31	17	10	22.6 ± 2.8	22 ± 3.2	–	–	Sucralose	96 mg/d	Water
Stamataki et al. (2020)	UK	Pa/Ra	Healthy with normal BMI	M/F (F:22, M:6)	14	14	12	25 ± 6	25 ± 4	–	–	Stevia (drop)	5 drops twice a day	Usual diet

IG: intervention group; CG: control group; DB: double-blinded; SB: single-blinded; PC: placebo-controlled; CO: controlled; RA: randomized; NR: not reported; F: Female; M: Male.

of these studies (16, 22) administered a combined NNS containing 54% aspartame, 22% acesulfame K, 23% cyclamate, and 1% saccharin. The third study used a mixture of aspartame and acesulfame K for the intervention group (Bonnet et al. 2018). Furthermore, four studies did not report the type of artificial sweetener used in the intervention. (Campos et al. 2015; Madjd et al. 2017; Peters et al. 2016; Tate et al. 2012). There are four studies that used water as the control intervention (Bueno-Hernández et al. 2020; Madjd et al. 2017; Peters et al. 2016; Tate et al. 2012). Additionally, eight studies utilized a placebo, where some used sucrose as the placebo (Al-Dujaili et al. 2017; Higgins and Mattes 2019; Raben et al. 2011; Raben et al. 2002), while others used non-absorbable compounds such as Talcum or Silicon dioxide (Hsieh et al. 2003; Leon et al. 1989; Maki et al. 2008; Silva et al. 2006). Furthermore, four studies employed other nutritive sweeteners or no intervention as the control intervention (Bonnet et al. 2018; Campos et al. 2015; Higgins, Considine, and Mattes 2018; Romo-Romo et al. 2020), and four studies did not report the intervention used in the control group (Barriocanal et al. 2008; Chan et al. 2000; Stamataki et al. 2020; Stern et al. 1976).

The RCTs were conducted on a range of populations, including healthy individuals (Al-Dujaili et al. 2017; Barriocanal et al. 2008; Bonnet et al. 2018; Bueno-Hernández et al. 2020; Higgins, Considine, and Mattes 2018; Leon et al. 1989; Romo-Romo et al. 2020; Stamataki et al. 2020), patients with type 1 or type 2 diabetes (Barriocanal et al. 2008; Madjd et al. 2017; Maki et al. 2008; Stern et al. 1976), hypertensive patients (Chan et al. 2000; Hsieh et al. 2003), overweight or obese subjects (Bonnet et al. 2018; Campos et al. 2015; Higgins and Mattes 2019; Peters et al. 2016; Raben et al. 2011; Raben et al. 2002; Tate et al. 2012), and patients with hyperlipidemia (Silva et al. 2006). Using the Cochrane Collaboration's tools for assessing study quality, 19 of the included RCTs were deemed to be of "good quality" (Al-Dujaili et al. 2017; Barriocanal et al. 2008; Bonnet et al. 2018; Bueno-Hernández et al. 2020; Chan et al. 2000; Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Hsieh et al. 2003; Leon et al. 1989; Madjd et al. 2017; Maki et al. 2008; Peters et al. 2016; Raben et al. 2011; Raben et al. 2002; Romo-Romo et al. 2020; Silva et al. 2006; Stamataki et al. 2020; Stern et al. 1976; Tate et al. 2012), while only one RCT was considered to be of "weak quality" (Campos et al. 2015) (see Table 2).

Findings from the systematic review

Of the 13 RCTs that evaluated the effects of NNS and stevia-based sweeteners on BW (Al-Dujaili et al. 2017; Bonnet et al. 2018; Campos et al. 2015; Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Leon et al. 1989; Madjd et al. 2017; Maki et al. 2008; Peters et al. 2016; Raben et al. 2002; Stamataki et al. 2020; Stern et al. 1976; Tate et al. 2012), three studies reported a significant reduction in BW following intervention with NNS and stevia compared to the placebo group (Peters et al. 2016; Raben et al. 2002;

Table 2. Quality assessment.

studies	Random sequence generation	Allocation concealment	Selective reporting	Other sources of bias	Blinding (participants and personnel)	Blinding (outcome assessment)	Incomplete outcome data
Stern et al. (1976)	L	U	L	H	L	L	H
Leon et al. (1989)	L	L	L	U	L	L	L
Chan et al. (2000)	L	U	L	H	L	L	L
Raben et al. (2002)	L	U	L	L	H	H	L
Hsieh et al. (2003)	L	L	L	L	L	L	L
Silva et al. (2006)	L	L	L	L	L	L	L
Barriocanal et al. (2008)	L	U	L	H	L	L	L
Maki et al. (2008)	L	U	L	H	L	L	L
Raben et al. (2011)	L	U	L	L	H	H	L
Tate et al. (2012)	L	L	L	L	H	H	L
Campos et al. (2015)	U	U	L	H	U	U	H
Peters et al. (2016)	L	L	L	L	H	H	H
Al-Dujaili et al. (2017)	L	U	L	L	U	U	L
Madjd et al. (2017)	L	L	L	L	H	H	L
Bonnet et al. (2018)	L	U	L	L	L	L	L
Higgins, Considine, and Mattes (2018)	L	L	L	H	U	U	H
Higgins and Mattes (2019)	L	L	L	L	H	H	H
Romo-Romo et al. (2020)	L	L	L	L	H	H	H
Bueno-Hernández et al. (2020)	L	L	L	L	L	L	L
Stamataki et al. (2020)	L	L	L	L	H	H	L

Stamataki et al. 2020). In the study by Higgins et al. it was found that three out of four intervention groups had a significant reduction in BW after consuming aspartame, rebaudioside A, and sucralose compared to the placebo group (sucrose). However, in the fourth intervention arm, there was no significant difference observed between the saccharin group and the placebo (sucrose) group (Higgins and Mattes 2019). Furthermore, Madjd et al. study found that NNS intervention resulted in a significant increase in BW compared to the control group with water intervention (Madjd et al. 2017). However, other studies found no significant effect on BW.

Out of the 11 RCTs that explored the effects of NNS or stevia-based sweeteners on BMI (Al-Dujaili et al. 2017; Barriocanal et al. 2008; Bueno-Hernández et al. 2020; Campos et al. 2015; Chan et al. 2000; Higgins and Mattes 2019; Hsieh et al. 2003; Madjd et al. 2017; Raben et al. 2002; Silva et al. 2006; Stamataki et al. 2020), only two studies revealed a significant reduction in BMI following NNS or stevia intervention compared to placebo (Raben et al. 2002; Stamataki et al. 2020), while the others found no significant effect.

Among 3 RCTs surveying the effects of NNS or stevia-based sweetener on FM changes (Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Raben et al. 2002), one study demonstrated a significant reduction in FM after NNS intervention compared with placebo (Raben et al. 2002). In Higgins et al. study, the intervention group with sucralose showed a decreasing effect in FM compared with the placebo (sucrose) group, while there was no significant effect of NNS on FM in the other groups (saccharine, aspartame, and rebaudioside A) (Higgins and Mattes 2019). In addition, no significant effect of NNS (aspartame) on FM was reported compared with the placebo group in the other study (Higgins, Considine, and Mattes 2018).

Among 3 RCTs investigating the effects of NNS or stevia-based sweetener on FFM changes (Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Raben et al. 2002), one study reported a significant reduction in FFM in comparison with placebo (Raben et al. 2002). Moreover, based on Higgins et al. study, intervention with sucrose, saccharine or aspartame caused a significant increase in FFM at the end of the study. According to this study, consumption of sucrose led to a significantly greater amount of FFM compared with sucralose and rebaudioside A groups, whereas this incremental effect was not significant compared with saccharine and aspartame groups (Higgins and Mattes 2019). Furthermore, another study reported no significant effect of NNS intervention (aspartame) on FFM compared to placebo (Higgins, Considine, and Mattes 2018).

Among 5 RCTs assessing the effects of NNS and stevia-based sweeteners on WC (Bonnet et al. 2018; Madjd et al. 2017; Peters et al. 2016; Stamataki et al. 2020; Tate et al. 2012), only one study showed a significant decrease in WC with NNS consumption compared to placebo (Peters et al. 2016), while the other studies did not find any significant effect on WC.

Among 3 RCTs surveying the effects of NNS on serum leptin level (Higgins, Considine, and Mattes 2018; Raben et al. 2011; Romo-Romo et al. 2020), only one study demonstrated a significant lower serum leptin level after NNS intervention compared to placebo (Raben et al. 2011), whereas the other studies did not report a significant effect.

Findings from the meta-analysis of the effect of artificial- and stevia-based sweeteners on body weight

In total, 13 RCTs with a total sample size of 1451 subjects were included in the analysis (Al-Dujaili et al. 2017; Bonnet et al. 2018; Campos et al. 2015; Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Leon et al. 1989; Madjd et al. 2017; Maki et al. 2008; Peters et al. 2016; Raben et al. 2002; Stamataki et al. 2020; Stern et al. 1976; Tate et al. 2012). Combining 17 effect sizes from these studies indicated that the intervention with artificial and stevia-based sweeteners,

compared to controls, resulted in a significant reduction in body weight [weighted mean difference (WMD): −1.02, 95% CI: −1.57, −0.46 kg] (Figure 2). However, there was evidence of high between-study heterogeneity (I^2 = 71.2%, p < .001) (Figure 2). The results of subgroup analyses are shown in Table 3.

Findings from the meta-analysis of the effect of artificial- and stevia-based sweeteners on BMI

In total, 11 RCTs investigating the effects of artificial- and stevia-based sweeteners on BMI with a total sample size of 980 participants were included in the current meta-analysis (Al-Dujaili et al. 2017; Barriocanal et al. 2008; Bueno-Hernández et al. 2020; Campos et al. 2015; Chan et al. 2000; Higgins and Mattes 2019; Hsieh et al. 2003; Madjd et al. 2017; Raben et al. 2002; Silva et al. 2006; Stamataki et al. 2020). Combining 17 effect sizes from these articles showed that there was no significant difference in BMI changes between the artificial sweeteners or stevia groups and the control group [WMD: −0.16, 95% CI: −0.35, 0.02] (Figure 3). The evidence indicated that there was low heterogeneity between the studies (I^2 = 28.9%, p = .128) (Figure 3).

Findings from the meta-analysis of the effect of artificial- and stevia-based sweeteners on FM

A total of 3 RCTs investigating the effects of NNS or stevia-based sweetener on FM changes with 436 subjects were included in the analysis(Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Raben et al. 2002). Combining 7 effect sizes from eligible studies showed a significant reduction in FM after intervention with NNS or stevia-based sweeteners groups compared to placebo groups [WMD: −1.09, 95% CI: −1.90, −0.29] (Figure 4). In addition, the evidence demonstrated a low between-study heterogeneity (I^2 = 0.0%, p = .803) (Figure 4).

Findings from the meta-analysis of the effect of artificial- and stevia-based sweeteners on FFM

Totally 3 RCTs investigated the effects of NNS or stevia-based sweetener on FFM changes with 436 individuals were analyzed (Higgins, Considine, and Mattes 2018; Higgins and Mattes 2019; Raben et al. 2002). After a combination of 7 effect sizes from eligible studies, with NNS or stevia-based sweetener intervention a significant reduction in FFM was seen in comparison with controls [WMD: −0.83, 95% CI: −1.42, −0.23] (Figure 5).

Findings from the meta-analysis of the effect of artificial- and stevia-based sweeteners on waist circumference

In total, 5 RCTs assessing the effects of NNS or stevia-based sweetener on WC changes with 680 subjects were included in the current analysis (Bonnet et al. 2018; Madjd et al. 2017; Peters et al. 2016; Stamataki et al. 2020; Tate et al. 2012). Combining 5 effect sizes from these articles showed no significant difference in WC changes between artificial sweeteners or stevia and control groups [WMD: −1.03, 95% CI: −2.77, 0.72] (Figure 6). There was evidence of high between-study heterogeneity (I^2 = 68.0%, p = .014) (Figure 6). The results of subgroup analyses are available in Table 3.

Findings from the meta-analysis of the effect of artificial- and stevia-based sweeteners on serum leptin levels

In total, 3 RCTs investigating the effects of NNS or stevia-based sweetener on serum leptin level changes with

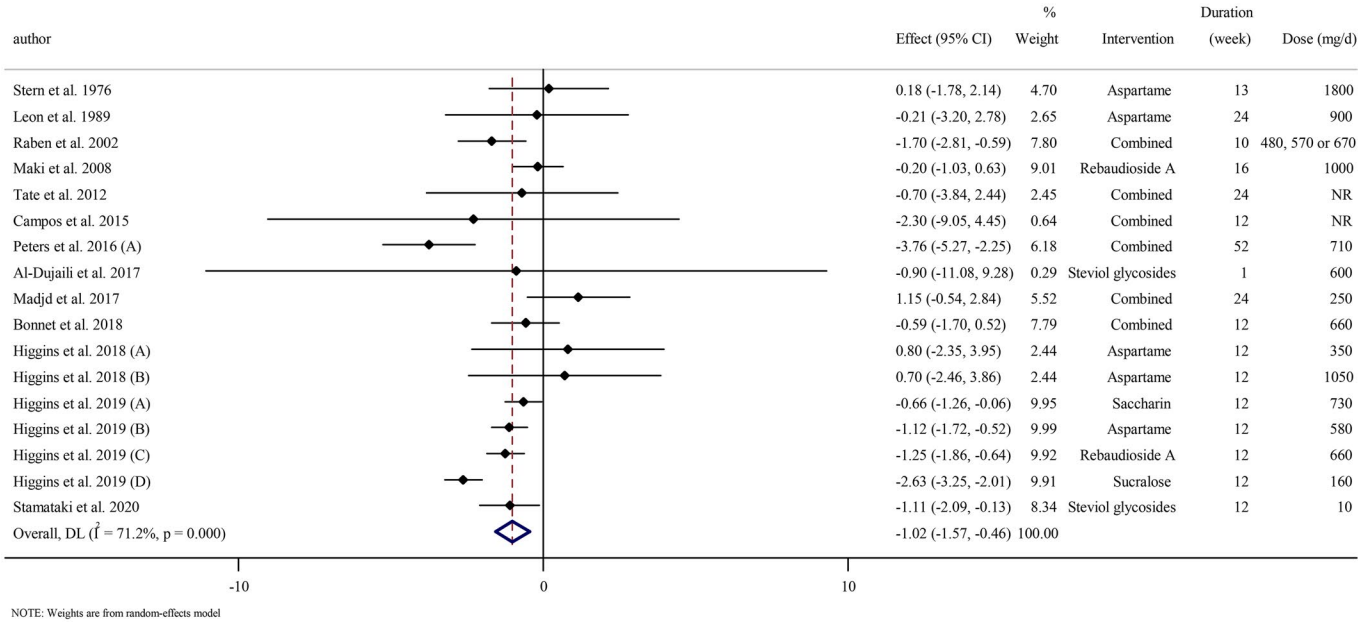

Figure 2. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the effect of NNS intake on BW.

Table 3. Subgroup analyses of artificial sweeteners on anthropometric indices and leptin.

				Heterogeneity		
	NO	WMD (95%CI)	*p* within group	*p* heterogeneity	I^2	*p* between groups
Subgroup analyses of artificial sweeteners on body weight.						
Overall effect	17	−1.01 (−1.57, −0.45)	**<.001**	<.001	71.2%	
Trial duration (week)						
<24	13	−1.02 (−1.55, −0.49)	**<.001**	<.001	67.0%	.954
≥24	4	−0.94 (−3.66, 1.77)	.497	<.001	84.2%	
Intervention Dose (mg/d)						
≥710	11	−1.01 (−1.53, −0.49)	**<.001**	.010	57.1%	.756
<710	4	−0.72 (−2.46, 1.00)	.412	<.001	87.0%	
Intervention type						
Aspartame	5	−0.88 (−1.42, −0.33)	**.001**	.407	0.0%	<.001
Combined	6	−1.22 (−2.68, 0.23)	.099	.001	75.8%	
Steviol glycoside	2	−1.10 (−2.08, −0.13)	**.026**	.968	0.0%	
Rebaudioside A	2	−0.76 (−1.79, 0.26)	.145	.046	74.8%	
Saccharin	1	−0.66 (−1.26, −0.05)	**.032**	–	–	
Sucralose	1	−2.63 (−3.24, −2.01)	**<.001**	–	–	
Intervention source						
Artificial	9	−0.99 (−1.73, −0.26)	**.008**	<.001	73.9%	0.948
Natural	4	−0.88 (−1.46, −0.31)	**.002**	.246	27.6%	
Baseline BMI (kg/m^2)						
Normal (18.5/24.9)	4	−0.72 (−1.42, −0.02)	**.043**	.507	0.0%	.362
Overweight (25/29.9)	5	−1.54 (−2.44, −0.64)	**.001**	<.001	81.1%	
Obese (>30)	5	−0.95 (−2.23, 0.33)	.145	<.001	82.4%	
Subgroup analyses of artificial sweeteners on BMI.						
Overall effect	17	−0.16 (−0.35, 0.02)	.084	.128	28.9%	
Trial duration (week)						
<24	14	−0.42 (−0.65, −0.19)	**<.001**	.876	0.0%	<.001
≥24	3	0.34 (0.02, 0.66)	**.033**	.913	0.0%	
Intervention Dose (mg/d)						
≥710	10	−0.12 (−0.38, 0.13)	.343	.137	33.8%	.986
<710	6	−0.19 (−0.47, 0.07)	.153	.137	40.2%	
Intervention type						
Aspartame	1	−0.38 (−1.27, 0.51)	.406	–	–	.284
Stevioside	3	0.28 (−0.08, 0.66)	.133	.959	0.0%	
Combined	3	−0.26 (−0.66, 0.12)	.178	.003	82.4%	
Steviol glycoside	5	−0.31 (−0.64, 0.01)	.059	.799	0.0%	
Rebaudioside A S	1	−0.39 (−1.30, 0.52)	.404	–	–	
Saccharin	1	−0.17 (−1.07, 0.73)	.713	–	–	
Sucralose	3	−0.41 (−1.07, 0.25)	.225	.420	0.0%	
Intervention source						
Artificial	6	−0.57 (−0.92, −0.21)	**.002**	.514	0.0%	.043
Natural	9	−0.07 (−0.31, 0.16)	.525	.448	0.0%	
Baseline BMI (kg/m^2)						
Normal (18.5/24.9)	6	−0.19 (−0.47, 0.09)	.192	.541	0.0%	.123
Overweight (25/29.9)	6	−0.60 (−0.97, −0.23)	**.001**	.522	0.0%	
Obese (>30)	2	0.20 (−0.27, 0.69)	.395	.126	57.2%	
Subgroup analyses of artificial sweeteners on WC.						
Overall effect	5	−1.02 (−2.77, 0.71)	.248	.014	68.0%	
Subgroup analyses of artificial sweeteners on FM.						
Overall effect	7	−1.09 (−1.89, −0.28)	**.008**	.803	0.0%	
Subgroup analyses of artificial sweeteners on FFM.						
Overall effect	7	−0.82 (−1.42, −0.23)	**.006**	.956	0.0%	
Subgroup analyses of artificial sweeteners on leptin.						
Overall effect	4	−2.16 (−4.98, 0.64)	.131	.016	71.1%	

CI: confidence interval; BMI: body mass index; FM: fat mass; FFM: free fat mass; WMD: weighted mean differences.

185 subjects were included in the current analysis (Higgins, Considine, and Mattes 2018; Raben et al. 2011; Romo-Romo et al. 2020). Combining 4 effect sizes from these articles showed no significant difference in serum leptin level changes between artificial sweeteners or stevia and control groups [weighted mean difference (WMD): −2.17, 95% CI: −4.98, 0.65] (Figure 7). In addition, there was evidence of high between-study heterogeneity (I^2 = 71.1%, p=.016) (Figure 7). The results of subgroup analyses are visible in Table 3.

Sensitivity analysis

Results from sensitivity analysis for BMI indicated that the overall results were significantly affected by the exclusion of studies conducted by Chan et al. (WMD: −0.20 kg/m^2, CI 95%: −0.39, −0.01) (Chan et al. 2000), Hsieh et al. (WMD: −0.24 kg/m^2, CI 95%: −0.44, −0.04) (Hsieh et al. 2003) and Madjd et al. (WMD: −0.23, CI 95%: −0.43, −0.03) (Madjd et al. 2017). Furthermore, after removing the study by Raben et al. (WMD:0.73, CI 95%: −1.78,

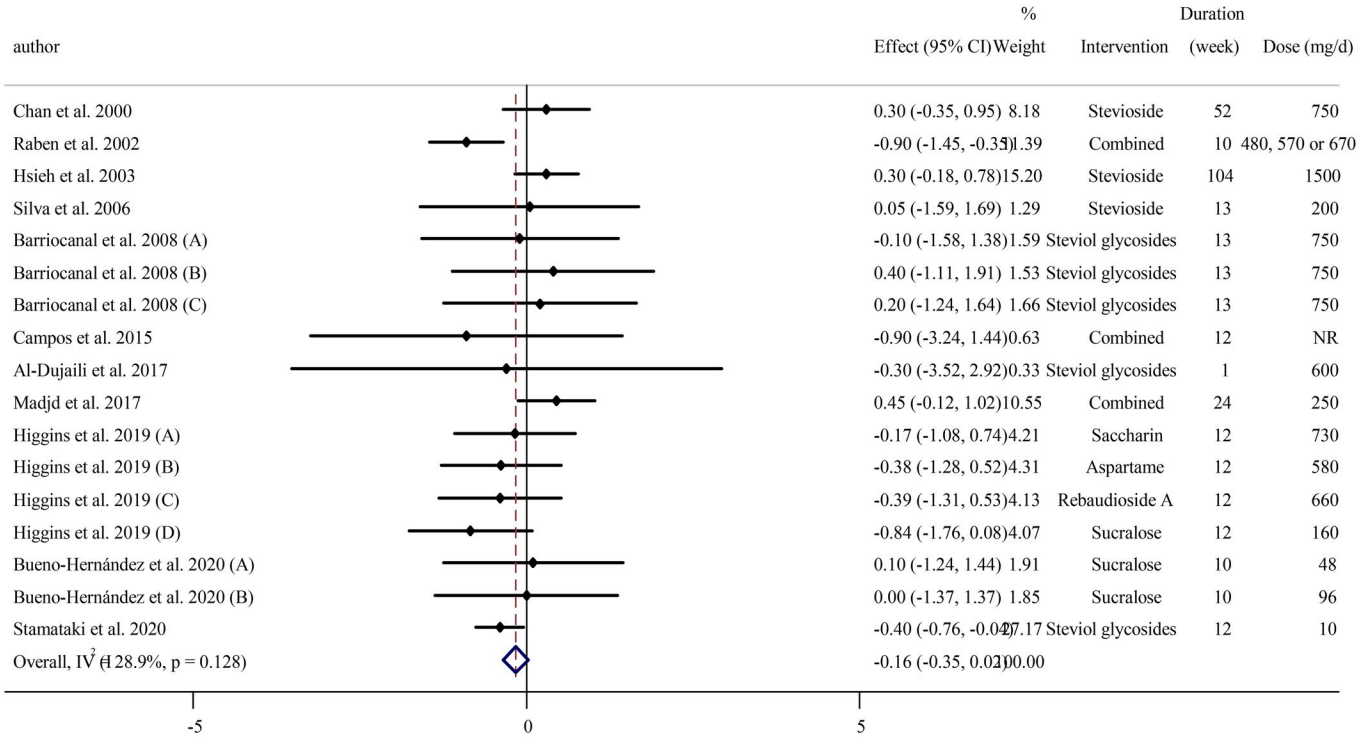

Figure 3. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the effect of NNS intake on BMI.

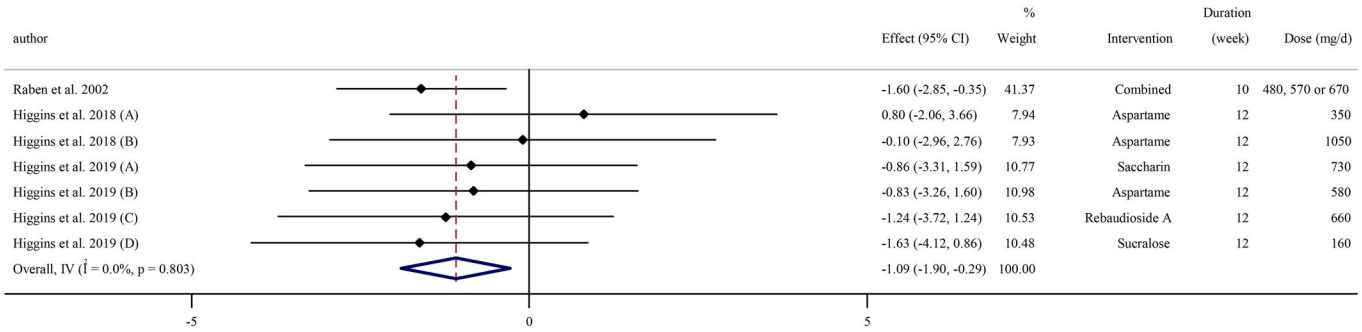

Figure 4. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the effect of NNS intake on FM.

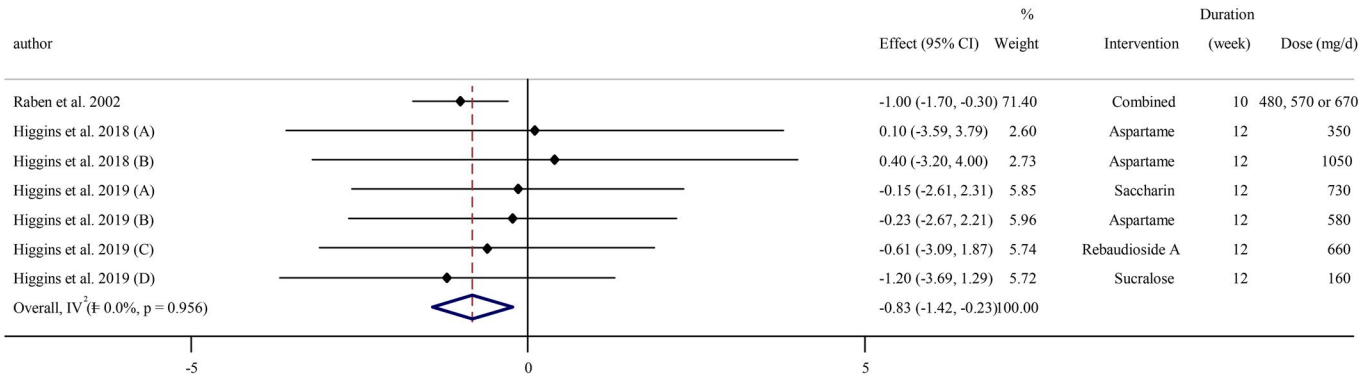

Figure 5. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the effect of NNS intake on FFM.

0.31) (Raben et al. 2002), the overall results for FM (WMD: −0.73 kg, CI 95%: −1.78, 0.31) and FFM (WMD: −0.39 kg, CI 95%: −1.50, 0.71) were significantly altered. Additionally, the elimination of the study by Romo-Romo et al. (2020), changed the overall result of leptin (WMD: −3.34, CI 95%: −6.45, −0.23).

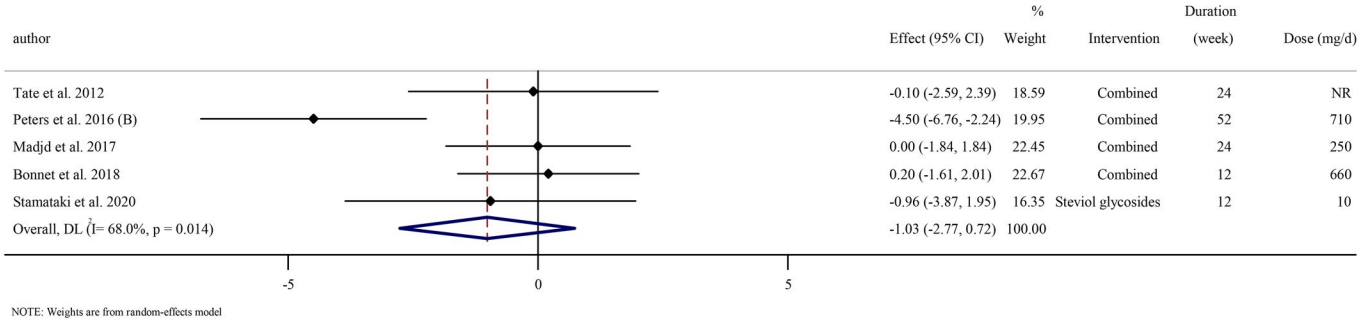

Figure 6. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the effect of NNS intake on WC.

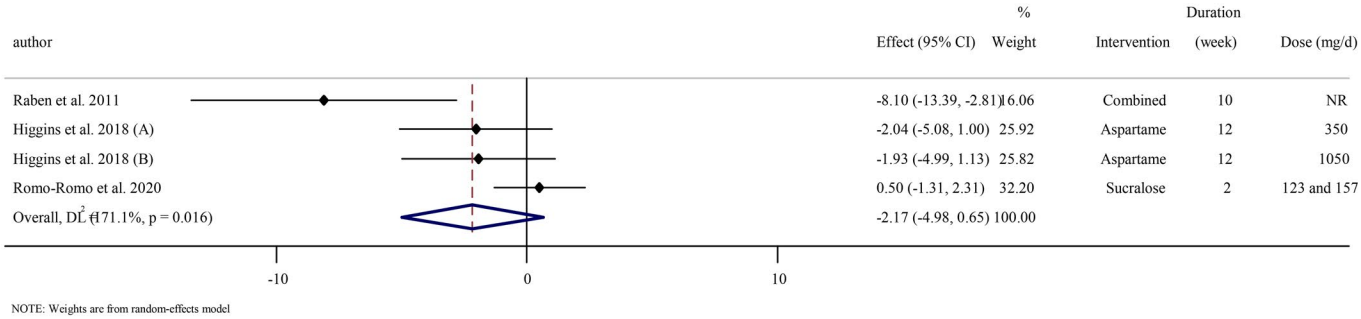

Figure 7. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the effect of NNS intake on serum leptin level.

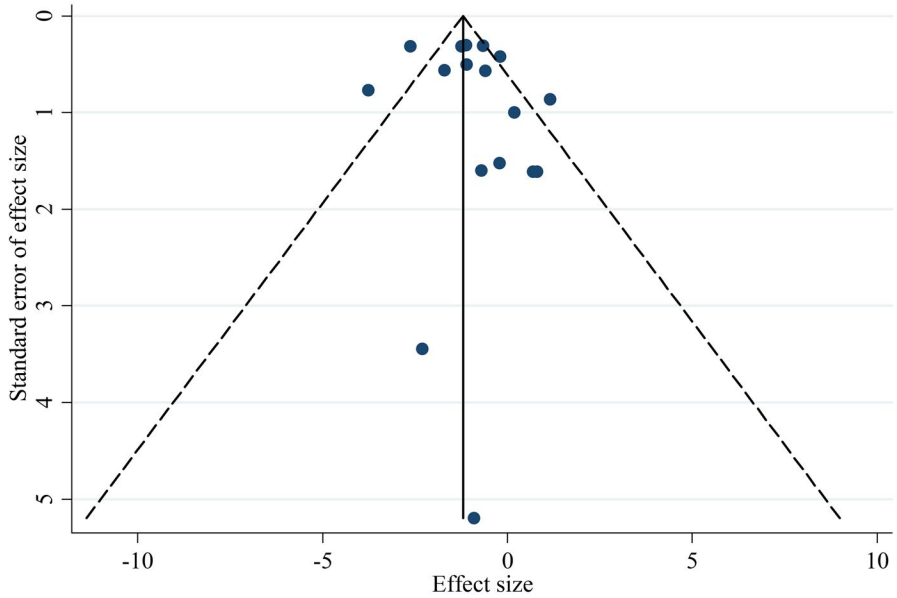

Figure 8. Funnel plot for the effect of NNS intake on BW with pseudo 95% confidence limits.

Publication bias

The results of Egger's regression test did not indicate any evidence of publication bias for body weight ($p = .554$), BMI ($p = .432$), and WC ($p = .453$). However, significant publication bias was observed for FM ($p = .042$), FFM ($p = .031$), and leptin ($p = .006$). On the other hand, the publication bias analysis based on Begg's test did not reveal any significant publication bias for body weight ($p = 1.000$), BMI ($p = 1.000$), WC ($p = .221$), FM ($p = .368$), FFM ($p = .368$), and leptin ($p = .308$). The funnel plots are depicted in Figures 8–13.

Meta regression

The meta-regression test results indicated that there was no linear relationship between the changes in body weight (Coefficient = −0.04, $p = .145$) and BMI (Coefficient = 0.008, $p = .62$) and the duration of intervention, as illustrated in Figures 14 and 15.

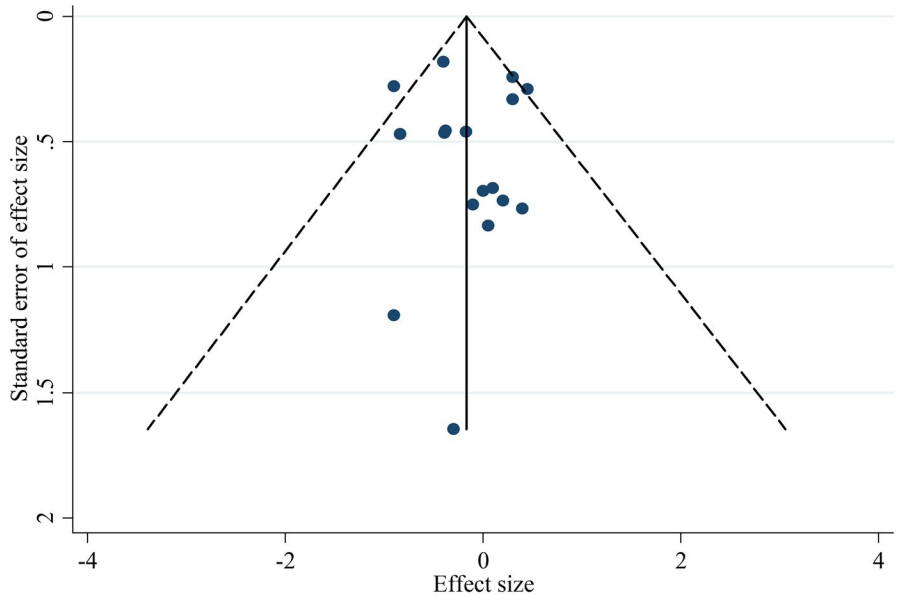

Figure 9. Funnel plot for the effect of NNS intake on BMI with pseudo 95% confidence limits.

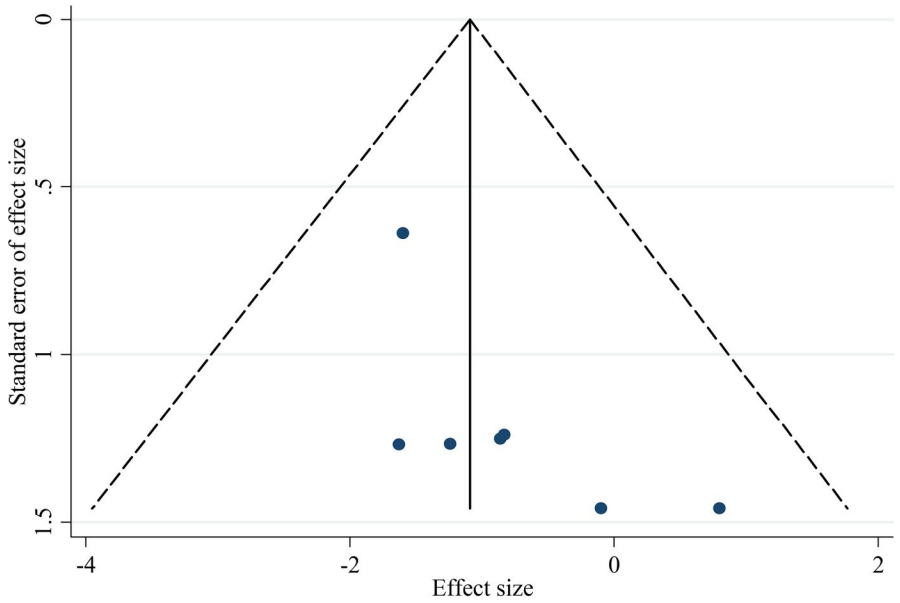

Figure 10. Funnel plot for the effect of NNS intake on FM with pseudo 95% confidence limits.

Grading of evidence

To assess the certainty of the evidence, the GRADE protocol was utilized. Based on the findings presented in Table 4, the evidence pertaining to Body weight was deemed to be of low quality due to significant limitations in inconsistency and indirectness. Similarly, the evidence concerning BMI was also assessed as being of low quality due to serious issues of indirectness and imprecision. The quality of evidence for FM and FMM was likewise determined to be low, as these measures exhibited serious limitations in terms of indirectness and publication bias. In the case of WC, the evidence was assessed as being of very low quality due to serious limitations in inconsistency, indirectness, and imprecision. Lastly, the evidence related to leptin was found to have serious limitations in all criteria, except the Risk of Bias, resulting in its assessment as very low quality.

Discussion

The current study systematically reviewed and quantitatively synthesized existing scientific evidence from RCTs on the effects of NNS consumption on anthropometric indices including body weight, BMI, fat mass, fat-free mass, waist circumference, and serum leptin. The results of our analysis indicated that the consumption of NNSs is associated with a decrease in body weight, fat mass, and fat-free mass. However, our study did not find a significant effect of artificial- and stevia-based sweeteners on BMI, waist circumference, and serum leptin levels.

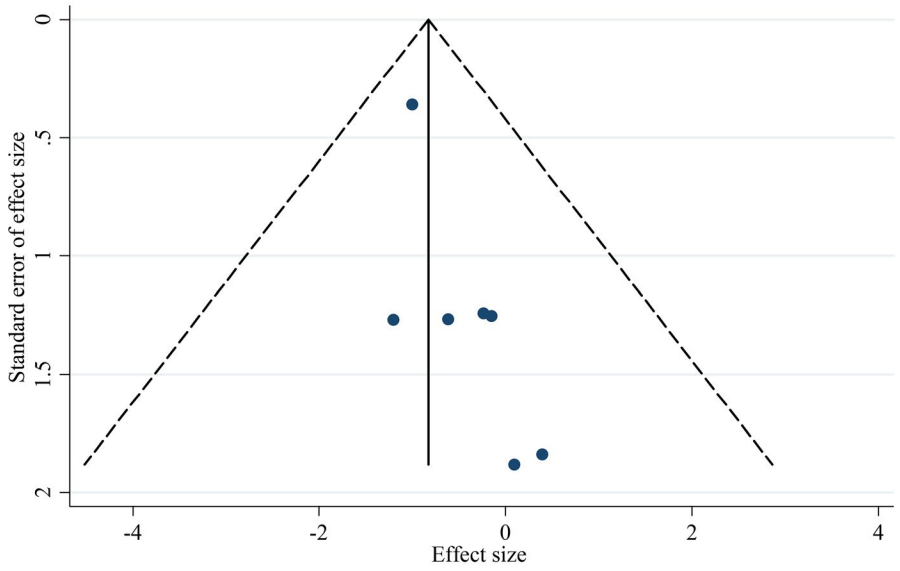

Figure 11. Funnel plot for the effect of NNS intake on FFM with pseudo 95% confidence limits.

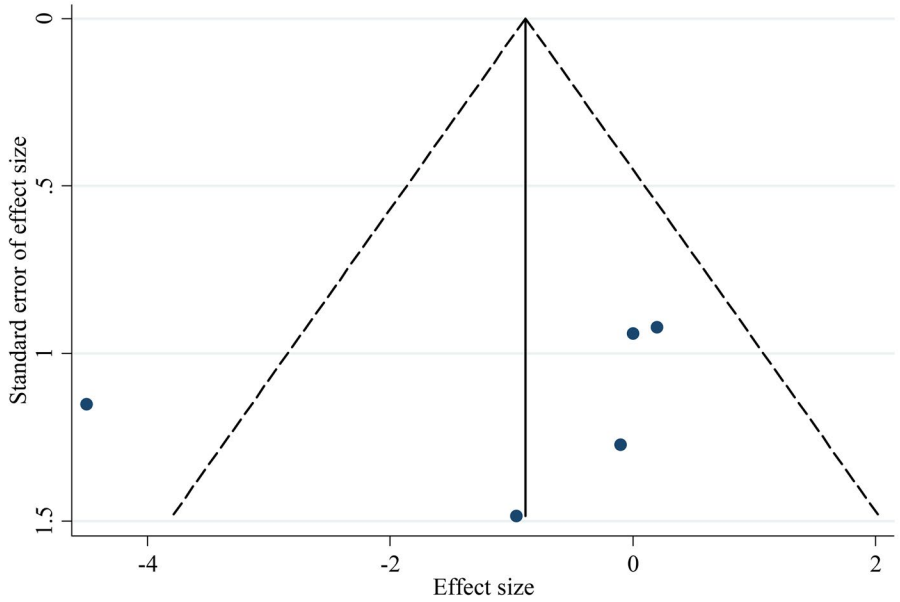

Figure 12. Funnel plot for the effect of NNS intake on WC with pseudo 95% confidence limits.

Several RCTs have investigated the individual and combined effects of low-calorie sweeteners on body weight. A meta-analysis of these RCTs has revealed a statistically significant but modest reduction in body weight associated with the use of low-calorie sweeteners (Miller and Perez 2014). Our study's findings are consistent with this conclusion, as we observed a significant reduction in body weight with the use of artificial- and stevia-based sweeteners compared to controls. This weight loss may be attributed to the substitution of high-calorie drinks and foods with low-calorie sweeteners. In contrast to the findings of our study, Higgins et al. reported that the consumption of NNS had an incremental effect on weight. (Higgins and Mattes 2019). The consumption of small amounts of saccharin, rebaudioside A, and sucralose has been shown to induce changes in the composition of the colonic microbiota. These modifications may affect the efficiency of energy uptake and expenditure, resulting in alterations in body weight (Magnuson et al. 2016; Suez et al. 2015).

The results of our meta-analysis of RCTs suggest that there is no significant difference in BMI changes between individuals who consumed artificial sweeteners or stevia and those in control groups. A meta-analysis attained conflicting conclusions about the effect of low-calorie sweeteners on BMI (Miller and Perez 2014). Cohort studies investigating the relationship between low-calorie sweetener use and BMI have reported conflicting results, with some studies reporting an increase (Azad et al. 2017; Miller and Perez 2014) in BMI and others finding no significant change (Rogers et al. 2016). The RCTs investigating the impact of NNS on BMI have reported a decrease (Miller and Perez 2014) or no change effect (Azad et al. 2017) in BMI. However, Mattes

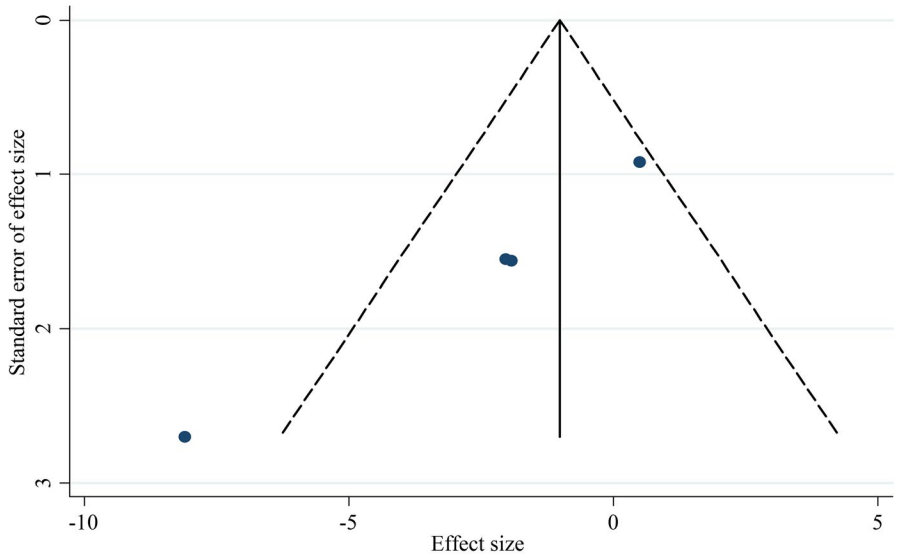

Figure 13. Funnel plot for the effect of NNS intake on serum leptin level with pseudo 95% confidence limits.

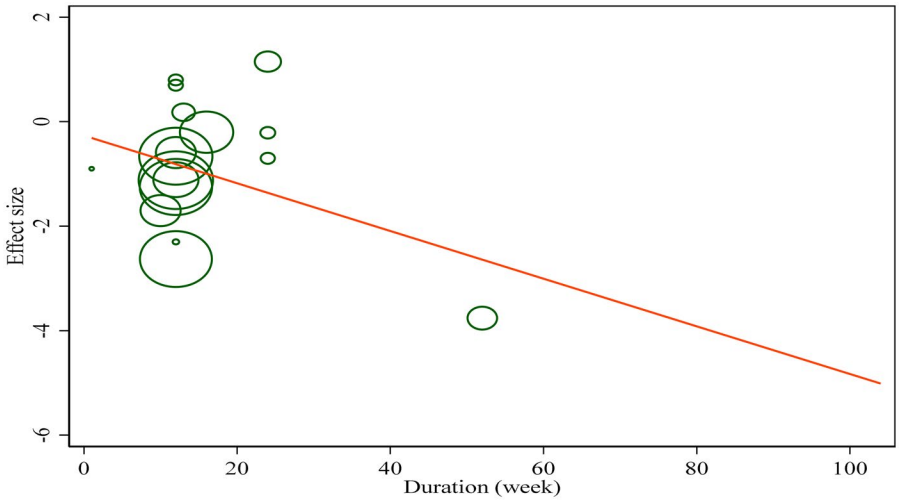

Figure 14. Random-effects meta-regression plots of the association between NNS intake and weighted mean difference of BW.

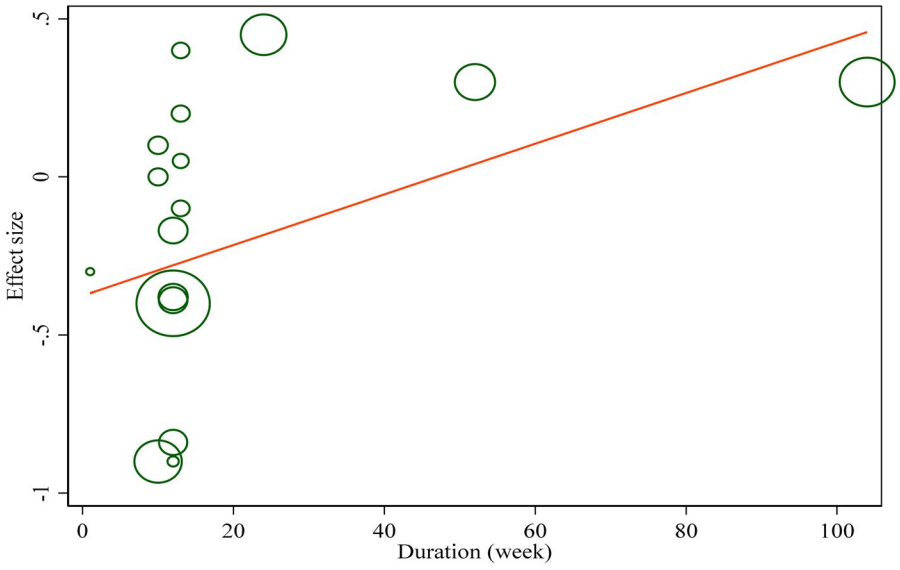

Figure 15. Random-effects meta-regression plots of the association between NNS intake and weighted mean difference of BMI.

Table 4. GRADE profile of artificial sweeteners on lipid profile.

	Quality assessment					
Outcomes	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Quality of evidence
Body weight	No serious limitations	Serious limitations[a]	Serious limitations[d]	No serious limitations	No serious limitations	⊕⊕◯◯ Low
BMI	No serious limitations	No serious limitations	Serious limitations[d]	Serious limitations	No serious limitations	⊕⊕◯◯ Low
WC	No serious limitations	Serious limitations[b]	Serious limitations[d]	Serious limitations	No serious limitations	⊕◯◯◯ Very low
FM	No serious limitations	No serious limitations	Serious limitations[d]	No serious limitations	Serious limitations[e]	⊕⊕◯◯ Low
FFM	No serious limitations	No serious limitations	Serious limitations[d]	No serious limitations	Serious limitations[f]	⊕⊕◯◯ Low
Leptin	No serious limitations	Serious limitations[c]	Serious limitations[d]	Serious limitations	Serious limitations[g]	⊕◯◯◯ Very low

[a]The test for heterogeneity is significant, and the I^2 is moderate, 74.6%.
[b]The test for heterogeneity is significant, and the I^2 is moderate, 80.5%.
[c]The test for heterogeneity is significant, and the I^2 is moderate, 80.5%.
[d]Studies conducted subcect with varous conditons.
[e]There is publication bias (p = .042).
[f]There is publication bias (p = .031).
[g]There is publication bias (p = .006).

and Popkin reported an increase in body weight and BMI among healthy individuals with a sedentary lifestyle who consumed NNS (Mattes and Popkin 2009). Yang's research also reported a significant increase in body weight and BMI among healthy subjects who consumed NNS over an extended period of time (Yang 2010). One explanation for the increase in body weight and BMI observed in individuals consuming NNS for an extended period is the potential for these sweeteners to increase cravings for sweet foods and subsequently stimulate appetite (Malik, Schulze, and Hu 2006). The inconsistencies in results obtained from various studies investigating the impact of low-calorie sweeteners on body weight and BMI may be attributed to several factors, including differences in inclusion criteria, variations in the type of low-calorie sweeteners used, study participants, study duration, and sample size.

In addition to human studies, animal studies investigating the effect of artificial- and stevia-based sweeteners on anthropometric indices have also yielded conflicting results. For instance, a study conducted by Rosales-Gómez et al. found that supplementation with NNS resulted in decreased food intake but led to an increase in body weight (Rosales-Gómez et al. 2018). In contrast to the findings of Rosales-Gómez et al. Uebanso et al. reported no significant increase in body weight in mice supplemented with sucralose (Uebanso et al. 2017). Uebanso et al. noted that mice supplemented with stevia and sucralose tended to consume large amounts of water. Additionally, their findings suggested that rats showed a preference for stevia over saccharin (Sclafani et al. 2010). Curry and Roberts observed a reduction in body weight in rats that were orally ingested with stevia (Curry and Roberts 2008). Consumption of stevia resulted in a decrease in dietary sugar intake, which could potentially aid in weight loss (Thomas and Glade 2010).

Our meta-analysis found a significant reduction in fat mass and free fat mass following intervention with NNS or stevia-based sweeteners compared to placebo. While evidence from prospective cohort studies reported no significant association with fat mass, evidence from randomized controlled trials demonstrated significant benefits of low-calorie sweeteners on fat mass (Hunter et al. 2019). The reason for the inconsistent results may be related to differences in study populations across the included studies, which involved children, adolescents, and adults. For instance, a randomized controlled trial reported that consumption of saccharin caused an increase in body weight, but had no significant effect on fat mass (Higgins and Mattes 2019). A parallel design intervention study reported that after supplementing with sucrose for 10 wk, there was an increase in body weight and fat mass, whereas there was a decrease or no change in these variables after artificial sweetener supplementation (Raben et al. 2002). Actually, the composition of macronutrients present in the diet of the sucrose group was similar to the total fat and carbohydrate of dietary recommendations (Fogelholm et al. 2012). The likely reason for the increase in body weight in the sucrose group is that the majority of the sucrose was consumed in fluid form. Fluids do not provide the same satiety as solid foods, which may have caused individuals to overconsume more energy from solid foods (DiMeglio and Mattes 2000). A six-month randomized intervention study found no statistically significant difference in fat mass among individuals who consumed sweetened beverages compared to those who did not (Maersk et al. 2012). Moreover, the study acknowledged that the lack of statistical significance may have been attributed to the small sample size used in the study.

In our systematic review and meta-analysis, the effects of NNS or stevia-based sweetener on waist circumference changes were analyzed. The results revealed no significant difference in waist circumference changes between individuals consuming artificial sweeteners or stevia and those in the control groups. This finding is consistent with the results of a randomized controlled crossover study (Koyuncu and Balci 2014).

Our study also examined the impact of artificial- and stevia-based sweeteners on serum leptin level. The results indicated no statistically significant difference in serum leptin level changes between individuals consuming these sweeteners and those in the control groups. A cross-sectional study reported a positive correlation between sugar-sweetened

beverage consumption and leptin concentration, whereas no association was found between artificially sweetened beverages and leptin concentration (Lana, Rodríguez-Artalejo, and Lopez-Garcia 2014). Artificially sweetened beverages are made with non-caloric, high-intensity sweeteners, while sugar-sweetened beverages contain fructose that may be responsible for the elevated leptin concentrations. Consistent with our findings, a randomized controlled trial reported that a two-week consumption of sucralose did not affect the leptin concentration (Romo-Romo et al. 2020).

Overall, given that the consumption of NNSs does not have a significant impact on certain anthropometric indices, such as waist circumference and BMI, which are important risk factors for cardio-metabolic disorders, it is advisable to combine the consumption of NNSs with regular exercise, lifestyle modifications, and a healthy diet for optimal effectiveness.

Strength and limitations

In this meta-analysis, we employed a rigorous search strategy to identify relevant studies and examined various types of artificial- and stevia-based sweeteners. We conducted subgroup analyses to compare the effects of different sweeteners. However, our review has some limitations. For instance, some studies included in our meta-analysis did not specify the type of non-nutritive sweeteners used, which could have affected the results of our subgroup analyses. Additionally, the effects of NNS could be influenced by the participants' genetic background and polymorphisms, but data on this were lacking. Last but not least, the control group interventions in the studies were highly diverse, including interventions involving water, nutritive sweeteners, non-absorbable compounds, or the absence of any intervention. Consequently, this diversity may have led to a potential confounding of the true effect of non-nutritive sweeteners (NNSs) in the intervention group. Therefore, the interpretation and conclusions of the results should be considered with caution.

Conclusion

The present study found that consumption of artificial- and stevia-based sweeteners may lead to reduced body weight, fat mass, and free fat mass. However, there was no significant association between the consumption of these sweeteners and reductions in BMI, waist circumference, and serum leptin levels. Further research is needed to clarify the effects of non-nutritive sweeteners on anthropometric measurements.

Acknowledgment

This study is related to project No. 1400/65033 from the Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. We also appreciate the "Student Research Committee" and "Research & Technology Chancellor" in Shahid Beheshti University of Medical Sciences for their financial support of this study.

Authors contributions

The study was conceptualized by A.H., M.M., and S.A.G. Data extraction from the included articles was performed by M.M. and S.A.G. following a systematic search. O.A. conducted the analysis of the extracted data. The manuscript was written by M.M., A.H., and K.P. Subsequent revisions of the manuscript were made by M.M., S.A.G., O.A., and A.H., and the study was supervised by A.H. All authors have approved the final version of the manuscript.

Ethical approval

The study protocol was approved by the ethics committee of the Shahid Beheshti University of Medical Sciences (ethical code: IR.SBMU.RETECH.REC.1400.837).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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