Do non-nutritive sweeteners affect the metabolic health of infants and children?
A systematic review of randomized controlled trials and prospective cohort studies

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ABSTRACT

Background: Non-nutritive sweeteners (NNS) have recently gained enormous popularity due to their perceived health benefits in weight loss and management; however, their long-term impact on human health is unknown, particularly when exposure occurs during early development. We conducted a systematic review of prospective cohort studies and randomized controlled trials (RCTs) to evaluate the association between NNS exposure in the prenatal period, infancy and childhood (age $\leq$ 12 years) and metabolic health outcomes.

Methods: A comprehensive peer-reviewed search strategy was used to search the Medline (OVID) database from inception to December 2014. Citations were screened in duplicate to identify RCTs and prospective cohort studies evaluating metabolic outcomes after NNS exposure during gestation or childhood. The primary outcomes were change in weight-for-length (WFL) z-score in infants and change in body mass index (BMI) z-score in children; secondary outcomes included growth velocity, birth weight, incidence of overweight/obesity, change in central and total adiposity, and incidence of adverse metabolic effects.

Results: From 4591 citations reviewed, 12 studies met our inclusion criteria; 3 RCTs and 9 prospective cohort studies (total n = 83,426 participants). Studies were heterogeneous in the type and duration of NNS exposure and outcomes reported. Two of the nine prospective cohort studies identified significant associations between NNS exposure and increase in BMI or fat mass in children, yet six studies reported no association. Two RCTs evaluated NNS exposure in children and found significant but contradictory associations with weight gain. In two prenatal studies, NNS exposure was not associated with infant birth weight; however, no subsequent metabolic outcomes were evaluated. No studies reported on incidence of metabolic syndrome, insulin resistance, or type 2 diabetes mellitus.

Conclusions: There is limited and inconsistent evidence for the metabolic effects of early-life NNS exposure. Further research is required to fully understand the impact of NNS exposure during gestation, infancy, and childhood.

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Introduction

Artificial Sweeteners and the Obesity Epidemic

Obesity and its associated co-morbidities, including type 2 diabetes (T2D) and cardiovascular disease, continue to be among the most important public health epidemics worldwide. Over 30% of American children and 69% of adults are considered overweight or obese (1-3), and the estimated annual medical cost of obesity is $147 billion in the United States (4) and up to $7.1 billion in Canada (5). Identifying strategies that help regulate body weight is crucial to managing the obesity epidemic. The consumption of high-calorie, sugar-sweetened foods is strongly associated with obesity, T2D and cardiovascular disease risk factors (3, 4, 6, 7), which has prompted health care organizations and practitioners alike to recommend population-wide reductions in the intake of added sugars (8, 9). In light of this, sugar replacements or "non-nutritive sweeteners" (NNS) have gained enormous popularity due to their low caloric value and perceived health benefits for weight reduction, weight maintenance and normalization of blood sugar levels (8, 10).

Commonly Used Artificial Sweeteners

NNS, also known as low-calorie sweeteners, artificial sweeteners, high-intensity sweeteners, or non-caloric sweeteners are chemical or natural additives that produce the perception of sweet taste at low concentrations in foods and beverages (11). Common NNS used in foods, chewing gums, and beverages include aspartame, sucralose, saccharin, stevia, and sugar alcohols such as xylitol. These sweeteners have a higher intensity of sweetness per gram than caloric sweeteners such as sucrose or corn syrups,

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thus providing sweet taste with few or no calories (8). There are numerous NNS available worldwide, yet not all are approved internationally, with certain sweeteners being banned in individual countries due to lack of evidence for safety (12). The Acceptable Daily Intake (ADI) also varies between countries (12).

Health Effects of Artificial Sweeteners

The consumption of beverages and foods containing NNS continues to increase (8), yet their long-term impact on human health is still unknown, with some research identifying potentially adverse metabolic effects including weight gain, obesity, and increased fat mass (9, 11, 13). Several biological and physiological mechanisms have been proposed for these possible adverse metabolic effects, including glucose intolerance and impaired insulin response (14), changes in gut microbiota leading to increased intestinal sugar absorption (14), and increased appetite due to altered taste and metabolic signaling (15). In a 2014 study, saccharin, sucralose and aspartame consumption caused glucose intolerance in mice, and saccharin was shown to induce compositional and functional alterations to the intestinal microbiota (14). In a parallel epidemiologic analysis, investigators reported significant positive correlations between human NNS consumption and several metabolic-syndrome related clinical parameters, including weight gain, increased waist-to-hip ratio, and higher fasting blood glucose, glycosylated hemoglobin, and glucose intolerance (14). The timing of NNS exposure in the lifecycle may be important, as animal studies have shown that NNS consumption during pregnancy and lactation may predispose the offspring to develop obesity and metabolic syndrome later in life (16).

Current Research

Epidemiologic studies in children have found that NNS consumption may increase body mass index (BMI), while other studies have demonstrated no association (17). Additionally, numerous studies maintain that there was insufficient data to determine conclusively whether NNS had any adverse impact on human health, with further studies needed to investigate the topic (8, 11, 16). A recent systematic review and meta-analysis conducted by Miller and Perez evaluated the association of NNS and body weight composition in adults and children (10). Pooled results from RCTs demonstrated potential health benefits, including modest weight loss and weight maintenance, however observational studies showed a significant positive association with BMI. These findings demonstrate the importance of incorporating evidence from observational study designs to comprehensively evaluate the impact of NNS intake; however, observational studies are prone to bias and no quality assessments were performed in the Miller and Perez review. In addition, the search strategy used in this review was not comprehensive, and missed key publications relevant to the analysis (18). Lastly, no metabolic outcomes beyond body weight and composition were evaluated, crucial age subsets such as gestation and infancy were not assessed, and concerns have been raised about the concluding recommendations for use of low calorie sweetened beverages in children (18).

The purpose of the current systematic review is to identify, appraise and synthesize data from prospective randomized trials and observational cohort studies documenting the metabolic effects of NNS exposure occurring during gestation, infancy and childhood.

Methods

Populations, Interventions, Comparators, Outcome Measures, Settings and Study Designs

The primary research question for this systematic review was "Does exposure to NNS during gestation, infancy or childhood have adverse metabolic effects compared to nutritive sweeteners or habitual diet?" RCTs and prospective cohort studies of NNS exposure in pregnant women, infants (birth to 2 years), or children (> 2 to ≤ 12 years) were included in this review. An upper age limit of 12 years was used for children, as this is when puberty often begins; an age when biological, physical and metabolic changes can start taking place in a child (19). All NNS, including aspartame (e.g. Equal®, Nutrasweet®), saccharin (e.g. Sugar Twin®, Sweet'N Low®), sucralose (Splenda®), stevia, and sugar alcohols such as xylitol, were considered in foods, beverages, or as additives to foods or beverages. Eligible comparators were caloric or nutritive sweeteners, placebo, or the participant's regular diet. A minimum study duration of ≥ 2 weeks for RCTs and ≥ 6 months for observational studies was chosen to allow sufficient time for our primary outcomes to develop (RCTs) and to ensure that NNS exposures preceded the outcome of interest (observational studies). In addition, this time period was consistent with previously published systematic reviews and meta-analysis that followed a similar study design (10, 20, 21). Follow up for studies documenting prenatal NNS exposure and reporting outcomes at birth was considered as 9 months. Inclusion and exclusion criteria are listed in Table 1. The primary outcome evaluated in infants was change in weight-for-length (WFL) z-score and the primary outcome in children was change in BMI z-score (Table 2). Secondary outcomes evaluated in both infants and

children included growth velocity, incidence of overweight/obesity, and change in total adiposity (% body fat by dual-energy x-ray absorptiometry (DEXA) or skinfold thickness). Additional secondary outcomes for children only included change in central adiposity (waist circumference, waist:hip ratio or waist:height ratio), incidence of type 2 diabetes/impaired glucose tolerance, and incidence of adverse metabolic effects (metabolic syndrome, insulin resistance, glycosylated hemoglobin HbA1C%). Birth weight was a secondary outcome evaluated for studies of NNS in pregnant women. A summary of primary and secondary outcomes is listed in Table 2.

Search Strategy

A sensitive Medline (OVID) search strategy was developed in consultation with an information specialist to identify studies on NNS exposure and to capture research where previous reviews have been inadequate (18). Since some studies reporting NNS consumption in children did not include NNS-specific terms in their searchable fields (title, abstract and keywords), a broad search strategy was applied to capture studies that were overlooked in previous reviews. The search strategy was peer-reviewed and included the following terms, among others: non-nutritive sweeteners, aspartame, neotame, saccharin, sucralose, cyclamate, xylitol, stevia, mannitol, carbonated beverages, calories, food frequency, and sweetening agents. The full MEDLINE search strategy is provided in Appendix I. RCTs were identified using a validated methodological filter; observational designs were identified using a combination of indexing and keyword terms. Searches were conducted from database inception date to December 2014 and no language limits were applied. Reference management was performed in EndNote

(version X7) and search results were exported to the web-based systematic review software, DistillerSR (22) for screening and data extraction.

Study Selection

For inclusion, studies were required to be: a) prospective, randomized controlled trials with NNS intervention, or prospective observational cohort studies reporting NNS exposure and b) NNS exposure reported in pregnant women, infants or children under 12 years of age. Exclusion criteria included: a) non-human studies; b) reviews/commentaries; c) trials that used quasi-randomized, cross-over, or cluster randomized designs; d) trials where NNS could not be examined independently of other intervention components; e) trials of insufficient duration (< 2 weeks); f) observational studies using cross-sectional or retrospective design; g) observational studies of insufficient duration (< 6 months)[1]; and h) no outcomes of importance to the review reported or available via contact with trial authors.

A two-step process for study selection was used. Level 1 screening focused on titles and abstracts; two reviewers independently screened the titles and abstracts (when available) of search results to determine if a study met the general inclusion criteria. Each report was classified as: include, exclude, unclear, or duplicate of another citation. The full text of all reports classified as "include" or "unclear" were retrieved for full text review. In Level 2 screening, two reviewers independently assessed the full text article of each report retrieved from Level 1 using a standardized form that outlined the pre-

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[1] Prenatal exposure was assumed as a 9 month follow up duration

determined inclusion and exclusion criteria. Disagreements were resolved by discussion between the two reviewers or by third-party adjudication, as needed.

Data Abstraction and Management

A standardized data extraction form was developed and deployed in DistillerSR (22). The form was pilot tested on a sample of studies prior to finalization. Reviewers extracted data from study reports independently with disagreements resolved through consensus or by a third member of the review team. The following data were extracted from each study: bibliographic data including author, journal, date; year of publication; country and language of publication; funding source(s); study design; study population (including main study inclusion and exclusion criteria) and baseline characteristics (age, sex, BMI z-score, WFL z-score, central and/or total adiposity, metabolic conditions); NNS intervention and comparator (for randomized studies) or NNS exposure and confounders/covariates (for non-randomized studies); type, dose and duration of NNS exposure; duration of follow up; and metabolic outcomes of interest (as described above). If multiple follow ups were reported for an individual study, the longest follow up was included in analysis.

For non-randomized studies, NNS effect estimates were extracted in two possible formats: (1) ratio comparing the highest vs. lowest category of NNS intake (extreme quantiles or as defined by study authors), and/or (2) linear association quantifying effects per unit NNS intake (intake unit as defined by study authors). Adjusted effect estimates were extracted; if multiple adjusted estimates were reported for a single outcome, the

estimate from the statistical model including the largest number of covariates was extracted.

Assessment of Methodological Quality

Methodological quality was assessed following guidance from the Cochrane Handbook of Systematic Review on Interventions (23).

RCTs were assessed using the Cochrane Collaboration Risk of Bias (RoB) tool (23, 24). This tool consists of six domains (sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and "other" sources of bias) and a categorization of the overall RoB. Information regarding methodological quality was used to guide sensitivity analyses and explore sources of heterogeneity. Each separate domain was rated "yes," "unclear," or "no." The overall assessment was based on the responses to individual domains. If one or more individual domains were assessed as having a high RoB, the overall score was rated as having a high RoB. The overall RoB was considered low only if all components were rated as having a low RoB. The RoB for all other studies was rated as unclear.

Prospective cohort studies were evaluated using the Newcastle Ottawa Scale (NOS) (25). This tool evaluates the internal validity of observational studies based on a 'point system' in which a study is judged on three broad perspectives: the selection of study groups, the comparability of the groups, and the ascertainment of the outcome of interest. Together, the response options for individual domains are combined to produce overall quality judgment for the study. The NOS tool requires certain evaluation criteria to be customized by the investigators. For the purpose of this review, "adequate follow

up duration" was classified as ≥1 year and "adequate retention" as >70% (worth 1 point each under outcome ascertainment), and designated two "critical confounders" (worth one point each under comparability of study groups): body composition at baseline (BMI, WFL, waist circumference, or other measure of body composition), and diet (some measure of total energy or sugar intake, or a diet pattern or quality score).

Meta-analysis

Where appropriate, data from included studies was pooled and meta-analyzed using an inverse variance fixed effects model with Comprehensive Meta Analysis Software (Version 3, Biostat, Inc.). Studies were grouped according to mean age of NNS exposure, with each of the following reviewed separately: prenatal, infancy (birth to 2 years), and childhood (> 2 to ≤ 12 years).

Results

The initial literature search produced 4,951 citations; after titles and abstracts were screened 199 potentially eligible articles were identified. Of these, a total of twelve studies (total n = 83,426 participants), heterogeneous in the type and duration of NNS exposure and outcomes reported, were included in the final review (Figure 1) (9, 26-36). Included studies are summarized in Table 3.

Study Characteristics and Study Populations

Nine of the twelve articles were prospective cohort studies, published in peer review journals between 2001 and 2013 (9, 26, 27, 29-31, 33, 34, 36). The majority of these

observational studies were published in the United States (26, 27, 29, 30, 33, 34, 36),

with two published in Europe (9, 31). Eligibility criteria varied, with three studies

restricting participation to specific BMI criteria (29, 33, 36) and the remaining six studies

recruiting a general population cohort with no specific health-related requirements for

participation (9, 26, 27, 30, 31, 34). Five studies analyzed NNS exposure and health

effects in both male and female children (9, 26, 27, 29, 30, 33), two studies analyzed

exposure in only female children (34, 36), and one study evaluated health impact in

pregnant women (31). The enrollment age of infants and children in these studies ranged

from intrauterine (31) to 11 years (36).

Three of the twelve included studies were RCTs (28, 32, 35), published between

2010 and 2013 in peer-reviewed journals. One RCT (28), published by de Ruyter et al. in

the Netherlands, evaluated NNS consumption on obesity prevention and weight

maintenance in children. Taljaard et al. (35) evaluated children in South Africa with a

focus on malnutrition and the effects of a multi-micronutrient fortified beverage on

growth and cognition. Both of these studies provided artificially sweetened beverages

(ASB) to male and female children, with an average age of 8 years (28, 35). Neither

study had health-related inclusion criteria, yet the baseline BMI of enrolled participants

were quite different between studies, with one study evaluating mostly healthy and

normal weight children (28), and the other investigating mainly underweight and

malnourished children (35). The third RCT was published by Nakai et al. (32) in Japan

and evaluated xylitol chewing gum in women during pregnancy with a primary goal of

improving oral health in mothers and infants (32).

NNS Interventions and Exposures

All of the observational studies exclusively evaluated ASBs (soft drinks specifically or beverages in general), with no clear indication of type of NNS. Most commonly, ASBs contain aspartame, sucralose, saccharine, or acesulfame potassium, alone or in combination (31). Four of these studies gathered dietary information and NNS exposure data with validated food frequency questionnaires (26, 30, 31, 33), three studies utilized food diaries (9, 34, 36), and the remaining two used a 24 hour recall (27) and a 3 day food record (36). In all studies, a trained dietitian or nutrition database was used to analyze dietary data. NNS units were reported as servings (9, 26, 30, 31, 36), weight (34), or volume (27, 29, 33, 34) of ASB consumed. Three of the nine studies reported specific intake categories, which were defined as ranges of intakes including 0 to ≥3 servings per day (26), never or <1 serving per month to ≥2 servings per day (30), and never to ≥1 servings per day (31). Serving sizes between studies were heterogeneous, varying from cans, glass/mug, and ounces consumed. Duration of follow up ranged from 12 weeks (36) to 10 years (34).

Two of the RCTs included in this analysis provided NNS to children in the form of ASBs (28, 35). One of these studies looked exclusively at sucralose exposure, comparing 25 mg of sucralose versus 20.6 grams of sucrose per day (35). The second study compared a combination of 34 mg of sucralose and 12 mg of acesulfame potassium versus 26 grams of sucrose (28). The third RCT provided artificially sweetened chewing gum, and compared 3.83 grams of xylitol per day versus no chewing gum (32). Duration of these studies ranged from 8.5 (35) to 18 months (28). Compliance to study intervention

was validated in all three RCTs through schoolteacher and parental supervision (28, 35), urine analysis (28), or daily adherence diaries (32).

Primary Outcomes

Three prospective cohort studies identified a positive association between NNS exposure and BMI z-score or BMI (9, 26, 27). Berkey et al. (26) evaluated NNS intake in both males and females, finding a significant association with weight gain in boys only (β (SE): *males* 0.12 (0.05), *p* 0.02; *females* 0.05 (0.04), *p* 0.16). Comparable results were found by Johnson et al. (9), who reported positive associations for BMI z-score and fat mass accumulation (β (95% CI) 0.26 (-0.004 to 0.52), *p* 0.05), but did not perform sex-stratified analyses. Blum et al. (27) reported a positive cross sectional association between NNS intake and BMI z-score, however longitudinal data showed no significant association with BMI change over time. The remaining five longitudinal studies reported no association between NNS and change in BMI or BMI z-score (29, 30, 33, 34, 36); yet Ludwig et al. (30) did find a negative association between NNS intake and obesity incidence (OR 0.44, p 0.03). Major confounders considered were comparable across all the prospective cohort studies, and included baseline BMI, baseline body composition, total energy intake, age, sex, ethnicity and physical activity or inactivity. Even after contacting study authors, meta-analysis was not possible for the observational studies included in this review due to the limited number of studies, variation in outcomes reported, and differences in the way these outcomes were reported.

Two RCTs evaluated NNS exposure in children and found significant but contradictory associations with weight gain (28, 35). De Ruyter et al. (28) evaluated an

ASB containing sucralose and acesulfame potassium in 641 healthy male and female children, with 18.4% of participants being overweight at baseline. After the 18-month intervention, both weight gain and fat accumulation were significantly lower in children consuming NNS (difference in change from baseline of BMI z score compared to controls: -0.13, 95% CI -0.02 to -0.06). Taljaard et al. (35) analyzed a multi micronutrient-fortified beverage, sweetened with sucrose or sucralose, on growth and cognition in 414 underweight children. Consumption of the sucralose ASB was associated with a significant increase in weight-for-age-z-score (β +0.07; 95% CI 0.14 to 0.002, p 0.026). Sucrose was used as a comparator to ASB in both of these studies. BMI z-score data from these two trials were meta-analyzed, and the pooled effect estimate for NNS was -0.12 (95%CI -0.19 to -0.05).

Secondary Outcomes

Infant birth weight was evaluated as a secondary outcome in this review. Maslova et al. (31) investigated ASB consumption and risk of child asthma and allergic rhinitis in 60,466 pregnant women between the ages of 21-39. They found no association between NNS intake and infant birth weight, however effect estimates were not adjusted for confounding factors since birth weight was reported as a covariate rather than a primary outcome. A RCT developed by Nakai et al. (32) evaluated xylitol chewing gum for prevention of maternal transmission of Streptococci mutans in 107 pregnant women in Japan. Similar to Maslova et al., they reported no effect of NNS consumption on infant birth weight, although birth weight was not a planned outcome measurement in this study, so it is unclear whether the study was adequately powered for this comparison.

Other secondary outcomes that we aimed to explore in infants and children, including Type 2 Diabetes, insulin resistance, and glycosylated hemoglobin, have not been evaluated in current literature.

Quality Assessment

Quality assessment results are summarized in Table 4A. Using the 9-point Newcastle-Ottawa scale, quality scores for prospective cohort studies ranged from 6 (33) to 9 (30), with the majority of studies scoring 7 or 8 (9, 26, 27, 29, 31, 34, 36). All studies controlled for selection of the non-exposed cohort and demonstrated reliable assessment of NNS exposure. Of the 9 longitudinal studies included, most controlled for elements of baseline diet (n=8), had adequate assessment of metabolic outcomes (n=8), included an appropriate follow up period of >1 year (n = 8), and controlled for body composition at baseline (n=7). Several studies, however, evaluated specific populations of children that do not represent the general population, such as nurses' offspring (26), participants recruited from a healthy lifestyle program (27), children born at high or low risk of obesity (29), or those with particular socioeconomic status, race or gender (33, 34, 36). A number of studies demonstrated poor or unclear adequacy to follow up and/or a drop out rate greater than 30% (9, 27, 29, 33), which also lead to decreased quality scores. Interestingly, the two studies judged to have the highest quality scores (9, 30), reported somewhat contradictory findings. Ludwig et al. (30), found no association between NNS intake and BMI change, but reported a negative association between NNS intake and obesity incidence (OR 0.44, p 0.03), while Johnson et al. (9) reported a positive association with NNS intake and fat mass.

Risk of Bias assessment results are summarized in Table 4B. Two of the three RCTs were judged to be at unclear risk of bias due to unclear selective outcome reporting (28), and unclear random sequence generation and allocation concealment (35). The third RCT (32) was judged to have a high overall risk of bias due to high risk of selective outcome reporting; the primary outcomes in this study were maternal and infant oral health, with infant birth weight being an unplanned measurement.

Discussion

The current systematic review provides a comprehensive synthesis and evaluation of RCTs and prospective cohort studies of NNS exposure during the prenatal period, infancy and childhood (age $\leq$ 12 years). From 4,591 citations reviewed, 12 studies met the inclusion criteria; 3 RCTs and 9 prospective cohort studies (total n = 83,426 participants). Two RCTs demonstrated significant but contradictory associations between NNS exposure and weight gain in children; however important differences exist between these two trials. De Ruyter et al. studied children in a developed country with a focus on weight maintenance and obesity prevention (28), while Taljaard et al. evaluated the impact of micronutrient-fortified beverages, sweetened with sugar or NNS, on growth and cognition in malnourished and underweight children in a developing country (35). The majority of observational studies (29-31, 33, 34, 36), which involved a total of 65,049 children, found no correlation between NNS consumption and weight change. However, two prospective studies, involving a total of 18,369 children, demonstrated a positive association between NNS exposure and weight gain (9, 26, 27). As noted above,

these prospective cohort studies were quite heterogeneous in population, type and duration of NNS exposure.

Comparison of Results with Other Studies

Recent epidemiological studies demonstrate inconsistent findings for the effects of artificial sweetener use in infants and children (17). As discussed previously, in 2014 Miller and Perez (10) published a meta-analysis of RCTs and prospective cohort studies that evaluated the association of NNS and body weight composition in adults and children. Results from RCTs demonstrated potential health benefits, including modest weight loss and weight maintenance, however observational studies showed a significant positive association with BMI. This review addressed limitations of the Miller and Perez review by utilizing a more comprehensive search strategy (18). Strict inclusion and exclusion criteria were implemented, with appropriate duration and follow up periods, and followed an *a priori* review protocol; two features that the Miller and Perez review were criticized for (18). In 2009, Brown et al. (17) conducted a systematic review of 18 human studies that evaluated the effects of artificial sweeteners on food intake, weight, and metabolic health in children. Inconsistent findings were reported; results from prospective cohort studies supported an association between ASB intake and weight gain in children, yet RCTs failed to show metabolic effects. Our review is a timely update to the 2009 Brown review, encompassing additional studies (28, 31-33, 35) for analysis. Moreover, with our unique focus on NNS exposure in gestation and infancy, two prenatal studies were included in our review (31, 32). Despite our study design modifications, search strategy improvements, and evaluation of new studies, we have found that there is

not enough evidence to clearly determine whether an association exists between early-life

NNS exposure and metabolic health.

Opportunities for Future Research

As the obesity prevalence in children continues to increase, small dietary changes in this

population are being encouraged (37, 38). Despite increasing availability and

consumption of NNS-containing food products (39), there is limited evidence to support

recommendations for safe intake levels in children. Only two RCTs to date (28, 35) have

sufficient duration in children under the age of 12 to show causal effects of NNS

exposure, yet these studies reported contradictory associations. With RCTs providing the

strongest evidence for causation, more trials, as well as extended follow up of current

studies, are required to progress the understanding of NNS exposure in metabolic health

in children. Longitudinal observational studies are also required, particularly when long-

term consumption and/or effects are of interest, since RCTs are often not feasible under

these circumstances. Additionally, observational studies are often a superior method of

studying sensitive populations that are challenging to randomize, for example pregnant

women. To date, only nine prospective studies have investigated the metabolic effects of

NNS exposure in gestation, infancy, and children under the age of 12, yielding limited

and inconsistent evidence. Moreover, all cohorts evaluated ASB consumption yet NNS

are widespread in many foods; better exposure assessments are required. Additional

prospective cohort studies are imperative to investigating long-term associations of NNS

intake in this under-studied population.

As previously discussed, NNS exposure has been linked to metabolic

derangements in adults (11), yet our findings indicate that no data from long-term studies

exist to evaluate the metabolic health impact in infants and children. Suez et al. (14)

demonstrated the development of glucose intolerance in four out of seven healthy human

volunteers aged 28-36 after consumption of the ADI of saccharine over a 7 day time

period (14). Other epidemiological data have demonstrated that NNS consumption may

increase the risk for diabetes, metabolic syndrome and cardiovascular disease in adults

(11-13). With the growing understanding of the potential metabolic role of NNS in

adults, further research is crucial to identify the impact in children.

Findings from the review also highlight the gap in current research and the need

for evaluation of NNS exposure in the gestational and neonatal period, as it is becoming

more clear that predisposition to metabolic disease may be acquired or "programmed"

early in life (16). This has been demonstrated in animal studies where a high sucrose diet

in the perigestational period induces hyperinsulinemia, impaired glucose tolerance, and

increased adiposity in offspring (40, 41). Additionally, NNS consumption in animals

during pregnancy predisposes offspring to later-life development of obesity and

metabolic syndrome (16). Some of these findings have been confirmed in human studies,

where mothers with adverse metabolic health issues, such as gestational diabetes (GDM),

high caloric intake, or hyperlipidemia, have offspring who are at an increased risk of

developing metabolic conditions later in life (16, 42). However, no human studies to date

have investigated the effect of prenatal NNS exposure on metabolic health. Maslova et

al. (31) identified an association between ASB intake in pregnancy and increased

incidence of asthma and allergic rhinitis in offspring, but metabolic outcomes were not

evaluated. Ultimately, these studies emphasize the sensitivity of the perinatal period and the impact of maternal nutrition on fetal outcomes. As no human studies to date have evaluated the metabolic impact of NNS exposure in the intrauterine and neonatal period, research in this area is required to inform nutritional recommendations for pregnant women and infants.

Strengths and Weaknesses

The strengths of this review include the broad search strategy applied. Not only was it peer reviewed, but it was also expanded to include multiple search terms that may have been overlooked in previous studies. Additionally, our study focused on a unique exposure period, including pregnancy and infancy, which has not been specifically investigated in previous systematic reviews of NNS effects. The results clearly highlight the need for studies on NNS exposure in these populations. There are several ways in which this systematic review should be extended. Future directions include an expanded search of additional databases, including Embase (OVID), The Cochrane Central Database of Controlled Trials (Wiley), Scopus and ISI Web of Science, and the World Health Organization's International Clinical Trials Registry Platform (ICTRP). In addition to electronic searching, abstracts and relevant conference proceedings in EMBASE and Scopus, as well as a grey literature sources could be searched for completeness.

Conclusion

Results from this systematic review indicate that there is limited evidence for the effects of early-life NNS exposure on metabolic health, with no studies to date investigating this association during gestation or infancy, and conflicting evidence from studies in children. Further research is required to fully understand the impact of NNS exposure during gestation, infancy, and childhood.

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References

1. Obesity in adults: Prevalence, screening, and evaluation [Internet]. [cited November 29, 2014].

2. Dietz WH, Robinson TN. Clinical practice. Overweight children and adolescents. N Engl J Med. 2005;352(20):2100-9.

3. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. Jama. 2014;311(8):806-14.

4. Finkelstein E, Trogdon J, Cohen J, Dietz W. Annual Medical Spending Attributable To Obesity: Payer-And Service-Specific Estimates. Health Affairs. 2009;28(5):w822-w31.

5. Canada PHAo. Obesity in Canada. 2011.

6. Dhingra R, Sullivan L, Jacques PF, Wang TJ, Fox CS, Meigs JB, et al. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation. 2007;116(5):480-8.

7. Strauss RS, Pollack HA. Epidemic increase in childhood overweight, 1986-1998. Jama. 2001;286(22):2845-8.

8. Gardner C, Wylie-Rosett J, Gidding SS, Steffen LM, Johnson RK, Reader D, et al. Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. Diabetes care. 2012;35(8):1798-808.

9. Johnson L, Mander AP, Jones LR, Emmett PM, Jebb SA. Is sugar-sweetened beverage consumption associated with increased fatness in children? Nutrition. 2007;23(7-8):557-63.

10. Miller PE, Perez V. Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. The American journal of clinical nutrition. 2014;100(3):765-77.

11. Swithers SE. Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. Trends in endocrinology and metabolism: TEM. 2013;24(9):431-41.

12. Tandel KR. Sugar substitutes: Health controversy over perceived benefits. Journal of pharmacology & pharmacotherapeutics. 2011;2(4):236-43.

13. Romaguera D, Norat T, Wark PA, Vergnaud AC, Schulze MB, van Woudenbergh GJ, et al. Consumption of sweet beverages and type 2 diabetes incidence in European adults: results from EPIC-InterAct. Diabetologia. 2013;56(7):1520-30.

14. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 2014;514(7521):181-6.

15. JE B, AJ H. Paradoxical effects of an intense sweetener (aspartame) on appetite. Lancet. 1986;1:1092-3.

16. Araujo JR, Martel F, Keating E. Exposure to non-nutritive sweeteners during pregnancy and lactation: Impact in programming of metabolic diseases in the progeny later in life. Reproductive toxicology (Elmsford, NY). 2014;49c:196-201.

17. Brown RJ, de Banate MA, Rother KI. Artificial sweeteners: a systematic review of metabolic effects in youth. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity. 2010;5(4):305-12.

18. Pan A, Hu FB. Question about a recent meta-analysis of low-calorie sweeteners and body weight. The American journal of clinical nutrition. 2014;100(6):1604.

19. Jessup A, Harrell JS. The Metabolic Syndrome: Look for it in Children and Adolescents, Too! Clinical Diabetes. 2005;23(1):26-32.

20. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. The American journal of clinical nutrition. 2006;84(2):274-88.

21. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies2013 2013-01-15 23:31:43.

22. Distiller SR 2014: Evidence Partners.

23. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions Versions 5.0.1: The Cochrane Collaboration2008.

24. Julian P, Douglas G, Peter G, al. e. The Cochrane Collaboration's tool for assessing risk of bias in randomized trials. BMJ. 2011:343.

25. GA Wells BS, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analysis 2014 [March 5, 2015].

26.   Berkey CS, Rockett HR, Field AE, Gillman MW, Colditz GA. Sugar-added beverages and adolescent weight change. Obes Res. 2004;12(5):778-88.

27.   Blum JW, Jacobsen DJ, Donnelly JE. Beverage consumption patterns in elementary school aged children across a two-year period. J Am Coll Nutr. 2005;24(2):93-8.

28.   de Ruyter JC, Olthof MR, Seidell JC, Katan MB. A trial of sugar-free or sugar-sweetened beverages and body weight in children. N Engl J Med. 2012;367(15):1397-406.

29.   Kral TV, Stunkard AJ, Berkowitz RI, Stallings VA, Moore RH, Faith MS. Beverage consumption patterns of children born at different risk of obesity. Obesity (Silver Spring). 2008;16(8):1802-8.

30.   Ludwig DS, Peterson KE, Gortmaker SL. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. Lancet. 2001;357(9255):505-8.

31.   Maslova E, Strom M, Olsen SF, Halldorsson TI. Consumption of artificially-sweetened soft drinks in pregnancy and risk of child asthma and allergic rhinitis. PLoS ONE. 2013;8(2):e57261.

32.   Nakai Y, Shinga-Ishihara C, Kaji M, Moriya K, Murakami-Yamanaka K, Takimura M. Xylitol gum and maternal transmission of mutans streptococci. J Dent Res. 2010;89(1):56-60.

33.   Newby PK, Peterson KE, Berkey CS, Leppert J, Willett WC, Colditz GA. Beverage consumption is not associated with changes in weight and body mass index among low-income preschool children in North Dakota. J Am Diet Assoc. 2004;104(7):1086-94.

34.   Striegel-Moore RH, Thompson D, Affenito SG, Franko DL, Obarzanek E, Barton BA, et al. Correlates of beverage intake in adolescent girls: the National Heart, Lung, and Blood Institute Growth and Health Study. J Pediatr. 2006;148(2):183-7.

35.   Taljaard C, Covic NM, van Graan AE, Kruger HS, Smuts CM, Baumgartner J, et al. Effects of a multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: a randomised, double-blind, controlled intervention. Br J Nutr. 2013;110(12):2271-84.

36.   Williams CL, Strobino BA, Brotanek J. Weight control among obese adolescents: a pilot study. Int J Food Sci Nutr. 2007;58(3):217-30.

37.   Definition; epidemiology; and etiology of obesity in children and adolescents [Internet]. [cited December 2, 2014].

38. Rodearmel SJ, Wyatt HR, Stroebele N, Smith SM, Ogden LG, Hill JO. Small changes in dietary sugar and physical activity as an approach to preventing excessive weight gain: the America on the Move family study. Pediatrics. 2007;120(4):e869-79.

39. Q Y. Gain weight by "going diet"? Artificial sweeteners and the neurobiology of sugar cravings. Yale Journal of Biology and Medicine. 2010;83:101-8.

40. Rawana S CK, Zhong S, Buison A, Chackunkal S, Jen CKL. Low dose fructose ingestion during gestation and lactation affects carbohydrate metabolism in rat dams and their offspring. Journal of Nutrition. 1993;123(12):2158-65.

41. Samuelsson AM, Matthews PA, Jansen E, Taylor PD, Poston L. Sucrose feeding in mouse pregnancy leads to hypertension, and sex-linked obesity and insulin resistance in female offspring. Frontiers in physiology. 2013;4:14.

42. Yajnik CS, Deshmukh US. Maternal nutrition, intrauterine programming and consequential risks in the offspring. Reviews in endocrine & metabolic disorders. 2008;9(3):203-11.

Tables

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	1. Prospective, randomized, controlled trial of NNS intervention, or Prospective observational cohort study reporting NNS exposure 2. NNS exposure reported in pregnant women, infants or children under 12 years of age

Exclusion Criteria	1. Non-human studies.
	2. Reviews / commentaries.
	3. Trials:
	   a. Quasi-randomized, cross-over, or cluster randomized design
	   b. NNS effects cannot be examined independently of other intervention components
	   c. Insufficient duration of intervention (< 2 weeks)
	4. Observational Studies:
	   a. Not prospective (cross-sectional or retrospective design)
	   b. Insufficient duration of follow up (< 6 months)*
	5. No outcomes of importance to the review were reported, or available via contact with trial authors.
	
	*Follow up for studies documenting prenatal NNS exposure and reporting outcomes at birth will be considered as 9 months

Table 2. Primary and Secondary Outcomes

	Population*	
	Infants	Children
Primary Outcome		
Change in WFL z-score	X	
Change in BMI z-score		X
Secondary Outcomes		
Growth velocity	X	X
Birth weight	X	
Incidence of overweight/obesity	X	X
Change in total adiposity (% body fat by DEXA or skinfold thickness)	X	X
Change in central adiposity (waist circumference, waist:hip ratio or waist:height ratio)		X
Incidence of Type 2 Diabetes / Impaired Glucose Tolerance (IGT)		X
Incidence of adverse metabolic effects (metabolic syndrome, insulin resistance, glycosylated hemoglobin HbA1C %)		X

BMI, body mass index; WFL, weight-for-length; DEXA, dual-energy x-ray absorptiometry; GFR, glomerular filtration rate.
*Mean age of study population at outcome assessment: infants (birth to 2 years), children (>2 to <12 years)

Table 3. Summary of included studies: population characteristics, NNS exposures, metabolic health outcomes and reported associations.

Observational studies: prospective cohort studies

Study, Country, Enrolment year	Subjects, Health related inclusion criteria	Age at NNS exposure	Follow up duration	% Overweight at baseline; Mean BMI z-score at baseline	Method of dietary assessment	Form of NNS exposure	NNS comparisons made	Major confounders considered*	Outcomes reported	Associations reported
Berkey et al. (26) *Growing Up Today Study* USA, 1996	16,771 children (6688 girls, 5067 boys) No health inclusion criteria	9-14 years	2 years	Boys: 23.2% Girls: 17.5% BMIz not reported	Validated FFQ	ASB; soft drinks	3+ servings per day vs. 0; per daily serving	Baseline BMI, total energy intake, physical activity or inactivity, puberty, ethnicity, age, sex	Change in BMI	NNS associated with increase in BMI in boys, but not girls β (SE) per daily serving: Males: 0.12 (0.05); p 0.02 Females: 0.05 (0.04); p 0.16
Blum et al. (27) USA, 1992	166 children (92 girls, 74 boys) No health inclusion criteria	9.3 $\pm$ 1 years	2 years	29.3% Owt BMIz 0.47	24-hour dietary recall	ASB; soft drinks	Ounces per day	Baseline BMI, total energy intake, age, sex,	Change in BMI z-score	No longitudinal association, but concurrent NNS intake positively associated with BMI z-score
Johnson et al. (9) *Avon Longitudinal Study of Parents and Children* UK, 1991-1992	1,432 children No health inclusion criteria	5 – 9 years	4 years	15% Owt BMIz 0.27	Food diary	ASB; general beverages	Per daily serving	Baseline BMI, total energy intake, physical activity or inactivity, sex	Change in total adiposity (% body fat by DEXA)	NNS intake associated with increase in fat mass βE (95% CI) Age 5: 0.26 (-0.004 to 0.52); p 0.05

30

Kral et al. (29) USA, late 1990's	49 children Children born at high or low risk of obesity based on maternal BMI	3 – 6 years	3 years	% Owt not reported BMIz -0.4 for both low and high risk children	3-day weighted food record (2 weekdays, 1 weekend)	ASB; soft drinks	Mean daily intake (oz)	Baseline BMI, baseline body composition, total energy intake	Change in BMI z-score; change in central adiposity	No association between NNS intake and change in BMI z-score
Ludwig et al. (30) *Planet Health intervention and evaluation project* USA, 1995	780 children No health inclusion criteria	11.7 $\pm$ 0.8 years	19 months	27.4% Owt BMIz not reported	Validated FFQ Youth Frequency Questionnaire	ASB; soft drinks	Per daily serving	Baseline BMI; baseline body composition; total energy intake; physical activity of inactivity; puberty; race; age; sex	Change in BMI; incidence of owt or obesity	No association between NNS intake and change in BMI, but NNS intake negatively associated with obesity incidence (OR 0.44; p 0.03)
Maslova et al. (31) Denmark, 1996-2002	60,466 pregnant women No health inclusion criteria	21-39 years	7 years	27% Owt BMIz not reported	Validated FFQ	ASB; soft drinks	Never vs. >1 serving/day	Maternal age (infant birth weight was not a primary outcome so no other confounders considered)	Infant birth weight	No crude association between maternal NNS intake and infant birth weight
Newby et al. (33) *North Dakota Special Supplemental Nutrition Program for Women, Infants and Children (WIC)* USA, 1995-1998	1,345 children Exclusion of under weight children (BMI < 5th %)	2.9 $\pm$ 0.7 years	6-12 months	Boys: 23% Girls: 18% at risk of overweight BMIz not reported	Validated FFQ	ASB; soft drinks	Ounces per day	Baseline BMI, total energy intake, ethnicity, age, sex, socioeconomic status	Change in BMI; change in weight	No association between NNS intake and change in BMI or weight

Striegel-Moore et al. (34) *The NHLBI Growth and Health Study* USA, 1987	2, 371 girls No health inclusion criteria	9-10 years	10 years	% Owt not reported Mean BMI 18.8	Food diary	ASB; soft drinks	Average grams per day	Total energy intake, ethnicity, age	Change in BMI	No association between NNS intake and change in BMI
Williams et al. (36) USA, 2004	38 girls Obese (BMI $\geq$ 95th percentile)	12-13 years	12 weeks	100% Owt (health inclusion criteria) BMIz not reported	Food diary	ASB; soft drinks	Servings (12 oz) per week	Baseline BMI, sugar intake	Change in BMI	No association between NNS intake and change in BMI

NNS=non nutritive sweeteners; ASB=artificially sweetened beverages; FFQ=food frequency questionnaire; BMI=body mass index; DEXA=dual-energy X-ray absorptiometry; β=beta estimate; SE=standard error; p=p value; 95% CI= 95% confidence interval; Owt=overweight; oz=ounces; NHLBI= National Heart, Lung, and Blood Institute

*Major confounders include: baseline BMI, baseline body composition, total energy, sugar intake, diet quality, physical activity, puberty, race, age, sex, socioeconomic status

Intervention studies: Randomized controlled trials

Study, Country, Enrolment year	Subjects, Inclusion criteria	Age at baseline	Intervention Duration	% Overweight at baseline; Mean BMI z-score at baseline	Form, type and daily dose of NNS	Comparator	Outcomes reported	Associations Reported
de Ruyter et al. (28) *Double-blind Randomized Intervention Study in Kids (DRINK)* Netherlands, 2012	641 children No health-related inclusion criteria	8.2 ± 1.8 years	18 months	18.4% Owt BMIz 0.03	ASB; 34 mg sucralose + 12 mg acesulfame potassium	26 g sucrose beverage	Change in BMI z-score, weight to height ratio, fat mass, sum of 4 skinfolds, waist circumference, % body fat	Reduced weight gain and fat accumulation in NNS group Difference in BMI z-score change from baseline for NNS vs. Control: -0.13 (95% CI -0.20 to -0.06; *p* 0.001)
Nakai et al. (32) Japan, 2010	107 women Pregnant women with high salivary *Streptococci mutans*	30.6 years	13 months	Owt and BMIz not reported	Gum; 3.83 g xylitol	No intervention	Infant birth weight	No significant difference in infant birth weight
Taljaard et al. (35) *BeForMi Study* South Africa, 2013	414 children Participants from baseline screening with the poorest iron status	7.9 ± 1.4 years	8.5 months	Owt not reported, BMIz -0.58	ASB; 25 mg sucralose	20.6 g sucrose beverage	Change in BMI z-score, Weight-for-age z-score	No significant difference in BMI z-score, but higher weight-for-age z-score in NNS group (β value +0.07; 95% CI 0.14 to 0.002, *p* 0.03)

NNS=non nutritive sweeteners; ASB=artificially sweetened beverages; BMI=body mass index; β =beta estimate; SE=standard error; p=p value; CI= confidence interval; Owt=overweight

Table 4A. Summary of Quality Assessment
Prospective Cohort Studies using Newcastle-Ottawa Scale for Quality Assessment

Primary Article	Quality Score (Maximum 9) **	Selection of Study Groups				Comparability		Assessment		
		Represents average child population	Selection from average child cohort	Reliable NNS exposure record	Demonstration that outcome is NOT present at start of study*	Controlled for body composition at baseline	Controlled for elements of diet quality at baseline	Objective assessment of outcome	Follow up ≥1 year	Drop out <30%
Berkey 2004 (26)	7	No	Yes	Yes	n/a	Yes	Yes	No	Yes	Yes
Blum 2005 (27)	7	No	Yes	Yes	n/a	Yes	Yes	Yes	Yes	No
Johnson 2007 (9)	8	Yes	Yes	Yes	n/a	Yes	Yes	Yes	Yes	Unclear
Kral 2008 (29)	7	No	Yes	Yes	n/a	Yes	Yes	Yes	Yes	Unclear
Ludwig 2001 (30)	9	Yes	Yes	Yes	n/a	Yes	Yes	Yes	Yes	Yes
Maslova 2013 (31)	7	Yes	Yes	Yes	n/a	No	No	Yes	Yes	Yes
Newby 2004 (33)	6	No	Yes	Yes	n/a	Yes	Yes	Yes	No	No
Striegel-Moore 2005 (34)	7	No	Yes	Yes	n/a	No	Yes	Yes	Yes	Yes
Williams 2007 (36)	7	No	Yes	Yes	n/a	Yes	Yes	Yes	No	Yes

*Not applicable as all studies reported BMI change from baseline rather than incidence of a binary cardiometabolic outcome.
**Superior quality score highlighted darkest to lightest

Table 4B. Summary of Risk of Bias Assessment
Randomized Controlled Trials using the Cochrane Risk of Bias Tool

Primary Article	OVERALL	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Incomplete outcome data	Selective outcome reporting	Other sources of bias
de Ruyter 2012 (28)	Unclear	Low	Low	Low	Low	Unclear	Low
Nakai 2009 (32)	High	Low	Low	Low	Unclear	High	Low
Taljaard 2013 (35)	Unclear	Unclear	Unclear	Low	Low	Low	Low

Figures

4,591 citations identified from MEDLINE and screened

4,392 abstracts excluded (not relevant; non-human studies; reviews; inappropriate study design; inappropriate study population; insufficient duration)

199 full text articles assessed for eligibility

187 articles excluded:
    42 inappropriate study design
    88 inappropriate intervention or control
    5 inappropriate duration or follow up
    45 no outcomes of interest
    6 non-English publication
    1 unable to access

12 citations included in analysis
    9 Prospective cohort studies
    3 RCTs

Figure 1. PRISMA Flow Diagram

Study or Subgroup	NNS Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
deRuyter-2012	0.02	0.4	225	0.15	0.42	252	88.5%	-0.13 [-0.20, -0.06]
Taljaard-2013 (Control Beverages)	-0.65	0.98	100	-0.58	1.09	102	5.9%	-0.07 [-0.36, 0.22]
Taljaard-2013 (Micronutrient Beverages)	-0.52	0.97	100	-0.55	1.14	102	5.6%	0.03 [-0.26, 0.32]
Total (95% CI)			425			456	100.0%	-0.12 [-0.19, -0.05]

Heterogeneity: Chi² = 1.20, df = 2 (P = 0.55); I² = 0%
Test for overall effect: Z = 3.32 (P = 0.0009)

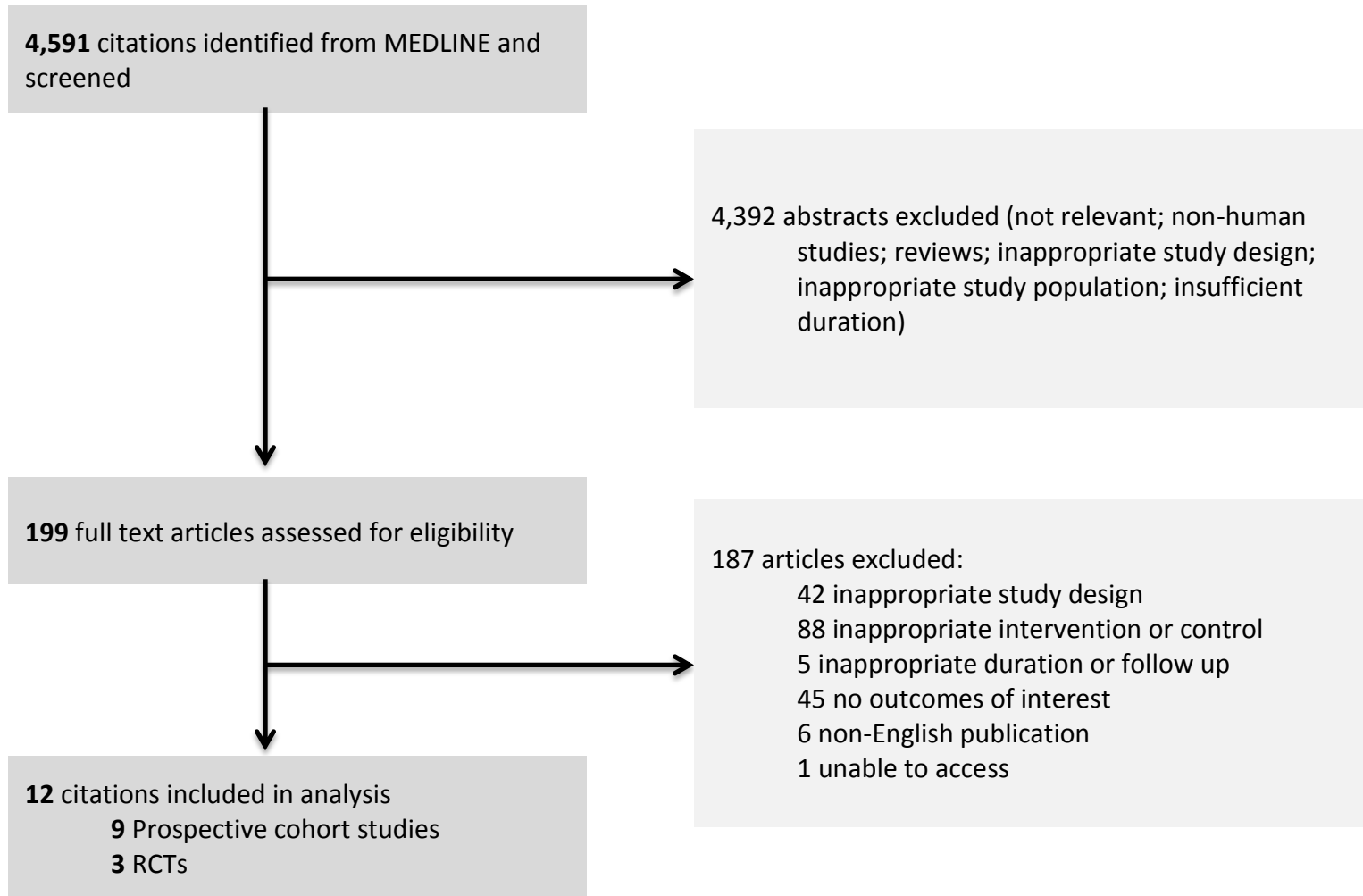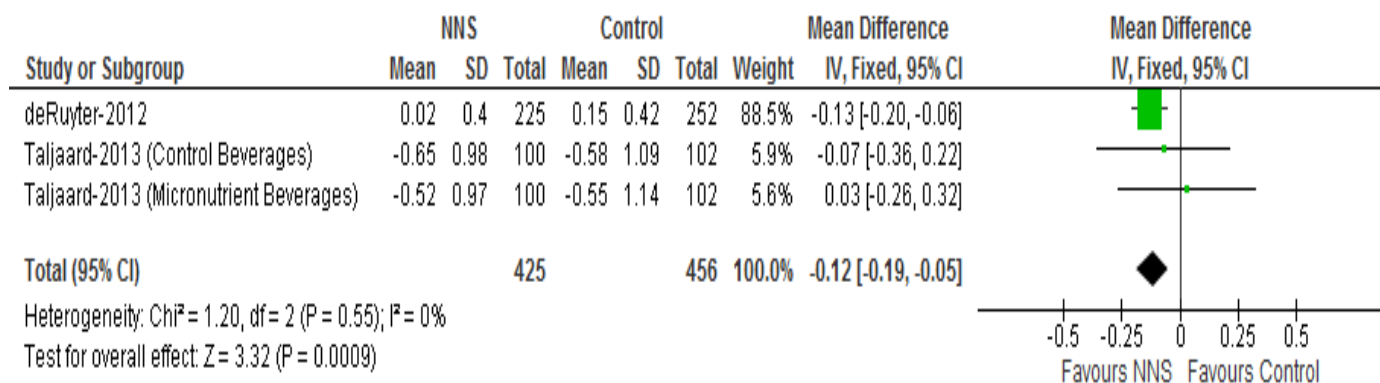**Figure 2. Meta-analysis of RCTs reporting NNS interventions during childhood and change in BMI z-score:** inverse variance fixed effect model.

Appendix I

PubMed/MEDLINE Search Strategy

1 non-nutritive sweeteners/ (18)
2 ((non-nutriti$ or nonnutriti$ or artificial$ or low calori$) adj3 sweeten$).ti,ab. (966)
3 intens$ sweetener?.ti,ab. (128)
4 (artificial sugar? or sugar free or sugarfree or reduced sugar? or sugar replac$ or sugar? substitute or sugar? substitutes or (artificial$ adj3 sweeten$)).ti,ab. (1693)
5 aspartame/ (816)
6 (aspartam? or aspartamum or canderel or (dipeptide adj (sweetener? or sweet)) or trisweet or tri-sweet or (equal adj brand?)).ti,ab. (1045)
7 (neotame or advantame).ti,ab. (62)
8 (asp-phe-ome or aspartylphenyl$ or (aspartyl$ adj3 (ester or phenylalanin$))).ti,ab. [http://chem.sis.nlm.nih.gov/chemidplus/rn/22839-47-0] (149)
9 Saccharin/ (2544)
10 (sac?hari$ or sac?harol or saxin or "sucre edulcor" or sucrette or sweeta or skose or zaharina or garantose or glucid or gluside or hermesetas or kandiset or natreen).ti,ab. (11689)
11 ((benzo$ adj2 sulfimide) or benzosulfimide?).ti,ab. (5)
12 (sucralose or splenda).ti,ab. (321)
13 (trichlorogalacto-sucrose or trichlorosucrose or (trichloro$ adj3 sucrose)).ti,ab. [http://chem.sis.nlm.nih.gov/chemidplus/rn/56038-13-2] (5)
14 (aspartame or saccharin or sucralose or acetosulfam$ or Acesulfam$ or acesulpham$ or "Ace-K").ti. [most commonly used in foods] (1671)
15 Cyclamates/ (495)
16 (cyclamate? or Sugar twin or sucaryl).ti,ab. (623)
17 (cyclamic acid or cyclamsaeure or cyclohexanesulfamic acid or cyclohexylaminesulf$ acid).ti,ab. [http://chem.sis.nlm.nih.gov/chemidplus/rn/100-88-9] (15)
18 (goldswite or hermesetas or milisucre or nozucar or nutrasweet).ti,ab. (20)
19 (necta sweet or sucaryl or sugar twin or "sweet and low" or "sweet n low" or "sweet'n low" or sweet twin or (equal adj2 sweetener?)).ti,ab. (27)
20 (Palatinit$ or Isomalt$).ti,ab. (1672)
21 (Acesul??am$ or acetosul??am? or "Ace-K" or sunetta or "sweet one" or "swiss sweet").ti,ab. (273)
22 xylitol/ (2024)
23 xylitol.ti,ab. (2517)

24   (kannit or lkinit or newtol or xylite or xyliton or eutrit).ti,ab. (38)

25   stevia/ (168)

26   Steviol glycoside?.ti,ab. (110)

27   (stevia or truvia or purevia or enliten).ti,ab. (334)

28   (thaumatin? or tagatose or D-tagatose).ti,ab. (804)

29   (Luo Han Guo fruit extract? or Siraitia grosvenorii or Swingle fruit extract? or monk fruit
extract? or nectresse or purelo or "monk fruit in the raw").ti,ab. (44)

30   Sweetening agents/ or Nutritive sweeteners/ [studies on sugar sweetened foods frequently
report data on artificially sweetened foods] (4470)

31   (sweetening adj2 (agent? or substance or additiv$)).ti,ab. (189)

32   (sweetener? or sweetened or sweetner?).ti,ab. (4754)

33   or/1-32 [Sweeteners] (25534)

34   Carbonated Beverages/ (1924)

35   ((calori$ free or (calori$ adj1 reduced) or diet or low calori$ or low cal or low sugar or non-
calori$) adj2 (beverage? or carbonated or cocktail? or coffee or coffees or cola or colas or "dr
pepper" or drink? or gingerale or ginger ale or iced tea? or lemonade? or limeade? or juice or juices
or pop or pops or punch or punches or refreshment? or root beer or smoothy or smoothies or soda
or sodas or sodapop? or soft drink? or softdrink? or sprite or "7-up" or tea or teas or water?)).ti,ab.
(1196)

36   ((diet or calori$ free or (calori$ adj1 reduced) or low calori$ or low cal or low sugar or non-
calori$) adj2 (candies or candy or chocolate? or frozen dinner? or food or foods or snack$)).ti,ab.
(1696)

37   or/34-36 [carbonated beverages frequently analysed for sugar content/nutritive; so consider
this an independent set to OR with Sweetener set] (4670)

38   (letter or editorial or interview or news).pt. (1415344)

39   ((letter or editorial or interview or news) and (randomized controlled trial or controlled clinical
trial)).pt. (6015)

40   38 not 39 [for exclusion] (1409329)

41   (randomized controlled trial or controlled clinical trial).pt. or randomized.ab. or placebo.ab. or
clinical trials as topic.sh. or randomly.ab. or trial.ti. (928209)

42   exp animals/ not humans.sh. (3972666)

43   41 not 42 [Cochrane RCT Filter 6.4.d Sens/Precision Maximizing] (855824)

44   Pragmatic Clinical Trial/ or Clinical Trial/ or Multicenter study.pt. (616092)

45   (randomi?ed or placebo? or randomly or RCT$1).ti,ab. (643090)

46   random assignment?.ti,ab. (1765)

47   ((singl* or doubl* or trebl* or tripl*) adj (mask* or blind* or dumm*)).ti,ab. (132024)

48   trial.ti. (131316)

49   (controlled adj2 (study or studies)).ti,ab. or (controlled adj2 (trial or trials)).ab. (181503)

50   (multicentre or multicenter or multi-centre or multi-center).ti. or ((multicentre or multicenter or multi-centre or multi-center) adj1 (study or studies or trial or trials)).ab. (51865)

51   random assignment?.ti,ab. (1765)

52   exp "Clinical trials as topic"/ (282845)

53   (or/44-52) not (or/40,42) [Trial terms to supplement Cochrane filter] (1137053)

54   epidemiologic studies/ (6028)

55   Observational Study/ or Cohort studies/ or Longitudinal studies/ or Follow-up studies/ or "national longitudinal study of adolescent health"/ or prospective studies/ or comparative study/ or Intervention studies/ (2492591)

56   "controlled before-after studies"/ (17)

57   Interrupted Time Series Analysis/ (7)

58   ((observational or cohort or longitudinal$ or followup or follow-up or prospectiv$ or comparative or intervention) adj3 (study or trial or trials or studies)).ti,ab. (584854)

59   (control adj3 (area or cohort? or compare? or condition or design or group? or intervention? or participant? or study)).ti,ab. (502217)

60   ("quasi-experiment$" or quasiexperiment$ or "quasi random$" or quasirandom$ or "quasi control$" or quasicontrol$ or ((quasi$ or experimental) adj3 (method$ or study or trial or design$))).ti,ab,hw. (105165)

61   ("time series" adj2 interrupt$).ti,ab,hw. (1127)

62   (time points adj3 (over or multiple or three or four or five or six or seven or eight or nine or ten or eleven or twelve or month$ or hour? or day? or "more than")).ti,ab. (9697)

63   (or/54-62) not (or/40,42) [Observational Designs] (2581192)

64   (or/33,37) and (or/43,53,63) [set 1] (4674)

65   remove duplicates from 64 (4641)