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# Metabolic and behavioural effects of prenatal exposure to non-nutritive sweeteners: A systematic review and meta-analysis of rodent models

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ABSTRACT

Little is known about possible effects of maternal non-nutritive sweetener (NNS) consumption on the metabolic health of a child. Animal models of maternal NNS consumption during pregnancy or weaning have yielded widely varying results, and there appears to be no clear consensus on the consequences for offspring body weight, glycaemic control or sweet preference choices. Moreover, heterogeneity in study design has hampered a clear focus for future research relevant to human health. In an effort to bring clarity, we have conducted a systematic review and meta-analysis (protocol no: CRD42018109509) in animal models (rat or mouse) of maternal NNS feeding (compared to water or basal diet) during pre-gestation, pregnancy or lactation. Four databases were searched from inception to 15th September 2018: PubMed, EMBASE, SCOPUS and Web of Science. We present maternal and offspring data from 24 included studies, which have been quantitatively analysed after study quality assessment, to identify relationships between maternal diet and offspring body weight (BW), feeding behaviour and glycaemic control. In 11 data sets, exposure to NNS reduced maternal BW during pregnancy, with no effect on litter outcomes. Meta-analyses on offspring BW during weaning (1123 offspring) and adulthood (646 offspring) identified small decreases in BW for both sexes. Subgroup analyses revealed reductions in BW of rat, but not mouse models. High dosage appears to be a potential factor for reduced palatability that could influence BW results; however, a lack of reported data limited our ability to confirm. Despite this, and the fact many papers were predisposed to bias, the balance of evidence suggests a maternal NNS diet during pregnancy or lactation did not increase the body weight in offspring.

## 1. Introduction

Consumption of non-nutritive sweeteners (NNSs) has dramatically increased over the last decade [1]. Widespread recommendations to reduce added sugar intake, heightened consumer awareness and implementation of sugar taxes have been catalysts for the greater consumption of NNS-supplemented food and beverages [2,3]. The use of NNSs as a partial or full sugar replacement conveniently sweeten foods whilst reducing energy content and are commonly marketed to consumers wanting to manage body weight. Recent systematic reviews report reductions in energy intake, body weight [4] and BMI [5] when NNS sweeteners were consumed in place of sugar. Nevertheless, links have been claimed between the consumption of NNSs and obesity, alterations to glycaemic control, poor appetite control and changes to the microbiome in humans and animals [6–9].

The prevalence of NNS consumption during pregnancy has risen by nearly 50% over the last 15 years, corresponding with trends in the general population [1]. Approximately one quarter of pregnant women reported drinking or eating NNS products in recent years. From a public health perspective, it is of significant interest to explore if possible adverse effects may be provoked during prenatal exposure through developmental programming. It is well known that maternal nutrition is an important factor for the long-term health of the child and dietary insults can interfere with interactions between genetic and environmental influences during prenatal life [10]. Some recent data identified NNS beverage consumption during pregnancy was associated with an increased risk of infant obesity and elevated BMI [11,12]. However, another study found no link to mid-childhood adiposity [13]. The reported observations could be either a direct effect of NNS on metabolism or a behavioural effect on eating; but of course, correlation studies

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are limited in their ability to determine causal relationships [14].

In the absence of prospective human trials, we analysed animal studies. A number of reviews [15,16] have explored maternal NNS consumption and metabolic implications in offspring. Some of the experimental studies in rat and mouse models focussed on offspring weight, behaviour and glycaemic outcomes [17–23]. Differing results have only generated further controversy in this intensely debated field. One such example was the observation that aspartame promoted increased offspring bodyweight following maternal consumption during pregnancy [19], where others identified no change [24–26]. In part, differences may be due to variability between study methodologies, animal species and strains. The choice of sweetener may introduce variations in results as individual NNSs may elicit different metabolic or sensory responses according to their distinct biological fate [27]. Moreover, the timing of NNS exposure complicates inter-study comparisons, as dams can be fed prior to mating, during pregnancy and/or lactation.

As such, we have conducted a systematic review to clearly collate and summarise the total body of evidence for metabolic and behavioural effects in offspring exposed prenatally to NNS diets. Based on all available evidence, we aim to identify areas for future research that may be relevant for human health, assist in the development of appropriate study designs and reduce unnecessary repetition of already performed animal studies.

## 2. Methods

We report our review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA 2009) Guidelines (S1. PRISMA Checklist). The protocol was developed with the SYstematic Review Centre for Laboratory animal Experimentation's (SYRCLE) Protocol template, Version 2.0 [28], and registered with PROSPERO (Protocol number : 42017109509; Available from http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018109509) on November 8th, 2018 and the Systematic Review Facility for preclinical studies (http://syrf.org.uk/protocols/) on November 1st, 2018.

The key research question is: what are the metabolic and behavioural effects in offspring exposed prenatally to non-nutritive sweeteners (NNS)?

### 2.1. Search strategy and study selection

The following databases were searched: PubMed (all years), EMBASE via OvidSP (1947 to present), Web of Science (all years) and SCOPUS (all years) on the 15th September 2018 and updated iteratively (final search 4th February 2019). An extensive search strategy was constructed using keywords, related synonyms and medical subject headings (MeSH). The final search strategy for each database can be found in S2. Table. Search Strategy. No date restrictions were applied. References extracted from each database were combined and duplicates manually removed in EndNote reference management software (EndNote™ X8) Screening for inclusion was performed by two independent reviewers. Inclusion followed our predefined criteria seen in Table 1. Only studies describing rodents (rat or mouse) consuming NNS as part of their *ad libitum* diet (i.e. NNS available in addition to or mixed with chow or drinking water) were included, to model human consumption. The NNS had to be fed to dams prenatally and/or during gestation and/or during lactation. No limitation was placed on dosage. We defined NNS as any artificially synthesised or natural sweetener that contributes a negligible energy content. They are classified as a broad range of sweetening compounds with differing molecular structures and vary in the way each is absorbed, metabolised and excreted. Included and excluded sweeteners and biological fates of common NNSs can be found in the Supplementary File 3.

**Table 1**
Eligibility criteria.

| *Inclusion criteria* |
|---|
| • Controlled interventional studies |
| • Rodent studies (rat or mouse) |
| • Maternal NNS diet during pre-gestation and/or gestation and/or lactation |
| • Free feeding models |
| *exclusion criteria* |
| • Non experimental studies |
| • Non English studies |
| • No offspring outcomes assessed |
| • No appropriate non-NNS control group for comparison; including nicotine or ethanol comparators for models of prenatal alcohol exposure |
| • Maternal dietary interventions with high fat total energy intake > 10%; including westernised, junk, cafeteria, obesogenic or high-fat high-sugar diets |
| • Intragastric or intraperitoneal feeding models |

Inclusion and exclusion criteria developed *in a priori* and used to assess papers during study selection.

### 2.2. Grouping of papers

For analyses, included papers were divided over four categories according to the primary outcome measure (category descriptors found in S4. Table). The first category comprised studies in which metabolic and behavioural measures of the offspring were the primary outcomes. The second category comprised studies in which the primary measures were offspring's sweet taste preference. The third category comprised studies in which the potential toxicological effects of compulsory consumption of a sweetener were of primary concern. The fourth category comprised studies in which neoplastic incidence in offspring was the primary concern.

### 2.3. Data extraction

Bibliographical details, experimental conditions, sample size, unit of measurement, animal characteristics (species, strain, source, maternal age and weight), maternal dietary intervention (NNS type, dosage, administration during pre-gestation and/or gestation and/or lactation periods, liquid or solid, free access or timed), additional offspring interventions, maternal and offspring outcomes and funding/sponsorship were extracted using a customised data extraction form (S5. Table). Maternal and offspring data for body weight, body composition, litter outcomes, food and fluid intake and glycaemic control were collected as mean and standard error of the mean (SEM) or standard deviation (SD) and entered into an excel spreadsheet. If required, a digital ruler (Pixel Ruler version 3.1) was used for extraction from graphs. If data were not reported or further information was required, attempts to contact the corresponding author were made. Where relevant, separate groups of animals receiving different treatments were treated as separate data sets. Experimental results were summarised as a percentage change of NNS-exposed animals compared to the control group.

### 2.4. Meta-analysis

Following completion of data extraction, it became evident that sufficient information was available to perform meta-analyses. Meta-analyses were then planned for maternal and offspring body weight, and for litter size. A separate protocol was registered with SyRF on the 28th November 2018 (http://syrf.org.uk/protocols/). Relevant data were extracted as mean and SD (SEM was converted to SD where necessary). Following recent meta-analyses of animal data [29–31], different groups of animals from the same paper were included as separate data sets, and thus effectively treated as independent experiments. We performed random-effects meta-analyses using R Version 3.5.1 (2018-07-02) "Feather Spray" for standardised mean differences (SMD) and

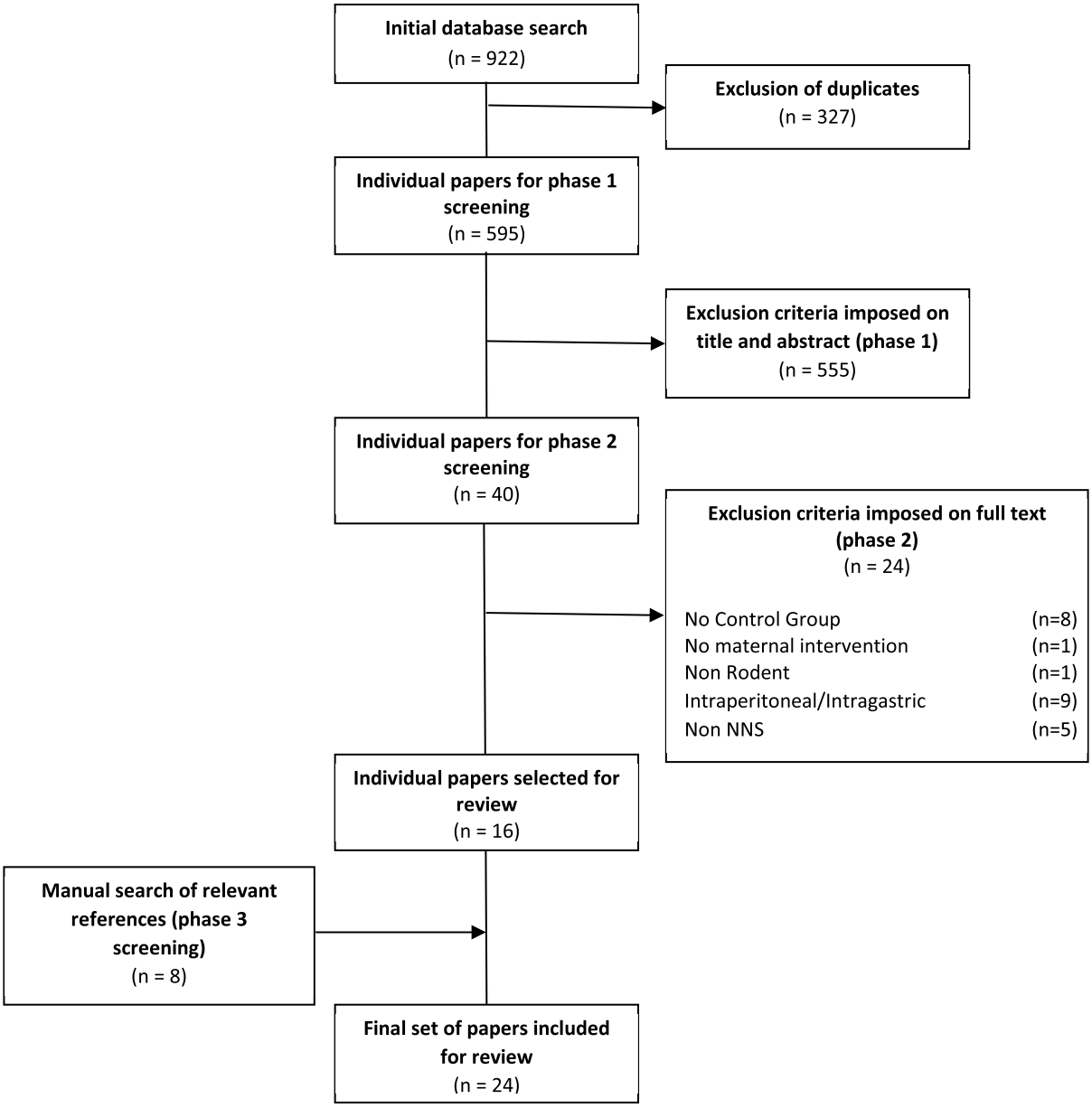


**Fig. 1.** Flow diagram of the paper selection.

corresponding 95% confidence intervals. The *metacont* function from the *meta* package was used for standard analyses; the rma function from the *metafor* package was used for the meta-regression. As several papers included data from multiple experimental groups compared to the same control group, a correction was made using the following equation: $N_{\text{corrected control}} = N_{\text{control}}$/no. of experimental groups. All data are presented as forest plots drawn using the forest function from the *metafor* package.

Maternal body weight was extracted for gestation day 21 (GD21). If such data were not available at this exact time-point, the next closest time-point within the gestational period was selected. Many studies included multiple experimental groups, therefore each group was considered as a separate data set in analyses. To assess the effect of maternal NNS exposure on litter size, the number of pups per litter was analysed for six papers, which yielded 15 separate experimental groups. Offspring body weight (BW) was extracted for weaning at PND21 and adulthood, at PND 30–140. Sub-group analyses on offspring and adult BW were performed for sex and species to explore heterogeneity. A meta-regression analysis was performed to analyse the effect of PND on offspring BW in adulthood. Additionally, we explored the possibility of bias to favourable results in publications funded by the artificial sweetener industry through sub-group analysis.

### 2.5. Study quality assessment

#### 2.5.1. Risk of bias

The included studies were assessed for internal validity using SYRCLE's Risk of Bias (RoB) Tool [32]. This 10-item validated tool has been adapted from the Cochrane RoB Tool [33] for use in systematic reviews on animal models and is recommended by SYRCLE, CAMARADES and NC3R. Rationale for each risk of bias category has been previously described [32]. Items relating to six types of bias: selection, performance, detection, attrition, reporting and other were judged as 'yes' (low risk of bias), 'no' (high risk of bias) or 'unclear' (unable to clearly assign risk of bias) using the reported signalling questions [32]. As industry-funding has previously been shown to be a potential bias toward positive reporting in artificial sweetener studies [34], an in-dustry funding source signalling question was added (item 10). Equally,

we could not assume that independently funded research carried no bias (e.g. white hat bias) [35], however assessment was outside the scope of this review.

#### 2.5.2. ARRIVE guidelines checklist

In addition to RoB, we evaluated reporting quality by assessing adherence to the Animal Research: Reporting of in Vivo Experiments (ARRIVE) Guidelines Checklist [36]. This checklist was developed in 2010 as a comprehensive tool to improve reporting in animal studies. Historically, poor reporting in animal studies has contributed to a lack of reproducibility, animal waste and failure in translation to human trials [37]. Assessment of reporting allows greater transparency for missing information and thus aids in evaluating the reliability of each study. We have modified and developed the ARRIVE Guidelines into evaluation descriptors and a reporting system (S6. Table. Arrive Descriptors) similar to evaluation tools previously described [38,39]. Briefly, the 20-item checklist has been extended to include 40 associated sub-items relevant to this review. Items are described as 'fully reported', partially reported or non-reported. For items with associated sub-items fully reported means that all sub-items were reported, partially reported that not all sub-items are fully reported, and not reported that none of the sub-items was reported.

## 3. Results

### 3.1. Study selection

Initial database searches yielded 922 papers, of which 595 remained after removal of duplicates. Screening based on exclusion criteria was applied to title and abstract (phase 1; 555 papers excluded) and to full text (phase 2; 24 papers excluded) which resulted in 16 included papers. During full-text screening, eight papers were excluded due to the absence of a non-NNS control group, one paper was excluded as no maternal intervention was applied, one paper was excluded as it was conducted in humans, nine papers were excluded because they were non free-feeding models (such as intragastric or intraperitoneal administration) and five papers were excluded as the intervention was not considered to involve a non-nutritive sweetener (e.g. D-tagatose and sugar alcohols). A full list of excluded papers and exclusion reasons, along with type of NNS is presented in the Supplementary files (S7. Table Excluded Papers). Searching reference lists yielded a further eight papers for inclusion. In total, 24 papers were identified for assessment in the systematic review (Fig. 1).

### 3.2. Study characteristics

Study characteristics varied for (rodent) species and strains, maternal age and weight, type and timing of NNS administered and dosage (See S8. Table. Study Characteristics). Mice (C57BL/6J and ICR) were used for the majority of metabolic and behavioural and sweet taste preference papers [17–23,40,41], only one study used Wistar rats [42]. Toxicology and neoplastic papers [24,25,43–52] commonly used rat models (Sprague Dawley, Hans Wistar and F344), although two studies (both by the same author) used Swiss mice to investigate potential carcinogenic effects of aspartame [26,53]. A variety of sweeteners (acesulfame potassium - 2 papers; advantame - 1 paper; arruva -1 paper; aspartame - 9 papers; rebaudioside A - 1 paper; saccharin - 7 papers; sucralose - 3 papers; sucralose mixed with acesulfame potassium - 1 paper) were administered in the animal's drinking water or mixed with food (See Table 2). In one study, the sweetener sucralose was provided in a restricted amount of drinking water to mimic human daily consumption [21]. Not always reported, the use of a variety of chow suppliers contributed to differences in composition (S9. Chow composition).

Considerable variation in maternal dosage was present, ranging from equivalent to human acceptable daily intake (ADI) [21] to several thousand fold greater than ADI [24]. It should be noted that toxicological studies commonly administered high doses as the experimental objectives included evaluation of a sweetener's safety for general use in foods. For several studies, [20,22,47,49,50,52,53], we could not calculate the dosage in mg/kg/day due to insufficient data. Timing and duration of maternal exposure also varied greatly, from 70 days pre-gestation to gestation and/or weaning. A summary of maternal dietary interventions and study design is shown in Table 2.

A complete list of all maternal and offspring outcomes for individual studies was tabulated prior to defining relevant metabolic and behavioural outcomes (S10. Table – Maternal and Offspring Outcomes).

### 3.3. Maternal outcomes

An overview of maternal results is shown in Table 3. From the 24 included papers, several dam groups were exposed to varying concentrations of NNS or the same concentration at differing time-points; consequently, 59 separate data sets were included for review. Maternal body weight, food and fluid intakes and litter effects were seldom reported for studies in the behavioural and metabolic, sweet taste preference and neoplastic categories. Additionally, several papers reported outcomes without providing data (as indicated in Table 3. with asterisk symbol *). No paper investigated maternal glycaemic outcomes, such as glucose or insulin levels or glucose tolerance.

#### 3.3.1. Maternal bodyweight and bodyweight gain

Nine papers gave rise to 29 data sets reporting on maternal body weight during gestation (14/29), lactation (3/29) or both (12/29) as seen in Table 3. Nine data sets reported a significant reduction in body weight (BW) compared to control fed dams (6-14%) when measured around gestation day 21 (GD21). One of these sets reported a decrease at both gestation and lactation and one showed no observable change during gestation but a significant increase during lactation. A meta-analysis performed on extractable data from four papers and eleven data sets identified a decrease in maternal BW measured at approximately GD21 (SMD = −0.34, 95% CI = −=0.59, −0.08, $I^2$ = =12%) with a low heterogeneity, as shown in Fig. 2. In over half of these groups (7/11), dams were exposed to NNS 14–70 days pre-pregnancy through to parturition [45,50], whereas the remainder were exposed during pregnancy only from gestational day 6–21 [23,43]. Of particular note, the NNS dosage for all groups exceeded human ADI by several hundred fold and the majority were classified as toxicology studies.

BW gain during pregnancy was measured in 15 experimental sets with six reporting significant reductions (8–19%) relative to non-NNS fed control dams. BW measured during the lactation period, in 15 experimental sets, generally detected no effect. The one paper reporting an increase [51] did not provide data.

#### 3.3.2. Maternal food and fluid intake

Food intake measured during the time of conception, gestation or lactation was reported in eight studies. There was variation in the duration of time that food consumption was measured, ranging from 3 to 7 days. Thus, we averaged to a mean intake of grams/animal/day for each individually housed dam. The majority of studies reported no change in food intake, although two high dosage papers [50,51] observed decreased chow consumption and increased water intake across the same time-period, suggestive of reduced NNS palatability. High concentrations of many NNSs present a bitter aftertaste or have other post-ingestive factors that may lead animals to display a pattern of avoidance [54,55]. One paper investigating rebaudioside A [45] demonstrated a nine per cent increase in food intake during the last week of lactation. Only five studies measured maternal fluid intake as an outcome; two observed no change in intake [40,47] and three reported increased water consumption [49–51].

**Table 2**
Study design and maternal dietary intervention summary. The comparator (control) was plain water where NNS was added to drinking water or standard chow where NNS was mixed with chow.

| Study ID/Year | Animal Species | Animal Strain | NNS type and dose | Equivalent dose mg/kg BW/day | Administration of NNS | Duration of maternal exposure PG | G | L | Additional offspring exposure |
|---|---|---|---|---|---|---|---|---|---|
| *Sweet taste preference studies* | | | | | | | | | |
| Choo 2018 | mouse | C57BL/6 | Sucralose 2ml of 6.7mM | 5.331 | oral via liquid ration daily | 28 | Y | PND1-14 | — |
| Li 2013 | mouse | ICR | Ace K 5, 12.5, 25 & 50mM | NR[b] | added to drinking water *ad libitum* | — | — | PND 4-21 | — |
| Zhang 2011 | mouse | ICR | Ace K 5g/kg preg only & lact only | 400-600 | mixed with standard chow *ad libitum* | — | GD6-21 preg group | PND 1-21 lact group | — |
| *Metabolic and behavioural studies* | | | | | | | | | |
| Zhang 2018 | mouse | C57BL/6 | Saccharin 2% (w/v) | NR | added to drinking water *ad libitum* | 21 | Y | Y | — |
| Olivier-VS 2019 | mouse | C57BL6 | Sucralose & Ace K (combined) ADIx1 & ADIx2 | ADIx1: 5 & 15 ADIx2: 10 & 30 (sucralose & Ace K respectively) | pipetted onto pellet daily | — | Y | Y | — |
| Collison 2016 | mouse | C57BL/6J | Aspartame 0.25g/L | 55.14 | added to drinking water *ad libitum* | 21 | Y | PND0-28 | weaning to 20 weeks age |
| Parlee 2014 | mouse | C57BL/6J | Saccharin 3% (w/v) | NR[b] | added to drinking water *ad libitum* | — | — | Y | — |
| Collison 2012a | mouse | C57BL/6J | Aspartame 0.25g/L | 55.14 | added to drinking water *ad libitum* | 21 | Y | Y | weaning to 20 weeks age |
| Collison 2012b | mouse | C57BL/6J | Aspartame 0.25g/L | 55.14 | added to drinking water *ad libitum* | 21 | Y | Y | weaning to 20 weeks age |
| von Poser Toigo 2015 | rat | Wistar | a) Aspartame 2g/L<br>b) Saccharin 1.35g/L | a) 300 – 400[a]<br>b) 200 – 270[a] | added to drinking water *ad libitum* | 30 | Y | — | — |
| *Toxicology studies* | | | | | | | | | |
| Brathwaite 2013 | rat | SD | Arruva (Monatin) 15000, 30000 & 50000ppm | 1259, 2564 & 4185 | mixed with standard chow *ad libitum* | — | GD6-21 | — | — |
| Otabe 2011 | rat | SD (CD) | Advantame 2000, 10000 & 50000ppm | 164, 833 & 4410 | mixed with standard chow *ad libitum* | 70 | Y | Y | weaning to 2nd generation |
| Curry 2008 | rat | Hans Wistar | Rebaudioside A 7500, 12500 & 25000ppm | 669, 1115 & 2273 | mixed with standard chow *ad libitum* | 70 | Y | Y | weaning to 2nd generation |
| Kille 2000 | rat | SD (CD) | Sucralose 0.3, 1.0 & 3.0% of diet | 500, 1825 & 5750 | mixed with standard chow *ad libitum* | 14 | Y | Y | weaning to 2nd generation |
| Reilly 1990 | rat | SD | Aspartame 500mg/kg | NR[b] | added to drinking water *ad libitum* | — | Y | Y | — |
| Holder 1987 | rat | SD | Aspartame 0.007, 0.036, 0.18 & 0.9% w/v | 14, 68, 347 & 1614 | added to drinking water *ad libitum* | 12 | Y | Y | weaning to PND38 |
| Brunner 1979 | rat | SD | Aspartame 2, 4 and 6% of diet | 1580, 3220 & 4970 | mixed with standard chow *ad libitum* | >14 | Y | Y | — |
| Lapointe 1979 | rat | SD | Sodium Saccharin 0.4mg/ml | NR[b] | mixed with standard chow *ad libitum* | 14 | Y | Y | — |
| *Neoplastic studies* | | | | | | | | | |
| Soffritti 2016 | mouse | swiss | Sucralose 500, 2000, 8000 & 16000ppm | NR[b] | mixed with standard chow *ad libitum* | — | GD12-21 | Y | weaning to natural death |
| Soffritti 2010 | mouse | swiss | Aspartame 2000, 8000, 16000 & 32000ppm | 247, 987, 1919 & 3909 | mixed with standard chow *ad libitum* | — | GD12-21 | Y | weaning to natural death |
| Soffritti 2007 | rat | SD | Aspartame 400 & 2000ppm | 20 & 200 | mixed with standard chow *ad libitum* | — | GD12-21 | Y | weaning to natural death |
| Cohen 1995 | rat | SD & F344 | Sodium Saccharin 0.4mg/ml | NR** | mixed with standard chow *ad libitum* | 14 | Y | Y | weaning to PND91 |
| Garland 1991 | rat | SD | Sodium Saccharin 7.5% of diet | 1283[a] | mixed with standard chow *ad libitum* | 28-42 | Y | Y | weaning to PND30 |
| Taylor 1980 | rat | SD (CD) | Sodium Saccharin 0.01, 0.1, 1.0, 5.0 & 7.5% of diet | NR[b] | mixed with standard chow *ad libitum* | 49 | Y | Y | weaning to 23 months age |

*Abbreviations:* PG – pre-gestation (days), G – gestation (days), L – lactation (days), Y – yes, GD – gestational days, PND – post natal days, NR – not reported, SD – Sprague Dawley, Ace K – Acesulfame Potassium

[a] Dosage (m/kg BW/day) not reported by authors; estimated dosage calculated based on average food/fluid consumption and body weight;

[b] Unable to calculate dosage (mg/kg BW/day) due to insufficient data

**Table 3**
Maternal results for body weight at conception, gestation and lactation, body weight gain, litter outcomes and food and fluid intakes at conception, gestation and lactation.

| Study ID/Year | Experimental Group | Species/ Strain | *n* | Conception | Gestation and Lactation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Age | BW | BW | BW Gain | Pups/ Litter | Litter size | Sex ratio | Food intake | Fluid intake |
| *Sweet taste preference studies* | | | | | | | | | | | | |
| Choo 2018 | Sucralose 5.3mg/kg BW/day | m/ C57BL/6 | — | PND 84 | — | — | — | — | — | — | — | — |
| Li 2013 | Ace K 5mM | m/ ICR | 7 | — | — | — | — | — | — | — | — | — |
| | Ace K 12.5mM | m/ ICR | 7 | — | — | — | — | — | — | — | — | — |
| | Ace K 25mM | m/ ICR | 7 | — | — | — | — | — | — | — | — | — |
| | Ace K 50mM | m/ ICR | 7 | — | — | — | — | — | — | — | — | — |
| Zhang 2011 | Ace K 5g/kg (*preg*) | m/ ICR | 6 | PND 56 | — | NS (G,L) | — | — | NS | NS | NS (G) | — |
| | Ace K 5g/kg (*lact*) | m/ ICR | 6 | PND 56 | — | NS (G,L) | — | — | NS | NS | NS (L) | — |
| *Metabolic and behavioural studies* | | | | | | | | | | | | |
| Zhang 2018 | Saccharin 2% (w/v) | m/ C57BL/6 | — | PND 77-105 | — | — | — | — | NS | NS | — | NS |
| Olivier-VS 2019 | Sucralose/Ace K ADIx1 | m/ C57BL/6 | 10 | PND 70 | — | — | — | — | NS* | NS* | — | — |
| | Sucralose/Ace K ADIx2 | m/ C57BL/6 | 10 | PND 70 | — | — | — | — | NS | NS | — | — |
| Collison 2016 | Aspartame 0.25g/L | m/ C57BL/ 6J | 7-12 | PND 63 | — | — | — | — | — | — | — | — |
| Parlee 2014 | Saccharin 3% (w/v) | m/ C57BL/ 6J | 5 | PND 70 | — | — | — | — | — | NS* | — | — |
| Collison 2012a | Aspartame 0.25g/L | m/ C57BL/ 6J | — | PND 63 | — | — | — | — | — | — | — | — |
| Collison 2012b | Aspartame 0.25g/L | m/ C57BL/ 6J | 7-10 | PND 63 | — | — | — | — | — | — | — | — |
| von Poser Toigo 2015 | Aspartame 2g/L | r/ WS | 4 | PND 120 | — | — | — | — | — | — | — | — |
| | Saccharin 1.35g/L | r/ WS | 4 | PND 120 | — | — | — | — | — | — | — | — |
| *Toxicology studies* | | | | | | | | | | | | |
| Brathwaite 2013 | Arruva 15,000 ppm | r/ SD | 25 | PND 83 | NS | NS (G) | NS(G) | NS | NS | NS | NS (L) | — |
| | Arruva 30,000 ppm | r/ SD | 25 | PND 83 | NS | NS (G) | ↓11 (G) | NS | NS | NS | NS (L) | — |
| | Arruva 50,000 ppm | r/ SD | 25 | PND 83 | NS | ↓7(G) | ↓19 (G) | NS | NS | NS | NS (L) | — |
| Otabe 2011 | Advantame 2000 ppm | r/ SD (CD) | 30 | PND 112 | NS* | NS (G,L) * | NS (G,L) * | NS | NS | NS | NS (C,G,L) | — |
| | Advantame 10,000 ppm | r/ SD (CD) | 30 | PND 112 | NS* | NS (G,L) * | NS (G,L) * | NS | NS | NS | NS (C,G,L) | — |
| | Advantame 50,000 ppm | r/ SD (CD) | 30 | PND 112 | NS* | NS (G,L) * | NS (G,L) * | NS | NS | NS | NS (C,G,L) | — |
| Curry 2008 | Reb A 7500 ppm | r/ HWS | 30 | PND 112 | — | NS (L)† | — | NS | NS | — | NS (C,G,L) | — |
| | Reb A 12,500 ppm | r/ HWS | 30 | PND 112 | — | NS (L)† | — | NS | NS | — | NS (C,G);↑9 (L) | — |
| | Reb A 25,000 ppm | r/ HWS | 30 | PND 112 | — | NS (L)† | — | NS | NS | — | NS (C,G);↑9 (L) | — |
| Kille 2000 | Sucralose 0.3% Fa | r/ SD (CD) | 30 | — | ↓6 | ↓6 (G) | ↓8 (G) | NS | NS | — | — | — |
| | Sucralose 1.0% Fa | r/ SD (CD) | 30 | — | ↓9 | ↓8 (G) | NS (G) | NS | NS | — | — | — |
| | Sucralose 3.0% Fa | r/ SD (CD) | 30 | — | ↓14 | ↓14(G) | NS (G) | NS | NS | — | — | — |
| | Sucralose 0.3% Fb | r/ SD (CD) | 30 | — | ↓6 | ↓6 (G) | ↓11 (G) | NS | NS | — | — | — |
| | Sucralose 1.0% Fb | r/ SD (CD) | 30 | — | ↓7 | ↓6 (G) | ↓12 (G) | NS | NS | — | — | — |
| | Sucralose 3.0% Fb | r/ SD (CD) | 30 | — | ↓8 | ↓13 (G) | ↓9 (G) | NS | NS | — | — | — |
| Reilly 1990 | Aspartame 500mg/kg | r/ SD | — | — | NS | NS (G,L) * | NS (G,L) | NS* | NS* | — | — | NS |
| Holder 1987 | Aspartame 0.007% (w/ v) | r/ SD | 10 | — | NS | NS (G,L) | — | — | NS* | — | — | — |
| | Aspartame 0.036% (w/ v) | r/ SD | 10 | — | NS | NS (G,L) | — | — | NS* | — | — | — |
| | Aspartame 0.18% (w/v) | r/ SD | 10 | — | NS | NS (G,L) | — | — | NS* | — | — | — |
| | Aspartame 0.9% (w/v) | r/ SD | 10 | — | NS | NS (G,L) | — | — | NS* | — | — | — |
| Brunner 1979 | Aspartame 2% diet | r/ SD | — | — | NS* | NS (G) | — | — | NS* | — | NS* | — |
| | Aspartame 4% diet | r/ SD | — | — | NS* | NS (G) | — | — | NS* | — | NS* | — |
| | Aspartame 6% diet | r/ SD | — | — | NS* | NS (G) | — | — | NS* | — | NS* | — |
| Lapointe 1979 | Na Saccharin 0.4mg/ml | r/ SD | 5 | — | — | — | NS (G,L) | — | NS | — | NS* | ↑* |
| *Neoplastic studies* | | | | | | | | | | | | |
| Soffritti 2016 | Sucralose 500 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| | Sucralose 2000 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| | Sucralose 8000 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| | Sucralose 16,000 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| Soffritti 2010 | Aspartame 2000 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| | Aspartame 8000 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| | Aspartame 16,000 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| | Aspartame 32,000 ppm | m /swiss | 20-30 | PND 91 | — | — | — | NS | — | — | — | — |
| Soffritti 2007 | Aspartame 400 ppm | r /SD | 12-16 | — | — | — | — | — | — | — | — | — |
| | Aspartame 2000 ppm | r /SD | 12-16 | — | — | — | — | — | — | — | — | — |
| Cohen 1995 | Na Saccharin 0.4 mg/ml *Exp1* | r/ SD | 17 | PND 70 | NS | ↓8 (G) | — | NS | NS | NS | ↓* | ↑ |
| | Na Saccharin 0.4 mg/ml *Exp1* | r/ F344 | 5 | PND 70 | NS | NS (G) | — | NS | NS | NS | ↓* | ↑ |
| | Na Saccharin 0.4 mg/ml *Exp2* | r/ SD | 40 | PND 70 | NS | NS(G);↑(L) | — | NS | NS | NS | ↓* | ↑ |

(*continued on next page*)

**Table 3** (*continued*)

| Study ID/Year | Experimental Group | Species/ Strain | *n* | Conception Age | Gestation and Lactation BW | BW Gain | Pups/ Litter | Litter size | Sex ratio | Food intake | Fluid intake |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Garland 1991 | Na Saccharin 7.5% diet | r /SD | 30 | PND 70-84 | ↓10* | ↓ (G,L) * | NS(G);↑(L) * | NS | NS | NS | ↓(C); NS (G,L) | ↑ |
| Taylor 1980 | Na Saccharin 0.01% diet | r /SD (CD) | 48 | PND 70 | — | — | — | — | — | — | — | — |
| | Na Saccharin 0.1% diet | r /SD (CD) | 48 | PND 70 | — | — | — | — | — | — | — | — |
| | Na Saccharin 1.0% diet | r /SD (CD) | 48 | PND 70 | — | — | — | — | — | — | — | — |
| | Na Saccharin 5% diet | r /SD (CD) | 48 | PND 70 | — | — | — | — | — | — | — | — |
| | Na Saccharin 7.5% diet | r /SD (CD) | 48 | PND 70 | — | — | — | — | — | — | — | — |

*Abbreviations:* BW – body weight; n – sample size; m – mouse; r – rat; ICR – Institute of Cancer Research; WS – Wistar; HWS – Hans Wistar; SD – Sprague Dawley; SD (CD) – Sprague Dawley (caesarean derived strain); NS – not significant; *preg* – pregnancy group; *lact* – lactation group; C – conception; G – gestation day 21; L – lactation day 21; Ace K – acesulfame potassium; Reb A – rebaudioside A; Na saccharin – sodium saccharin; mg – milligram; g – gram; kg – kilogram; mM – millimolar; ml – millilitre; L – litre; ADI – acceptable daily intake; w/v – weight per volume; ppm – parts per million; Fa – parent generation a group; Fb – parent generation b group; *Exp1* – experimental group 1; *Exp2* – experimental group 2; (↓x) or (↑x) denotes reported percent change from the control group; (↓) or (↑) denotes reported change without magnitude; (—) not recorded; (L)†- taken on PND28; * - Data not provided.

### 3.3.3. Litter outcomes

Data relating to litter outcomes were extracted from 16 studies. Here, we describe the following litter outcomes associated with maternal NNS exposure: the number of live pups per litter, litter size and sex ratio. 15 experimental groups were included in the meta-analysis with no effect identified (SMD = −0.15, 95% CI = −0.35, 0.05, $I^2$ = =0%; Figure shown in S11. Litter Meta-analysis).

## 3.4. Offspring results

Detailed study characteristics, BW at birth, weaning and adulthood, BW gain and body composition for offspring are shown in Table 4. Multiple maternal interventional groups resulted in 59 offspring data sets being included. However, data were not always reported for each outcome. Only a small number of studies (5/24) investigated glycaemic control outcomes. These results are presented separately (Table 5).

### 3.4.1. Offspring birthweight

For the purpose of this study, we defined birthweight as an offspring's weight measured at either GD0, GD1 or during foetal morphological assessment. Data was extracted from 30 groups. However, there was insufficient information available on sample size, SD or SEM for meta-analyses. Only one experimental group reported a reduction in birthweight for males and females [43]. This was the highest concentration group in a toxicity study on arruva, a monatin salt isomer sweetener. All other groups reported no significant effect on birthweight.

### 3.4.2. Offspring bodyweight at weaning

Over half the studies (13/24) reported offspring's BW at weaning (PND21). Further data sets were obtained when separating groups by sex. Overall, 18 experimental groups gave rise to 1123 offspring included in the meta-analysis (Figure 3A). No significant effect in an offspring's BW at weaning was observed; however, high heterogeneity was noted (SMD = −0.31; 95% CI = − =0.67, 0.05; $I^2$ = =86%). Further exploration by subgroup analysis for sex (Fig. 3B) found a significant decrease in BW for both males (SMD = − =0.70; 95% CI = − =1.19, −0.21; $I^2$ = =72%;) and females (SMD = − =0.84; 95% CI = − =1.36, −0.32; $I^2$ = =68%;) when measured at PND21 (Fig. 3B). Studies with only combined-sex groups identified no effect (SMD = 0.28; 95% CI = − =0.09, 0.65; $I^2$ = =68%). When sub grouped by species (Fig. 3C), only rats displayed a small yet significant reduction in BW at weaning (SMD = − =0.54; 95% CI = − =1.00, −0.07; $I^2$ = =84%) whereas mice displayed no effect (SMD = 0.07; 95% CI = 0.13, 0.27; $I^2$ = =0%). Of note, mice data were extracted from behavioural preference studies of lower dose acesulfame potassium exposure during gestation and lactation, whereas the majority of rat data were extracted from toxicological studies of various sweeteners at significantly higher dosages.

### 3.4.3. Offspring bodyweight at adulthood

Data was available for the majority of papers (18/24) and the general meta-analysis included 646 offspring. Overall, we identified a significant reduction in offspring BW measured during adulthood following maternal exposure to NNS during pre-pregnancy, pregnancy and/or lactation (SMD = − =0.95; 95% CI = − =1.64, −026; $I^2$ = =92%) as seen in Fig. 4A. Sub-group analyses determined that females (SMD = − =2.44; 95% CI = − =4.52, −0.36; $I^2$ = =94%) maintained the BW reduction into adulthood, whereas the males (SMD = − =0.47; 95% CI = − =1.39, 0.44; $I^2$ = =86%) and combined-sex groups displayed no effect (SMD = − =0.92; 95% CI = − =2.14, 0.30; $I^2$ = =95%) (Fig. 4B.). Although the results may be skewed by data from one study reporting significant BW loss in females [20]. Sub-grouped by species, mice identified no significant

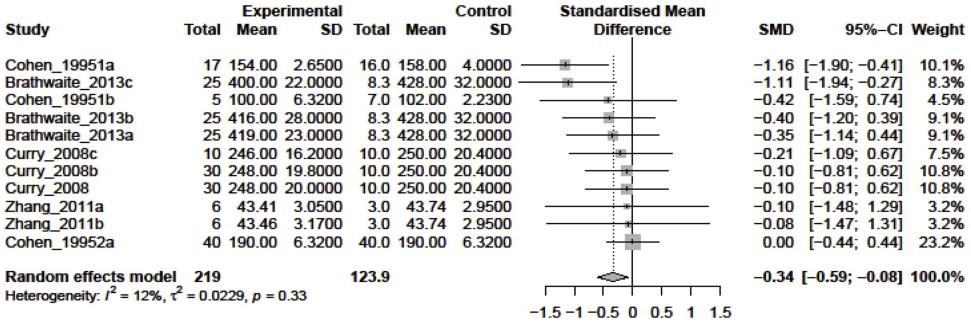


**Fig. 2.** Meta-analysis of maternal outcomes for body weight. Maternal BW measured at approximately GD21. Overall, standardised mean difference (SMD) and 95% confidence interval (CI; upper; lower limits) were estimated by random effects meta-analysis.

**Table 4**
Offspring outcomes for body weight at birth, weaning and adulthood, body weight gain and body composition.

| Study ID/Year | Experimental Group | Species/Strain | *n* | Body weight / Growth | | | | | | Body Composition |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bwt (~PND 0) | Weaning (~PND 21) | Adult | Age adult | BW gain | Age BW gain | |
| *Sweet taste preference studies* | | | | | | | | | | |
| Choo 2018 | Sucralose 5.3 mg/kg BW/day | m/ C57BL/6 | 21 | — | — | ↓7(M); NS (F) | PND 56 | — | — | — |
| Li 2013 | Ace K 5 mM | m/ ICR | 56 | — | NS | NS | PND 49 | — | — | — |
| | Ace K 12.5 mM | m/ ICR | 56 | — | NS | NS | PND 49 | — | — | — |
| | Ace K 25 mM | m/ ICR | 56 | — | NS | ↓5 | PND 49 | — | — | — |
| | Ace K 50 mM | m/ ICR | 56 | — | NS | ↓10 | PND 49 | — | — | — |
| Zhang 2011 | Ace K 5 g/kg (*preg*) | m/ ICR | 71 | NS | NS | NS (M) | PND 56 | — | — | — |
| | Ace K 5 g/kg (*lact*) | m/ ICR | 69 | NS | NS | NS (M) | PND 56 | — | — | — |
| *Metabolic and behavioural studies* | | | | | | | | | | |
| Zhang 2018 | Saccharin 2% (w/v) | m/ C57BL/6 | 15 | NS* | NS* | NS* | PND 90 | — | — | — |
| Olivier-VS 2019 | Sucralose/Ace K ADIx1 | m/ C57BL/6 | 30 | NS | NS | — | — | — | — | — |
| | Sucralose/Ace K ADIx2 | m/ C57BL/6 | 30 | NS | ↓10 | — | — | — | — | — |
| Collison 2016 | Aspartame 0.25g/L (M only) | m/ C57BL/6J | 18 | — | — | ↑9 (M) | PND 140 | — | — | ↑32(M)∞ |
| Parlee 2014 | Saccharin 3% (w/v) | m/ C57BL/6J | 25 | — | NS (M);↓11(F) | NS (M);↓10(F) | PND 98 | — | — | ↓(M);NS(F)¤ |
| Collison 2012a | Aspartame 0.25g/L | m/ C57BL/6J | 12 | — | — | NS | PND 119 | ↑36 (M); NS(F) | PND 42-119 | ↑167(M)∞ |
| Collison 2012b | Aspartame 0.25g/L | m/ C57BL/6J | 18 | — | — | NS | PND 119 | ↑11 (M); NS(F) | PND 42-119 | NS∞ |
| von Poser Toigo 2015 | Aspartame 2g/L | r/ WS | 16 | — | — | — | — | ↑190 (M); NS(F)‡ | PND 80-110‡ | NS‡^ |
| | Saccharin 1.35g/L | r/ WS | 16 | — | — | — | — | NS (M;F) ‡ | PND 80-110‡ | NS‡^ |
| *Toxicology studies* | | | | | | | | | | |
| Brathwaite 2013 | Arruva 15,000 ppm | r/ SD | 370 | NS (M;F) | — | — | — | — | — | — |
| | Arruva 30,000 ppm | r/ SD | 368 | NS (M;F) | — | — | — | — | — | — |
| | Arruva 50,000 ppm | r/ SD | 363 | ↓9(M); ↓8(F) | — | — | — | — | — | — |
| Otabe 2011 | Advantame 2000 ppm | r/ SD (CD) | 395 | NS* | NS* | NS* | — | NS* | — | — |
| | Advantame 10,000 ppm | r/ SD (CD) | 386 | NS* | NS* | NS* | — | NS* | — | — |
| | Advantame 50,000p pm | r/ SD (CD) | 354 | NS* | NS* | NS* | — | NS* | — | — |
| Curry 2008 | Reb A 7500 ppm | r/ HWS | 25-30 | NS (M;F) | NS (M;F) | — | — | — | — | — |
| | Reb A 12,500 ppm | r/ HWS | 25-30 | NS (M;F) | NS (M;F) | — | — | — | — | — |
| | Reb A 25,000 ppm | r/ HWS | 25-30 | NS (M;F) | ↓6(M); ↓8(F) | — | — | — | — | — |
| Kille 2000 | Sucralose 0.3% Fa | r/ SD (CD) | 339 | NS | NS† | NS (M); ↓8(F) | PND 70 | ↓7(M); ↓9(F) | PND 0-70 | — |
| | Sucralose 1.0% Fa | r/ SD (CD) | 370 | NS | NS† | NS (M); ↓9(F) | PND 70 | NS (M); ↓11(F) | PND 0-70 | — |
| | Sucralose 3.0% Fa | r/ SD (CD) | 364 | NS | NS† | NS (M); ↓11(F) | PND 70 | ↓7(M); ↓12(F) | PND 0-70 | — |
| | Sucralose 0.3% Fb | r/ SD (CD) | 364 | NS | NS† | — | — | — | — | — |
| | Sucralose 1.0% Fb | r/ SD (CD) | 370 | NS | NS† | — | — | — | — | — |
| | Sucralose 3.0% Fb | r/ SD (CD) | 435 | NS | NS† | — | — | — | — | — |
| Reilly 1990 | Aspartame 500 mg/kg | r/ SD | — | — | NS | — | — | — | — | — |
| Holder 1987 | Aspartame 0.007% (w/v) | r/ SD | — | — | NS†† | NS | PND 38 | — | — | — |
| | Aspartame 0.036% (w/v) | r/ SD | — | — | NS†† | NS | PND 38 | — | — | — |
| | Aspartame 0.18% (w/v) | r/ SD | — | — | NS†† | NS | PND 38 | — | — | — |
| | Aspartame 0.9% (w/v) | r/ SD | — | — | NS†† | NS | PND 38 | — | — | — |
| Brunner 1979 | Aspartame 2% diet | r/ SD | — | — | — | — | — | — | — | — |
| | Aspartame 4% diet | r/ SD | — | — | — | — | — | — | — | — |
| | Aspartame 6% diet | r/ SD | — | — | — | ↓30(M); ↓14(F) | PND 30(M); 44(F) | — | — | — |
| Lapointe 1979 | Na Saccharin 0.4 mg/ml | r/ SD | 111 | NS (M;F) | — | — | — | NS* | — | — |
| *Neoplastic studies* | | | | | | | | | | |
| Soffritti 2016 | Sucralose 500 ppm | m /swiss | 219 | — | — | NS | PND 56 | — | — | — |
| | Sucralose 2000 ppm | m /swiss | 140 | — | — | NS | PND 56 | — | — | — |
| | Sucralose 8000 ppm | m /swiss | 134 | — | — | NS | PND 56 | — | — | — |
| | Sucralose 16,000 ppm | m /swiss | 131 | — | — | NS | PND 56 | — | — | — |
| Soffritti 2010 | Aspartame 2000 ppm | m /swiss | | — | — | NS | PND 56 | — | — | — |
| | Aspartame 8000 ppm | m /swiss | — | — | — | NS | PND 56 | — | — | — |
| | Aspartame 16,000 ppm | m /swiss | — | — | — | NS | PND 56 | — | — | — |
| | Aspartame 32,000 ppm | m /swiss | — | — | — | NS | PND 56 | — | — | — |

(*continued on next page*)

**Table 4** (*continued*)

| Study ID/Year | Experimental Group | Species/Strain | *n* | Body weight / Growth | | Adult | Age adult | BW gain | Age BW gain | Body Composition |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Bwt (~PND 0) | Weaning (~PND 21) | | | | | |
| Soffritti 2007 | Aspartame 400 ppm | r /SD | — | — | — | NS | PND 112 | — | — | — |
| | Aspartame 2000 ppm | r /SD | — | — | — | NS | PND 112 | — | — | — |
| Cohen 1995 | Na Saccharin 0.4 mg/ml *Exp1* | r/ SD | 29 | NS (M;F) | NS (M;F) | ↓30(M) | PND 91 | — | — | — |
| | Na Saccharin 0.4 mg/ml *Exp1* | r/ F344 | 23 | NS (M;F) | — | NS | PND 91 | — | — | — |
| | Na Saccharin 0.4 mg/ml *Exp2* | r/ SD | 40 | NS (M;F) | ↓19(M); ↓18(F) | — | — | — | — | — |
| Garland 1991 | Na Saccharin 7.5% diet | r /SD | 287 | NS (M;F) | ↓40(M); ↓36(F) | ↓36(M); ↓39(F) | PND 30 | — | — | — |
| Taylor 1980 | Na Saccharin 0.01% diet | r /SD (CD) | 20 | NS (M;F)* | NS (M;F)* | NS (M;F)* | PND 182 | NS | PND 0-210 | — |
| | Na Saccharin 0.1% diet | r /SD (CD) | 20 | NS (M;F)* | NS (M;F)* | NS (M;F)* | PND 182 | NS | PND 0-210 | — |
| | Na Saccharin 1.0% diet | r /SD (CD) | 20 | NS (M;F)* | NS (M;F)* | NS (M;F)* | PND 182 | NS | PND 0-210 | — |
| | Na Saccharin 5% diet | r /SD (CD) | 20 | DNC | DNC | DNC | PND 182 | NS | PND 0-210 | — |
| | Na Saccharin 7.5% diet | r /SD (CD) | 20 | DNC | DNC | DNC | PND 182 | ↓7(M); NS (F) | PND 0-210 | — |

*Abbreviations:* Ace K – acesulfame potassium; ADI – acceptable daily intake; Age adult – age at time of adult BW measurement; age BW gain – number of days BW gain measured; BW – body weight; Bwt – birthweight; DNC – data not clear; *Exp1* – experimental group 1; *Exp2* – experimental group; Fa – parent generation a group; Fb – parent generation b group; g – gram; HWS – Hans Wistar; ICR – Institute of Cancer Research; kg – kilogram; L – litre; m – mouse; mg – milligram; mM – millimolar; ml – millilitre; n – sample size; Na saccharin – sodium saccharin; NS – not significant; ppm – parts per million; PND – post natal day; r – rat; Reb A – rebaudioside A; SD – Sprague Dawley; SD (CD) – Sprague Dawley (caesarean derived strain); w/v – weight per volume; WS – Wistar; (↓x) or (↑x) denotes reported percent change from the control group; (↓) or (↑) denotes reported change without magnitude; (—) not recorded; † -weight taken on PND25; †† - weight taken on PND26; ‡ -weight gain reported after 30 days of chocolate consumption *ad libitum*; ^ - abdominal fat pad weights; ∞ - white adipose tissue; ¤ - nuclear magnetic resonance (NMR) analysis

change (SMD = − −=0.50; 95% CI = − −=1.21, 0.21; $I^{2\ =}$ =92%) and rats showed a considerable decrease (SMD = − −=3.05; 95% CI = − −=5.10, −1.00; $I^{2\ =}$ =85%), although significant heterogeneity still existed (Fig. 4C). Variability in the timing of measuring adulthood BW ranged from 30 to 140 days of age and meta-regression modelling found this had no detectable effect on BW as an outcome, $p = 0.0727$. Sub-group analysis for industry funding as a source of potential bias showed that papers with industry funding generally found no effect on body weight (SMD = − −=0.50; 95% CI = − −=1.21, 0.21; $I^{2\ =}$ =92%) and papers with no industry funding generally found reduced body weights (SMD = − −=3.05; 95% CI = − −=5.10, −1.00; $I^{2\ =}$ =85%). See Fig. 4D.

### 3.4.4. Offspring bodyweight gain and body composition

Bodyweight gain was determined by measuring changes in body weight over a specified time-period. Four papers (17 datasets) reported on BW gain with three datasets reporting a reduction in males and females [46]. In direct contrast, two papers [17,18] reported males having substantial increases in offspring BW gain compared to control (11–36%) and a third reported a dramatic increase of 190% following consumption of novel food (chocolate) over a 30-day period [42]. Body composition was assessed either directly by nuclear magnetic resonance analysis [20] or indirectly by visceral fat mass following resection of abdominal fat pads [17–19,42]. Parlee et al. [20] reported elevated lean mass in male offspring at 8 and 13 weeks of age with concurrent fat mass reduction. No change was evident for females even though there was a reported decrease in female BW beginning postnatal week three. A substantial elevation in visceral fat was observed in male offspring at 20 weeks of age by the Collison group [17,19], corresponding with increases in BW and BW gain.

### 3.4.5. Offspring glycaemic control outcomes

Limited evidence from four papers provided data on fasting or random-fed glucose levels measured in blood or plasma in offspring. Collison et al. [17–19] reported substantial increases in glucose levels in both male (49–2%) and female (25–60%) mice exposed to aspartame from 21 days pre-gestation to the end of weaning. Here, the offspring were exposed to NNS after they were weaned from dams until approximately 20 weeks of age. Similarly, von Poser Toigo et al. [42] found an increase in fasting blood glucose levels (FBGL) in offspring of mothers fed aspartame (males 26%; females 29%); although in this case, exposure lasted from 30 days pre-gestation to the end of pregnancy and was investigated in rats. They also reported no significant changes in FBGL for a saccharin-exposed group. Differing from these results, Olivier-Van Stichelen et al. [41] observed a significant decrease in glucose levels in mice where dams were exposed to a combination of sucralose and acesulfame potassium during gestation and lactation, however FBGL was measured at a considerably earlier age (PND20) compared to the previous studies (PND112-133). Two papers [17, 19] performed random-fed insulin tolerance tests (ITT) on mice aged 19 weeks. Measured as total area under the curve, results displayed a sexually dimorphic response with males displaying increased insulin levels (20–26%) relative to the control group. (See Table 5).

### 3.4.6. Offspring food and fluid intake

Large variations were observed in measurement methodologies in the eleven papers reporting offspring food intake and nine papers offspring fluid intakes. Several provided data for weekly consumption from birth to cull [20,25,26,52,53], reported as either weekly or cumulative mean intake, whereas others measured at single [19,21] or dual time-points [17,18]. The majority of studies calculated the intake by dividing the overall cage consumption by the number of animals per cage [17,18,25,26,42,53], and commonly, the unit of measurement was not clear [17,20,21,52]. A full summary of food and fluid intakes is provided in Supplementary files (S12. and S13. Table. Offspring food and fluid intakes). With regard to food consumption, no significant

**Table 5**
Offspring outcomes for glycaemic control.

| Study ID | Experimental group | Species/strain | *n* | Age | FGL | RF GL | FPI | RF ITT AUCtotal |
|---|---|---|---|---|---|---|---|---|
| Olivier-VS 2019 | Sucralose/Ace K ADIx1 | m/ C57BL/6 | 30 | PND 20 | NS | — | — | — |
| | Sucralose/Ace K ADIx2 | m/ C57BL/6 | 30 | PND 20 | ↓9 | — | — | — |
| Collison 2016 | Aspartame 0.25 g/L (males) | m/ C57BL/6J | 18 | PND 133 | — | ↑10 (M) | NS(M) | ↑26 (M) |
| Collison 2012a | Aspartame 0.25 g/L | m/ C57BL/6J | 18 | PND 133 | ↑49 (M); ↑25 (F) | ↑20 (M); NS(F) | NS(M;F) | ↑21 (M); NS(F) |
| Collison 2012b | Aspartame 0.25 g/L | m/ C57BL/6J | 18 | PND 133 | ↑62 (M); ↑60 (F) | * | * | * |
| Vp Toigo 2015 | Aspartame 2 g/L | r/ WS | 16 | PND 112 | ↑26 (M); ↑29 (F) | — | — | — |
| | Saccharin 1.35 g/L | r/ WS | 16 | PND 112 | NS (M;F) | — | — | — |

*Abbreviations:* Ace K – acesulfame potassium; ADI – acceptable daily intake; Age – age of glycaemic control measurement; AUCtotal – area under the curve total; F – female; FGL – fasting glucose levels; FPI – fasting plasma insulin; g – grams; ITT – insulin tolerance test; L – litre; M – male; m – mouse; n – sample size; NS – not significant; PND – post natal days; r – rat; FR – random-fed; WS – Wistar; (↓x) or (↑x) denotes reported percent change from the control group; (↓) or (↑) denotes reported change without magnitude; (—) not recorded; *Data reported for same group of animals as Collison (2012a).

difference was reported in nine of the eleven studies, one observed a small decrease in the first three days post weaning that recovered by PND30 [51] and another reported males reduced their food intake across the first 24 weeks of life [52]. Interestingly, both studies exposed offspring to high concentrations of sodium saccharin (7.5% of diet) for up to 49 days pre-gestation and continued the intervention post-weaning through to adulthood. Similarly, fluid consumption data was not reported in 15 papers, and in the studies that did report it, the majority (8/9 papers) observed no effect relative to control.

#### 3.4.7. Sweet taste preference outcomes

Only three papers [21–23] reported outcomes relating to sweet taste acceptance and preference in offspring treated with NNS prenatally. As previously described by Myers and Sclafani [56], preference is considered the choice of one flavour over another and can be measured by brief behavioural assays, whereas acceptance is the absolute amount of a tastant consumed, which is commonly measured over longer periods such as 24 or 48 hours (h). For the purpose of this review, we will refer to 48-h two-bottle testing as 'acceptance' testing. The first two papers, authored by the same group, examined the developmental modulation in adult offspring's sweet taste responses following maternal exposure to acesulfame potassium when mixed in drinking water during either pregnancy or lactation [22] or lactation alone [23]. Their initial study reported a reduction in the preference threshold for acesulfame potassium in both pregnancy and lactation groups relative to control during a 48-h acceptance test, suggesting lower concentrations elicit a sweet taste [22]. Preference ratios were observed to be higher than control. Their subsequent study investigated multiple concentrations administered in chow and fed to dams during lactation [23]. In direct contrast, they reported an increase in the preference threshold, suggesting higher concentrations are required to elicit a sweet taste. Observed preference ratios were reduced, particularly in the low to moderate concentrations. Notably, acesulfame K dosage provided to dams significantly exceeded human ADI guidelines. The final paper investigating sweet taste preference [21] had two key differences from the other described work. First, the sweetener sucralose was fed to dams during both pregnancy and lactation and second, the dosage was more relevant to human ADI. This paper also included a second comparator group, being sucrose. The authors reported no difference in response to sweet taste preference during a brief access lickometer test and no change in total intake or preference ratio during a two-bottle 48-h acceptance test to either sucralose or sucrose.

### 3.5. Study quality assessment

#### 3.5.1. Risk of bias

The results of bias assessment are presented in Fig. 5. Results are reported as the percentage of papers per item categorised as low risk of bias (yes), high risk of bias (no) or unclear risk of bias.

Three items (1 to 3) related to selection bias. Only two (8%) of the included papers reported randomisation of animal allocation to create comparable groups using adequate methods, such as computer-generated randomisation or number tables. 11 papers reported using randomisation yet failed to describe the method, therefore were judged 'unclear'. The remaining 11 papers did not mention randomisation at all. Eight (33%) of the papers described similar baseline characteristics for maternal age and weight or weight matching of groups. None of the 24 included papers reported concealing allocation to treatment groups from the investigators. Performance bias relates to systematic differences in the management of care provided to experimental groups [32]. In animal studies, this involves husbandry and blinding of caregivers to an animals' interventional group. The potential for bias was judged high as none of the papers described randomly housing animals and only one paper (4%) described blinding investigators during the study (question 4 and 5).

Detection bias (question 6 and 7) may be introduced by differences in the way outcomes are assessed. Only three (13%) of included papers described randomly selecting animals for outcome assessment, and only one (4% of papers) described blinding the assessor.

Concerning attrition bias (question 8), over two thirds of studies indicated a reason for the observed attrition or exclusion of data, yet 25% were unclear. The majority of studies were assessed as free of selective outcome reporting by comparing methods and results within the paper (question 9). However, it must be noted that no paper made the study protocol available, limiting the reliability of this assessment. Sixteen (67%) papers were assessed as free from potential risk of bias due to industry funding sources (question 10) and one (4%) was unclear. Individual study results for risk of bias can be found in the Supplementary files (S14. Table RoB Results).

#### 3.5.2. Reporting adherence to ARRIVE guidelines

Generally, low to moderate adherence to the guidelines was observed in the majority of studies, with only one item (appropriate title) judged as fully reported. Of concern, two items were assessed as 100% partially reported: item 6 (study design) and item 18 (interpretation and scientific implications). This was predominantly due to lack of reporting of blinding measures, unit of analysis, animal model limitations and study implications for the 3R's in animal research.

Several important items were less than 60% fully reported, including experimental procedures (54% FR; 46% PR), experimental animals (21% FR; 79% PR); mostly due to lack of reported dam details, housing and husbandry (92% PR; 8% NR), sample size (4% FR; 83% PR; 13%NR), allocation of animals to experimental groups (4% FR; 58% PR; 38% NR), statistical methods (50%; 46% PR; 4% NR), baseline data (38% FR; 21% PR; 42% NR) and generalisability/translation (54% FR, 25% PR, 21% NR). Overall adherence of the ARRIVE guidelines per item and individual study assessment can be found in the supplementary files (S15 and S16. Table ARRIVE Adherence Results).

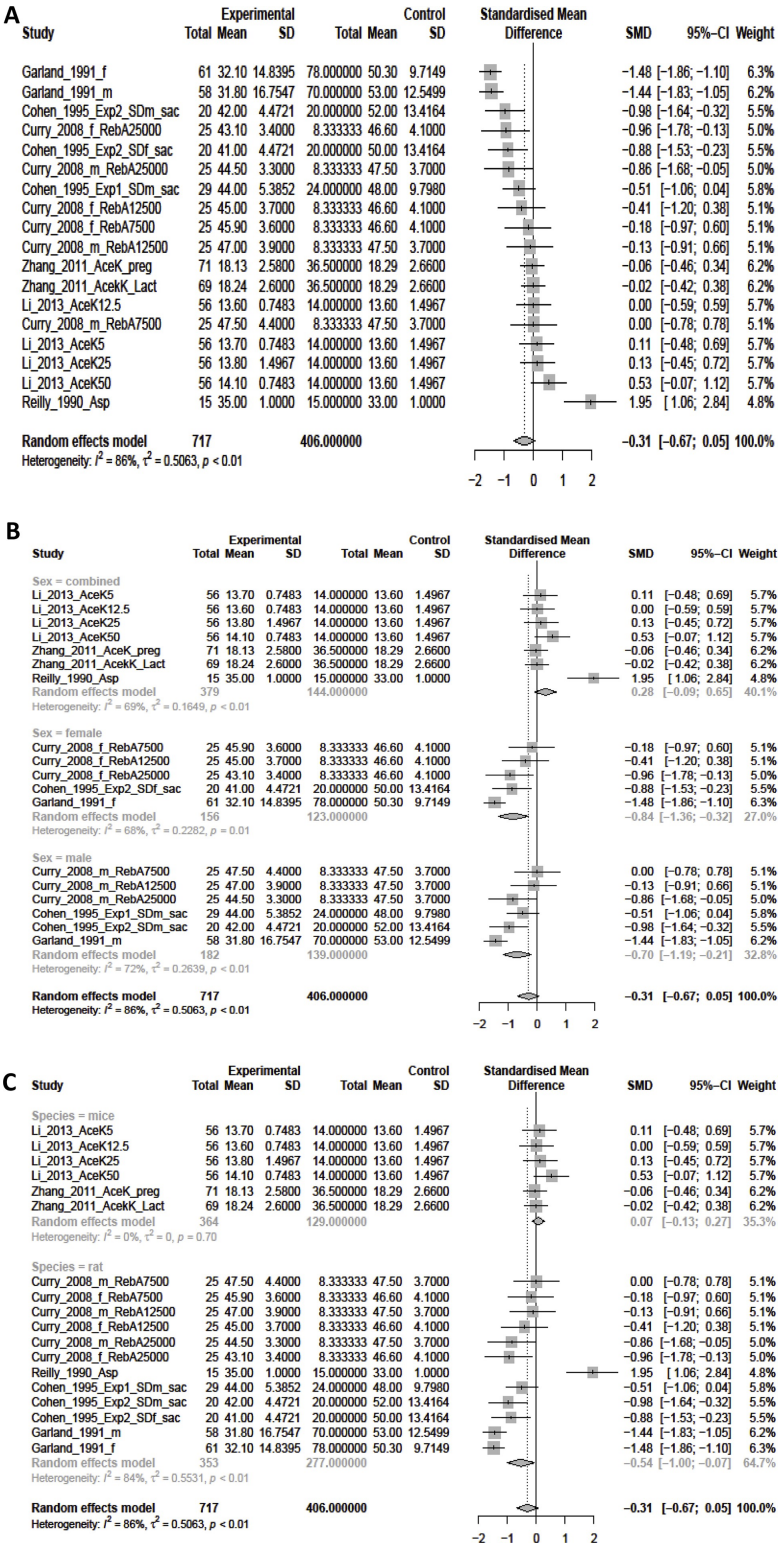


**Fig. 3.** Meta-analysis of wean bodyweight in 1123 included offspring exposed to maternal NNS diets. **A**. combined sex BW, **B**. offspring BW subgroup analysis for sex; separated by males, females and combined sex groups, **C**. offspring subgroup analysis for species; separated by rat and mouse. Estimated standardised mean differences (SMD) and 95% confidence interval (CI; upper; lower limits) were estimated by random effects analysis with heterogeneity expressed as $I^2$ and tau$^2$.

## 4. Discussion

This comprehensive systematic review and meta-analysis was conducted to identify metabolic and behavioural effects in offspring exposed to non-nutritive sweetened maternal diets in rodent models. Included were papers where NNS was administered *ad libitum* during pre-gestation and pregnancy, pregnancy alone or during lactation. We accepted papers that provided offspring with additional NNS exposure after weaning ceased, to capture relevant data.

Whilst meta-analyses show evidence for minor reductions to BW in dams and adult offspring, it was impossible to disassociate if reduced weight gain was a direct physiological action from NNSs or if

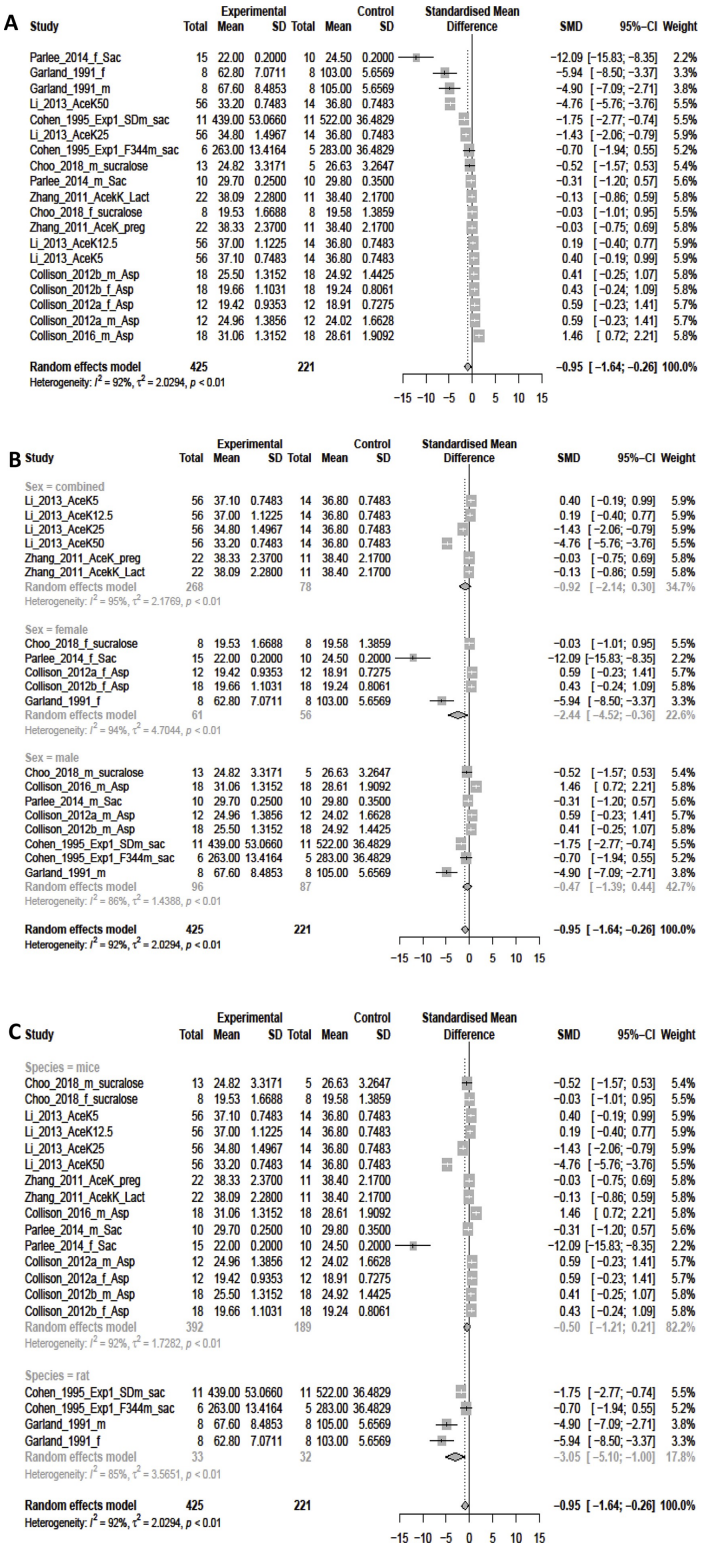


**Fig. 4.** Meta-analysis of adult bodyweight in 646 included offspring exposed to maternal NNS diets. **A**. Combined sex adult offspring BW, **B**. Subgroup analysis for sex; separated by males, females and combined sex groups, **C**. offspring subgroup analysis for species; separated by rat and mouse. **D.** Subgroup analysis for potential funding risk; separated by industry funded and not industry funded papers. Estimated standardised mean differences (SMD) and 95% confidence interval (CI; upper; lower limits) were estimated by random effects analysis with heterogeneity expressed as $I^2$ and tau$^2$.

contributions from unpalatable concentration was a factor. Nevertheless, the bulk of evidence suggest offspring weight did not increase. This outcome is important as it is well known that maternal and childhood obesity are linked to poor metabolic health [57–59]. For other metabolic and behavioural outcomes such as body composition, glycaemic control and sweet taste preference, no overall conclusions could be drawn due to a paucity of data. Moreover, there was significant risk of internal bias across the majority of studies, mainly due to a lack of reporting of randomisation and blinding.

The current analysis was extensive, yet challenged by significant

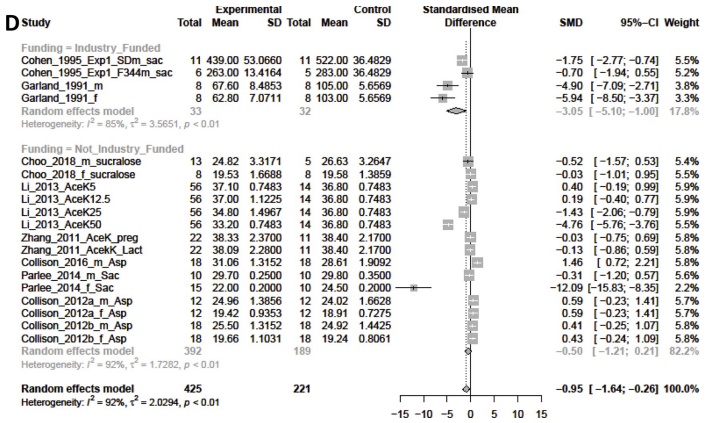


**Fig. 4.** (*continued*)

variability in study design. Given these limitations and the inconclusive nature of some results, the focus turned to present a broader summary of evidence, including gaps in the current literature and recommendations for the future. Several weaknesses were uncovered in the included studies, which have real potential to impact results. This included inappropriate choice of concentration, NNS type, animal model, timing of dietary administration and study design. Taken together, relevance for human metabolic health was difficult to interpret. Accordingly, guidelines have been proposed for key methodological considerations and priorities in determining prenatal effects of NNS in rodent models.

Whilst we recognise certain papers were specifically designed to inform health bodies on toxicological, reproductive and neoplastic effects during pregnancy by administering extreme concentrations, many papers focusing on metabolic and behavioural effects also used supra-physiological doses [20,23,40,42]. Despite strain differences, rodents in general tend to avoid higher concentrations of NNSs due to their bitter aftertaste [54,55], leading to decreased food or fluid consumption. As previously mentioned, the inclusion of high dosage data sets may have skewed our reported results towards bodyweight reduction. A lack of reported data on either BW, food/fluid intake or concentration meant we were unable to calculate dosage in mg/kg/day to determine if this may be the case. To eliminate this weakness in study design, future researchers should carefully select and report concentrations more relevant to levels used in the human food supply.

Nevertheless, a small number of studies reported an increase in the adiposity of male offspring following maternal consumption of aspartame at levels approximating human ADI in C57BL/6J mice [17–19] and high doses in Wistar rats [42]. Given these particular mouse and rat strains do not perceive aspartame as sweet [60,61], one would assume the observed adiposity was not related to behavioural responses, such as stimulated intake or altered preference for sweet foods. There may be other biological mechanisms to explain the reported findings. Some animal studies suggest alterations to gut hormone secretions [62], appetite regulation [8,63] or the microbiota [9] can have implications on

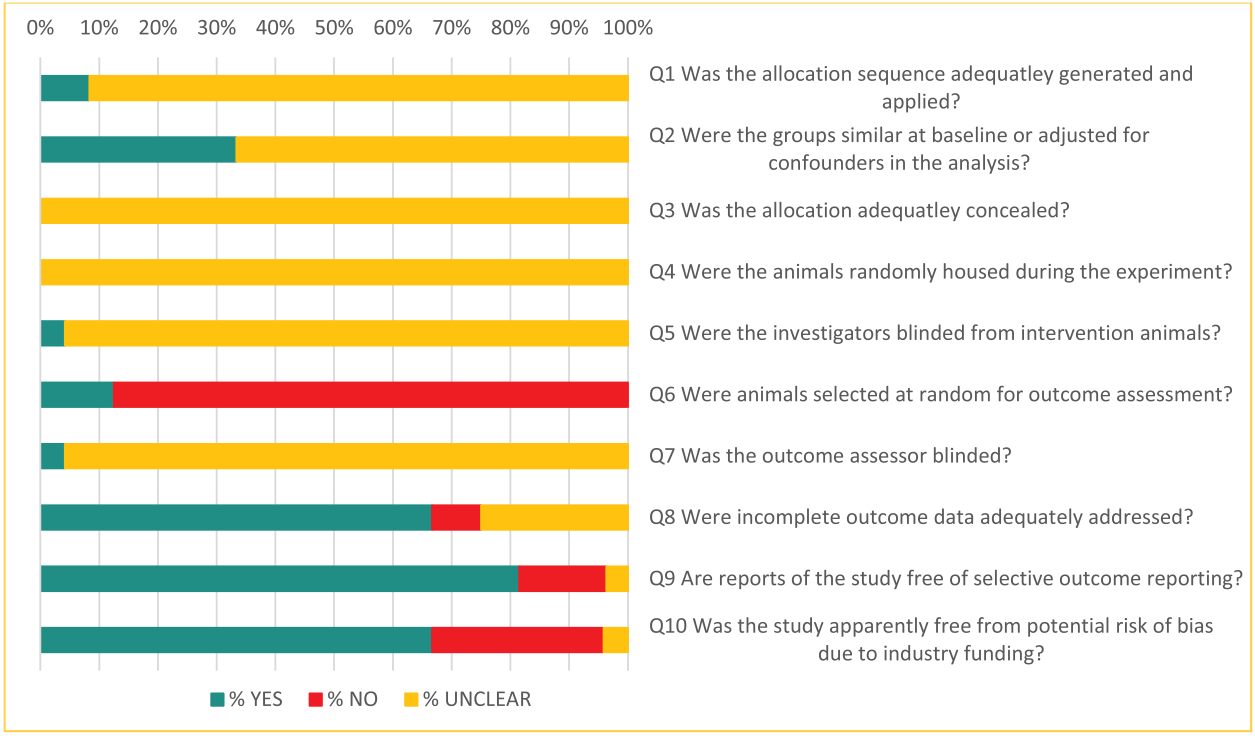


**Fig. 5.** Risk of Bias reported per item according to SYRCLE's tool [32]. Rationale and further detail for the above items was also reported in this tool. Percentages (0–100%) refer to the percentage of included studies with a particular risk of bias score. Two independent reviewers assessed each study for these items and classified as follows: 'YES' = low risk of bias; 'NO' = high risk of bias; 'UNCLEAR' = unable to estimate / assess risk of bias.

body weight and glycaemic control, although most of this work was conducted using saccharin. Further investigation must take into consideration that individual sweeteners have differing biological pathways, which may in turn display a variety of metabolic responses [64]. For example, some NNSs pass through the gastrointestinal tract unchanged, whilst others are rapidly metabolised or absorbed in the small intestines. (See S9.) This implies that perinatal impacts may also be a function of gastrointestinal permeability to the NNS under investigation and the animal model it presents.

Of the included studies, aspartame and saccharin dominated the investigations, yet these make up only a portion of NNSs in the human food supply and are rarely added to products as the sole sweetener. Furthermore, the majority of papers do not mimic the way humans consume NNS, specifically in sweetener combination or feeding patterns. For example, diet beverages often blend two or more NNSs to enhance the sweet taste profile. Yet effects of blended sweeteners are rarely studied in animal studies, let alone maternal investigations. Surprisingly we identified one maternal paper investigating consumption of blended sucralose and Acesulfame K pipetted onto pellets at physiological concentrations [41]. Of interest was that both sweeteners were detected in the mothers' breastmilk and pups' blood and/or faeces at similar, albeit low concentrations. This suggested NNS transmission occurred in perinatal life. Although the presence of NNS in breastmilk may not be sufficient evidence to predict a physiological effect, the authors observed extensive metabolic and gut microbial changes in offspring, suggesting increased potential risk for future metabolic disease. It would be of significant interest if these findings relate to other combinations of NNSs in beverages and foods; including stevia, which has been rapidly introduced into the food chain in recent years [1]. Moving forward, a valid animal model for consumption of commercially available NNS products, for translational relevance to humans, would be of value to assess in both maternal and non-maternal feeding paradigms.

Timing of dietary manipulation is an equally important aspect of study design in maternal feeding paradigms. This review identified half of the studies extended NNS exposure from prenatal to postnatal periods by administering additional NNS diets to offspring following weaning, the majority being toxicological and neoplastic papers [24–26,44–46,50–53]. However three experiments, focussing on metabolism, fed the offspring aspartame into adulthood (20 weeks of age) [17–19]. Confounding of offspring data due to direct and chronic exposure cannot be excluded. Moreover, this limited the ability to assess a temporal impact of NNS exposure during pre-gestation, gestation and/or lactation. These are critical periods of neonatal development when the rapid rate of DNA synthesis is vulnerable to nutritional influences, and timing may be as important as the insult [10,65]. Although our review focussed specifically on maternal NNS consumption, models of over-nutrition suggest the lactation period to be a crucial period for metabolic developmental programming, and effects may be further heightened when feeding occurs during both gestation and lactation [66,67]. The recommendation to cease exposure at parturition and/or weaning or to utilise a cross-fostering approach may sufficiently achieve the delineation of temporality if hypotheses include developmental programming.

Arguably, the most disappointing aspect of this review was identifying a lack of reporting of maternal results. An early focus was to identify a relationship between maternal diet and offspring effects. Yet our interpretation of the maternal metabolic state and offspring development was bounded by the fact extracted data did not always include a dam and her corresponding offspring. None of the included papers focussing on metabolism and/or behaviour, reported relevant maternal outcomes, such as body weight, body composition, feeding behaviour or glycaemic control [17–20,40,41]. This appears to be a consistent pattern in animal research, with previous systematic reviews of maternal feeding citing a paucity of maternal results with a singular focus on offspring outcomes [66,68,69]. Given the mounting human evidence observing that altered maternal metabolic state is associated with increased risk for childhood adiposity and poor glucose tolerance [11,57–59], it seems not only prudent, but overdue that future animal studies investigating offspring prenatally exposed to NNS also report relevant maternal outcomes.

Due to rising interest in the use of NNS as a sugar replacement, glycaemic control was considered a relevant outcome for this review. With only two high risk studies from the same group performing dynamic assessment for whole body insulin disposal [17,18] and a further three reporting fasting blood glucose levels [19,41,42], we identified a clear gap in the research. Results suggest male mice exposed to aspartame from pre-gestation to 20 weeks of age displayed reduced insulin sensitivity, along with elevated fasting glucose levels during random-fed insulin tolerance tests (ITT). However, we cannot ascertain each animal was in a similar 'fed' state at the commencement of the ITT ensuring consistent baseline values, as reporting on feeding methodology was unclear [17,18]. It is apparent further high quality studies with appropriately reported dynamic assessments are needed to link offspring glucose homeostasis and maternal NNS consumption.

An overarching concern in this review is the high risk of bias demonstrated for the majority of studies following critical appraisal. Additionally, there is potential bias in NNS research due to funding sources [34], which can promote over-estimations of interventional effects [70,71]. As such, future animal studies must ensure appropriate randomisation and blinding methods and adhere to reporting guidelines to improve methodological quality and reduce replication of unnecessary experiments.

This review has several strengths, including rigorous study quality

**Table 6**
Considerations for future experiments to improve quality and translation.

| | |
|---|---|
| NNS type and dosage | • Translational consideration is recommended in the selection of NNSs, i.e. widely consumed and ecologically relevant.<br>• The biological pathways of the chosen NNS/s and/or metabolites may determine physiological interactions and perinatal impact.<br>• Concentrations at human physiological levels are recommended for metabolic and behavioural studies. |
| Animal model | • Species and strain differences exist in perception of sweetness for individual NNSs. This may differ to human perception and post-ingestive responses. |
| Timing of intervention | • If primary hypotheses involve developmental origins of disease, consideration of timing is critical e.g., exposure pre-gestation, during pregnancy and/or lactation suggested for metabolic programming. Direct exposure in offspring after weaning may confound effects. |
| Study design | • Randomisation by computerised RNG or similar during allocation and outcome assessment is recommended.<br>• Blinding of allocation to groups and outcomes assessor is recommended, where possible.<br>• Power analysis for appropriate sample size.<br>• Relevant baseline characteristics for dams, e.g. weight, age, glycaemic values, sweet preference ratios etc.<br>• Litter (not individual offspring) used as the unit of analysis. |
| Potential outcomes to be measured | • Relevant maternal and offspring outcomes to identify potential relationships, e.g. dynamic assessment of glycaemic control, fat mass assessment as measure of adiposity, blood chemistry, microbiota. |
| Reporting | • Accurate reporting adhering to ARRIVE guidelines for improvement of transparency.<br>• Accurate reporting of randomisation and blinding.<br>• Selection of animal model and its relevance to other biological systems including humans. |

assessment, inclusive data capture across extensive primary outcomes, transparency on reporting methodology and inclusion of meta-analyses with subgroup analyses. To our knowledge, this is the first fully comprehensive systematic review summarising all available evidence on this highly relevant topic.

### Future recommendations

The current landscape appears to be shifting towards a greater understanding that each NNS has the potential to elucidate its own metabolic or sensory effect. We recommend future systematic reviews investigate maternal consumption of individual sweeteners in animal models, when sufficient evidence becomes available. In addition, we offer important considerations in design, quality and reporting of animal experiments concerning the impact of NNS during pregnancy that were identified in this systematic review (Table 6).

### Conclusions

While meta-analyses results suggest maternal NNS consumption during gestation and/or lactation reduced body weight in pregnant dams and their adult offspring, we could not determine if a physiological effect or if unpalatable concentrations contributed to this reduced weight gain. Regardless, the bulk of evidence suggests when aggregated together, NNSs do not increase offspring BW in rodents exposed during prenatal life, which is contrary to human observational findings [11,12]. Limited evidence was available for glycaemic outcomes and sweet taste preferences in offspring. This review advances our understanding on this complex topic by identifying prior study weaknesses and outlining key considerations for future investigations. We hope the information presented assists in guiding future translational research.

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### CRediT authorship contribution statement

**H.L. Morahan:** Conceptualization, Data curation, Formal analysis, Writing - original draft. **C.H.C. Leenaars:** Data curation, Formal analysis, Validation, Writing - review & editing. **R.A. Boakes:** Supervision, Writing - review & editing. **K.B. Rooney:** Conceptualization, Formal analysis, Supervision, Validation, Writing - review & editing.

### Declaration of Competing Interest

None.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2019.112696.

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