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The impact of non-nutritive sweeteners on fertility, maternal and child health outcomes: a review of human and animal studies

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There is significant evidence that an unhealthy diet greatly increases the risk of complications during pregnancy and predisposes offspring to metabolic dysfunction and obesity. While fat intake is typically associated with the onset of obesity and its comorbidities, there is increasing evidence linking sugar, particularly high fructose corn syrup, to the global rise in obesity rates. Furthermore, the detrimental effects of added sugar intake during pregnancy on mother and child have been clearly outlined. Guidelines advising pregnant women to avoid food and beverages with high fat and sugar have led to an increase in consumption of 'diet' or 'light' options. Examination of some human birth cohort studies shows that heavy consumption (at least one beverage a day) of non-nutritive sweetener (NNS) containing beverages has been associated with increased risk of preterm birth and increased weight/BMI in male offspring independent of maternal weight, which appears to be offset by breastfeeding for 6 months. Rodent models have shown that NNS exposure during pregnancy can impact maternal metabolic health, adipose tissue function, gut microbiome profiles and taste preference. However, the mechanisms underlying these effects are multifaceted and further research, particularly in a translational setting is required to fully understand the effects of NNS on maternal and infant health during pregnancy. Therefore, this review examines maternal sweetener intakes and their influence on fertility, maternal health outcomes and offspring outcomes in human cohort studies and rodent models.

Keyword: Sweeteners: Metabolic health: Maternal: BMI

The global rate of overweight and obesity continues to rise each year, with approximately 650 million adults and 340 million children and adolescents currently living with obesity[1]. Obesity is associated with an increased risk in weight-related complications such as type 2 diabetes and hypertension[2]. While these conditions are typically associated with adults, the alarming rise in cardiometabolic disease in children and adolescents is cause for concern both from an individual health and wellbeing perspective[3] but also an economic outlook, with current research showing that the estimated total lifetime cost of childhood overweight and obesity on the island of Ireland is now 7·2

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billion euro (COSI, 2020)[4]. Multiple factors contribute to the increasing rates of obesity including genetics, sedentary lifestyle and poor-quality diet. There is also clear evidence that exposure of the foetus to environmental stressors such as poor maternal nutrition during gestation can predispose offspring to obesity and its associated comorbidities[5–7]. With almost two-thirds of women of reproductive age currently overweight or obese, childhood obesity is likely to remain a major issue[8]. It is therefore essential that the underlying causes which pre-empt early life-induced health complaints are comprehensively investigated so that realistic therapeutic strategies, which incorporate an individual's life history, can be developed to reduce the healthcare burden which will inevitably arise at an earlier life stage as a result of the burgeoning childhood obesity epidemic.

Fat consumption has traditionally been attributed to obesity; however, it is now clear that foods and drinks with added sugars are contributing significantly to the obesity epidemic[9–11]. Furthermore, the detrimental effects of sugar consumption during pregnancy, on both mother and child, have been clearly outlined[12–14]. The WHO recommends that dietary intakes from added sugars are limited to no more than 10 % of total energy intake but this recommendation is generally not adhered to, with adult women consuming more added sugars compared to men and younger adults consuming higher intakes compared to older adults[15]. This has resulted in several policy initiatives to limit use of added sugars in food production including taxes on sugar-sweetened beverages (SSB), education campaigns and front-of-pack labelling. These policies have resulted in reformulation of foods and beverages to remove added sugars or replace them with non-caloric nutritive sweeteners (NNS)[16], which along with the general perception of NNS as a healthier alternative, has resulted in increased consumption and availability of artificial sweeteners via 'diet' beverages and foods. This trend, however, is more prevalent in women, particularly during pregnancy[17,18]. While several compounds have been approved, the most widely used are acesulfame-potassium and aspartame[19] with natural NNS such as stevia also becoming popular. A recent study found that sales of NNS have increased globally by 36 % and the overall sweetness of packaged foods has increased over time[16].

Despite being viewed as the 'healthier' option[20], there is much controversy in relation to the health effects of NNS with links to cancer[21,22] albeit in cell lines rather than humans, neurological effects in mice[23,24] and obesity and metabolic dysfunction in mice and human associative data[25–27]. Of the RCTs that have been conducted in relation to body weight and reduced energy intakes, those that used NNS as a replacement for SSB were more effective than those who compared with water, summarised in a meta-analysis and systematic review by Rios-Leyvraz and Montez[28]. Nonetheless, this has raised questions about the effects of NNS on long-term health with recent WHO guidelines released around use of NNS within daily acceptable daily intakes (ADI) limits as weight loss agents[29]. As NNS are assessed in relation to toxicology and ADI are recommended, there is a lack of information in relation to health outcomes below these thresholds and given the observation nature of the available evidence guidance on individual sweeteners is not possible.

Concerns are particularly evident during critical developmental periods where several sweeteners are known to cross the placenta and enter the foetal circulation[30–32]. To date, there have been no RCTs examining the impact of NNS during pregnancy on either pregnancy or offspring outcomes. Furthermore, NNS are present in breastmilk representing another source of exposure early in life[33], albeit at relatively low concentrations. Data in this area is reliant on observational cohort studies and animal models. The aim of this review is to examine the evidence of NNS consumption during pregnancy on maternal and offspring outcomes.

NNS and their physiological effects

Sweeteners can be classified as natural or artificial sweeteners and can be further classified as nutritive or non-nutritive sweeteners depending on their calorie content. NNS range from 30–13 000 times sweeter than sucrose[34]. The current list of artificial NNS approved for use in food and drinks in the EU are: acesulfame-k, aspartame, cyclamate, saccharin, sucralose, neohesperidine DC, steviol glycosides, neotame, aspartame-acesulfame salt and advantame. Each NNS is unique in its structure and how they are metabolised, therefore affecting physiological processes differently, and have varying properties, such as sweetness intensity and aftertaste effects[35]. The potential benefits and adverse effects of NNS on health outcomes such as metabolic and gut health vary widely as summarised in Table 1, with most research conducted in animal models.

NNS and their role in metabolic and gut health

NNS, like sucralose, interact with sweet taste receptors (T1R2 + T1R3) in the oral cavity, resulting in the activation of neural pathways to and within the brain that interpret and react to sweet stimuli. Sweet taste receptors are not exclusive to the oral cavity and are located throughout the body including the gastrointestinal tract (GIT), pancreas, adipose tissue, respiratory and reproductive system, with metabolic roles defined in the GIT system. The function of these extra-oral sweet taste receptors was previously unknown, however studies from animal models reveal they may play an important role in in nutrient sensing, monitoring changes in energy stores, and triggering metabolic and behavioural responses to maintain energy balance[56]. The activation of sweet taste receptors in the small intestine promotes glucose uptake and release of incretin hormones such as glucagon-like peptide, an important hormone for insulin release from the pancreas[26]. Furthermore, sweet taste signalling pathways in the GIT mediate the hormonal responses that orchestrate the hunger–satiety cycle. An area which has garnered interest is the ability of NNS to alter the gut

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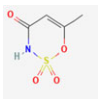
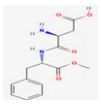
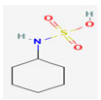
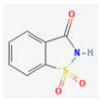
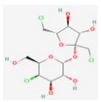
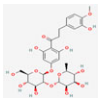
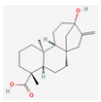
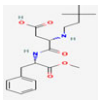
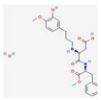
Table 1. NNS and their physiological effects

NNS	Chemical structure	Times sweeter than sugar	Physiological effects of NNS
Acesulfame-k		200×	Increased glucose intolerance and adipocyte size and function[36,37]*, altered gut microbiota composition[38,39]*, increased proinflammatory cytokine expression[40].
Aspartame		200×	Increased AUC insulin concentration, decreased glucose concentrations,[41] decreased plasma GLP-1[42], improved body composition*, altered gut microbiota composition[43] *
Cyclamate		30×	Increased food intake and weight gain[44]*
Saccharin		300×	Increased glucose tolerance, altered microbiota composition and increased proinflammatory cytokine expression[27,45]*, altered gut microbiota composition[45]*
Sucralose		600×	Significant improvement in HbA_{1c}, lipid profile, and BMI[46]. Increased glucose, insulin, and C-peptides in obese individuals[47], decreased blood glucose AUC and increased GLP-1 AUC,[48] decreased insulin sensitivity[49], Decreased acute insulin response and sensitivity and increased GLP-1 AUC[50].
Neohesperidin DC		3,000×	Reduced body weight gain and increased production of anti-inflammatory cytokines[51]*, improved fasting blood glucose altered gut microbiota composition[52]
Steviol glycosides		100–300×	Decreased plasma glucose and insulin levels compared to sucrose[53], reduced weight gain and improved insulin levels[44]*
Neotame		7000–13,000×	Altered gut microbiota composition[54]
Advantame		20,000×	Altered gut microbiota composition[54]*, low body weight and reduced weight gain[55]*

*Evidence from rodent studies.

microbiome which may have negative implications on health. However, it should be noted that this is an area that is far from conclusive and more research on the impact of NNS on the gut microbiome is warranted to truly understand how NNS may alter microbial profiles and whether or not this is significant within the context of human health. As inflammation is frequently associated with disruptions to the gut microbiota and differences in gut microbial composition, it is possible that NNS-mediated changes in microbial profiles may represent a mechanism through which NNS impact metabolic efficiency[57]. However, the role of sweeteners on metabolic health during pregnancy have been less well defined.

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Preconception – sweeteners and fertility

There is very little evidence in relation to effects of NNS on fertility outcomes in either human or animal studies. Chen *et al.*[58] examined 840 Chinese women from the Taipei Women and Infant Nutrition Birth Cohort, 164 of which had fertility issues. NNS intakes were characterised as 0–3 servings a month, 1–4 servings a week or ≥5 servings a week. They found that aspartame consumption, measured using a validated NNS FFQ, was associated with an increased risk for infertility with a dose-response observed. They also found that time to conceive was significantly longer in high (≥5 servings a week) NNS consumers. To investigate further, they followed this work up with an animal study which demonstrated increased oxidative stress and mitochondrial dysfunction in the reproductive system, particularly the granulosa cells of the ovary of Sprague-Dawley rats exposed to 30 mg/kg or 60 mg/kg aspartame for 12 weeks[59].

A series of rodent studies demonstrated several negative impacts of the NNS acesulfame-k on pregnancy and ovarian outcomes in C57/Bl6 mice. They demonstrated that exposure to acesulfame-k (7·5 mM solution in drinking water), but not fructose (34·7 mM solution in drinking water), during pregnancy, resulted in longer time to pregnancy after time-mating. Mice at 10 weeks post-partum who had been exposed to acesulfame-k during pregnancy and lactation had altered expression of genes involved in folliculogenesis and ovulation in the ovary[36,37]. To investigate further, they conducted another study that demonstrated offspring from C57/Bl6 dams exposed to either fructose or acesulfame-k during pregnancy had disrupted oestrus cycles accompanied by gene expression changes in the ovary, an effect that was more pronounced in fructose-exposed offspring[60], however, they stopped short of demonstrating an impact on fertility outcomes in these mice. An additional study which examined stevia (2–3 mg/kg Rebaudioside A) and aspartame (5–7 mg/kg) exposure in drinking water in an obese rat model demonstrated that stevia consumption reduced fertility in dams but all animals eventually went on to give birth[61]. Notably, the obesogenic diet included increased sucrose making it difficult to disentangle the contributions of regular sugar from NNS.

In relation to male fertility, a systematic search of the literature provided no evidence from human studies exploring the impact of NNS on fertility. The evidence from animal studies was collated in a systematic review which identified 9 papers in rodent models[62]. Of the 9 papers identified 4 showed negative impacts of NNS on sperm morphology and function. There was a large degree of heterogeneity in methodologies and study design making any firm conclusion on whether or not NNS have a negative impact on fertility impossible.

Pregnancy complications and sweetener intakes

Preterm birth

Preterm birth is classified as birth prior to 37 weeks of gestation. This can be further broken down into early (less than 26 weeks), moderate (28–32 weeks) and late (32–37 weeks) and can be either spontaneous or medically induced. It is estimated that one in ten infants are born prematurely. Despite the dramatic increase in survival rates, preterm birth remains one of the highest causes of infant mortality globally, particularly in low- and middle-income countries. There are also increased risks for disability, learning disabilities, hearing and vision problems as well as contributing to increased risk for cardiometabolic disease in later life[63]. The causes of preterm birth can be varied with infection/inflammation often implicated. Maternal pregnancy complications such as gestational diabetes and preeclampsia can often result in medically induced preterm birth.

Diet has also been implicated as a risk factor for preterm birth, however, studies can often be inconsistent and are generally derived from prospective cohort studies. Therefore, while associations can be drawn this does not necessarily indicate causality. A recent systematic review showed that Western style diets can increase the risk for preterm birth, while improving maternal diet via adherence to dietary patterns such as the Mediterranean diet or Dietary Approaches to Stop Hypertension (DASH) diet may be beneficial in preventing preterm birth[64]. This review did not specifically include information on nutritive sweeteners or NNS.

The evidence in relation to NNS consumption during pregnancy was limited to three cohort studies[65–67], the results of which have been consolidated in a meta-analysis[68]. No RCTs were identified, stratification based on the sex of the infant was not carried out and some inconsistencies were observed across the studies. Data was derived from FFQ and based solely on consumption of beverages that contained either sugar or NNS. Each study adjusted for common covariates associated with preterm birth such as maternal age, parity, smoking, socio-economic status, education and BMI. However, it must be noted that only one study accounted for previous preterm birth which is undoubtedly one of the biggest risk factors for preterm birth. Petherick *et al.* examined consumption of sugar-sweetened or artificially sweetened cola beverages in a British multi-ethnic cohort between 26 and 28 weeks of gestation and only found an association between the highest consumers of SSB (4 + servings/day) and preterm birth[67]. Halldorsson *et al.* examined NNS and SSB consumption in the Danish National Birth Cohort (*n* 59 334) at 25 weeks gestation and found that while there was no association with SSB, NNS consumption was associated with preterm birth even when adjusted for dose and maternal BMI[66]. Englund-Ogge *et al.* utilised the Norwegian mother and baby cohort at 22 weeks gestation (*n* 60 761) and found that both SSB and NNS were associated with increased risk of preterm birth even when adjusted for previous preterm birth[65]. These studies have demonstrated that consuming SSB and/or beverages containing NNS was associated with increased risk for preterm birth. Meta-analysis of these three studies showed an overall low certainty of evidence from 3 observational studies (*n* 129 009). There was an association between prenatal NNS (>1 serving/day) and preterm birth, which was associated with an 18 % increase in the risk compared with no NNS consumption.

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Similar to human studies there is a lack of data in relation to NNS exposure and preterm birth/gestational length in animal models. One study by Plows *et al.* showed that exposure to a high dose of acesulfame-k (12·5 mM solution in drinking water) during pregnancy resulted in a significant reduction in gestational length in C57/BL6 mice[37]. This study also showed that among the animals who became pregnant (identified by copulatory plug), there was a reduction in successful pregnancy in the acesulfame-k group.

The mechanisms which may underpin the effects of NNS on preterm birth have not been determined. However, several proposed mechanisms have been suggested. One of the major mechanisms which promote preterm birth is inflammation, indeed many murine models of preterm birth are induced through administration of inflammatory agents such as lipopolysaccharide or proinflammatory cytokines[69]. While inflammation is a key initiator of labour in term pregnancies, dysregulation of these processes can result in early delivery. While there is some evidence from mechanistic studies that NNS exposure can result in increased inflammation in organs such as the adipose tissue in humans[18] and,[70] skin[71] and liver[45] in animal models, there is no evidence in relation to how sweeteners influence inflammation in the reproductive organs.

Gestational diabetes

GDM is a major pregnancy complication which is linked to a range of biological and environmental factors. High BMI, increased gestational weight gain, advanced age, ethnicity and unhealthy dietary patterns all influence the onset of GDM. The link between increased added sugar intakes before and during gestation and increased risk for GDM has been established from a range of observational studies[58,72]. There are limited studies in relation to NNS consumption during pregnancy and GDM risk. Hrolfsdottir *et al.* examined an Icelandic cohort of pregnant women and found that the was a higher incidence of GDM in women who consumed beverages containing SSB and/or NNS > 5 times a week[73]. However, as the main aim of this study was to look at dietary patterns, SSB and NNS were grouped together, therefore making it impossible to disentangle the individual effects of added sugar *v.* artificially sweetened products.

Nicoli *et al.* recruited 376 pregnant women at a diabetes care clinic in Pisa, Italy, 102 of which were diagnosed with GDM according to the International Association of the Diabetes and Pregnancy Study Groups (IADPSG)/WHO 2013 criteria[74]. They evaluated NNS exposure using a 16-item semi-quantitative beverage questionnaire demonstrating that 47 % of the participants consumed NNS at least once a week. They found that GDM was more common in NNS consumers (33·3 %) *v.* non-consumers (19·6 %) with diagnosis more commonly attributed to fasting glucose concentrations rather than 1h and 2h post glucose load concentrations in high NNS consumers. After adjustment for known GDM risk factors such as prior GDM, family history of diabetes, and pre-pregnancy obesity, the risk of GDM was still evident in NNS consumers. As Nicoli *et al.*[74] recruited their participants from a diabetes care facility rather than a population-based cohort, it is possible that these women had a higher risk of developing diabetes and this is therefore reflected in the results. Donzar-Ezcurra *et al.* evaluated the association between SSB and NNS and GDM in 3396 women recruited to the Sun study based in Spain[72]. Approximately 5·2 % of their cohort were diagnosed with GDM. Similar to other studies they found that after adjusting for dietary and non-dietary factors, SSB was a significant risk factor for GDM and this risk increased with dose and was independent of pre-pregnancy BMI. However, they did not find any significant increase in the risk of developing GDM in high NNS consumers after adjusting for the same dietary and non-dietary factors.

Given the lack of evidence, it is difficult to make any conclusion on whether NNS increases risk for GDM. The reliance on observational studies also remains an issue, however, given the difficulty of undertaking RCTs in pregnant women given the potential for harm to both the mother and the growing foetus, gathering conclusive evidence in this area remains difficult. Furthermore, given the difficulty in generating appropriate animal models for GDM, deciphering potential mechanisms remains difficult.

Offspring outcomes

Human studies

There are now several decades of evidence in both human studies and animal models to support claims that the in-utero environment has a significant influence on long-term health outcomes in offspring. Given the relatively high rates of NNS consumption during pregnancy it is important to determine whether early life exposure to NNS can impact offspring growth and development. Unfortunately, there is a lack of RCTs examining whether high consumption of NNS during pregnancy can confer increased risk of overweight/obesity during childhood. However, there are several prospective cohort studies which have looked at association between NNS consumption during pregnancy and offspring outcomes, as summarised in Fig. 1.

Five studies from 3 cohorts examined this question. The first of these studies was carried out by Azad *et al.* using 2413 mother/infant dyads from the Canadian CHILD cohort[75]. This data was collected between 2009 and 2012 in the 2nd trimester of pregnancy using FFQs that recorded SSB and NNS beverage consumption. While they were not able to demonstrate an association between SSB during pregnancy and offspring outcomes they showed that compared to non-consumption, daily consumption of NNS in pregnancy was associated with a 0·20 unit increase in infant BMI *z*-score (adjusted 95 % CI, 0·02–0·38) and a 2-fold higher risk of the infant being overweight at 1 year of age (95 % CI, 1·23–3·88). Exploratory analysis found that the effects of NNS consumption on overweight infants were confined to those who were not breastfed for at least 6 months. Additionally, the effect of NNS also differed by sex, with the effects found only in male infants[75].

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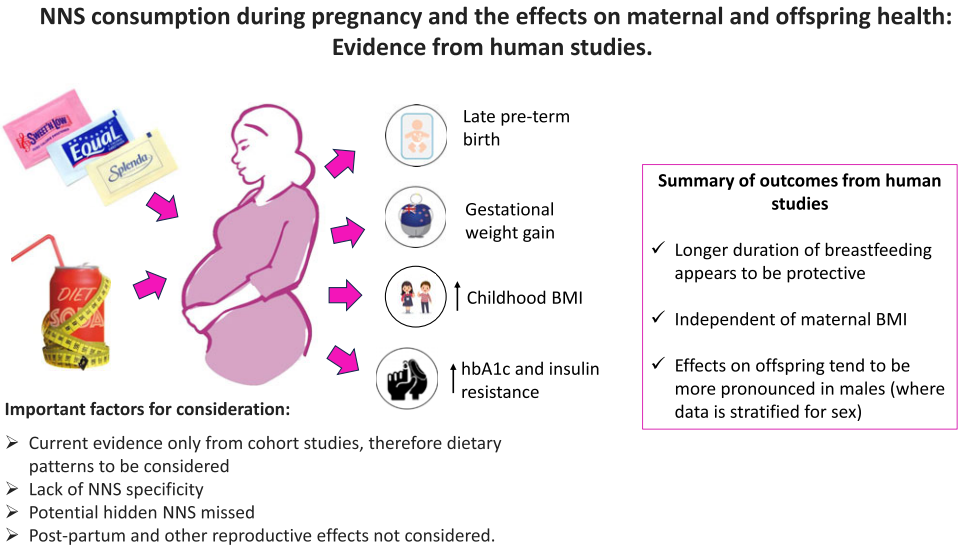

Fig. 1. NNS consumption during pregnancy and the effects on maternal and offspring health: Evidence from human studies.

This study was followed up with an in-depth examination of infant microbiome composition in a sub cohort of the CHILD study containing 50 infants from mothers who had consumed ≥5 servings of NNS beverages and 50 from non-consumers[76]. They confirmed that maternal NNS consumption was associated with higher BMI *z*-scores at 1 year. Using cluster-based analysis, they demonstrated that the effects of NNS on infant microbiome composition were smaller than other known factors such as breastfeeding, birth mode, ethnicity and antibiotic exposure. Succinate was confirmed as common microbial metabolite in the urine of offspring from the maternal high NNS consumers. This metabolite has been linked to microbial metabolism of fibre in the gut and high concentrations can result in inflammation and obesity[77]. Analysis showed that 29 % of the effects of NNS on BMI *z*-scores at 1 year of age were associated with succinate concentrations.

Supporting this, Azad *et al.* followed up on infants at 3 years of age[78]. Consistent with previous findings, results showed that infants born to mothers who consumed NNS from beverages during pregnancy had an increase in BMI *z*-score of offspring, with the increase in BMI remaining significantly higher at 3 years of age than in infants born to mothers who did not consume NNS from beverages during pregnancy, even after adjusting for potential confounders in infancy, pregnancy, and early life (adjusted 95 % CI; –0·03–0·40). Their analysis did however show that full adjustment for maternal and childhood lifestyle factors and maternal BMI at least partially accounts for these BMI changes. Despite observing a sex-specific effect of this interaction at 1 year of age, a sex-specific effect was not observed in this age group.

Zhu *et al.* carried out similar analysis using data from the Danish National Birth cohort study[79]. This study was recruited from 1996–2002 and data was collected from an FFQ administered at 25 weeks gestation, however, analysis was exclusively conducted in women with GDM. In contrast to Azad *et al.* no association was found between maternal consumption of NNS from beverages during pregnancy and offspring BMI *z*-scores at 1 year of age. They found however, that daily NNS beverage consumption was associated with a 1·57-fold increased risk of large for gestational age and a 1·93-fold increased risk of overweight/obesity in children at 7 years of age. These interactions were stable when adjusted for pre-pregnancy BMI, age and smoking. In stratified analysis, they determined that this effect was more pronounced in male children who were breastfed for less than 6 months. They also found a dose relationship with increased daily servings increasing the risk of BMI at 7 years. Additionally, they found that per serving per day substitution of SSB with NNS increased the risk of offspring overweight/obesity at 7 years. The authors noted that women who consumed NNS rather than SSB had differing demographic characteristics and higher loss to follow up in lower SES which may result in underestimation of the true effect sizes.

Contrary to the previous studies, Gillman *et al.*[80] did not find a significant association between NNS consumption during pregnancy and offspring outcomes at 7–8 years. This study analysed 1078 mother/child pairs from the US-based Project Viva study which was recruited between 1999–2002. They measured NNS and SSB intakes using a semi-quantitative FFQ where intakes were based on beverage consumption only. They did see a significant increase in BMI *z*-scores in children of mothers who consumed SSB during pregnancy. Dose, maternal BMI class, gender or ethnicity did not impact the association. In order to rule out the contribution of childhood consumption of SSB they stratified data based on maternal and child intakes and results showed that the effects were primarily driven by maternal consumption.

Further analysis of the Project Viva dataset[81] focused on BMI trajectory over time (birth to 18 years) rather than an isolated time-point. After characterising NNS data into quartiles they found that low *v.* high NNS consumption during pregnancy was not associated with birthweight but there was an association with increased

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BMI in infancy (0·20-unit change) and late childhood (0·21-unit change) and the strength of the association increased with age reaching 0·58-unit change by 18 years of age. Unlike the other studies, paternal factors were included in the analysis. They found that paternal BMI and education level attenuated the impact of maternal NNS consumption on increased BMI in adolescence. They did not account for the gender of the child in the analysis. Differences with Gillman *et al.* may be due to inclusion of data from both diet soda and sweetener packets rather than just diet soda, incorporation of multiple timepoints and consideration of dietary data from both 1st and 2nd trimesters of pregnancy. While the effect size is smaller than Azad *et al.*[78] and Zhu *et al.*[79] it is possible that differences in population demographics, habitual diet patterns and incorporation of dietary factors may influence the overall result. Studies examining the impact of NNS during pregnancy on offspring outcomes in human studies have been consolidated in a meta-analysis by Li *et al.* who have shown a significant increase in risk for increased BMI in offspring from 1 year of age to mid-childhood but not with birthweight[82]. However, it should be noted that the data extracted from the Project Viva study was from Plows *et al.*[37] rather than Gillman *et al.*[80] which may have influenced the final result towards increased NNS risk in the mid-childhood timepoint (7–8 years of age).

Offspring outcomes – mechanistic understanding from animal studies

Given the observational nature of human studies in this area, animal studies are often used to determine causality and decipher potential mechanisms. While this is extremely useful, findings must be interpreted with caution. Animal experiments provide proof of principle but often these model systems are out of the physiological range in terms of dosage/frequency of exposures used by humans and do not necessarily represent human dietary variation. Rodents also display a much faster BMR and a much higher turnover of both protein and RNA (both transfer and messenger RNA)[83]; for example, genomic responses to inflammatory stimuli in rodents are approximately 30–50 times faster than of humans[84]. There are also major differences in the timings of major biological processes such as gestational length, age at puberty etc. While both rodents and humans are altricial, rodents are less developed at birth with minimal adipose tissue deposition etc which may impact the interpretation of data in relation to early life developmental programming of adiposopathies and obesity. Taken together, this means that pharmacokinetics and indeed genomic/protein translational responses along with the rate of clearance of chemicals (including NNS), which can differ between species, further compounds the interpretation of animal studies in relation to human data. Rodents also birth in litters which doesn't represent the human situation. However, there are benefits of using rodent models including physiological similarities such placental structure and similar genetic make-up. Further, it is possible to control the diets of these animals allowing for understanding of the impact of specific nutrients such as nutritive and NNS[85]. It is also possible to understand the individual effects of each NNS which is difficult to determine in human studies. Several potential mechanisms for the actions of NNS exposure during pregnancy on maternal and offspring health have been examined in animal models.

Taste perception

Taste preference and perception are thought to play a major role in the predisposition to overweight and obesity. While genetic factors influence taste, experiential factors are also involved in the overall development of food preferences. Certainly, introduction to various tastes and flavours early in life through the amniotic fluid and breastmilk has the potential to determine food preference in the longer term. Therefore, it is possible that early life exposure to intensely sweetened foods/beverages may have the capacity to influence the preference for sweet foods later in life. The limited evidence available in this area is contradictory although this may be due to the differences in strain of mice and dose and type of sweetener used. Choo and Dando show no impact on taste preference in mice who were exposed in utero to the NNS sucralose[86]. While male and female offspring were assessed, pooled data is presented. However, supplemental data separated by sex showed no difference in sweet taste preference, but a significant increase in fungiform density (structures on the tongue which inform on taste perception) in male offspring in the NNS *v.* sucrose groups. Zhang *et al.* demonstrated that exposure to the NNS acesulfame-k resulted in transfer to both the amniotic fluid and breastmilk after oral exposure[32]. Exposure during pregnancy resulted in increased preference for sweet solutions (either acesulfame-k or sucrose) in the adult offspring. This study was only conducted in male offspring[32]. While sex-stratification was only carried out in two of the cohort studies[32,86], both showed that the effects of NNS exposure (specifically sucralose and acesulfame-k) in utero on BMI *z*-scores were greater in males. Therefore, it is possible that the effects of NNS on taste preference are sex-specific and contribute to the observed increase in weight gain in male offspring. This further highlights the need to examine both sexes in biomedical research, particularly studies examining in utero exposures.

To date, the relationship between child health outcomes and breastmilk NNS concentrations has not been established in humans. Zhang *et al.*[87] examined the impact of acesulfame-k exposure during the lactation period showing that while there were decreases in the threshold for sweet taste, there was increased preference at lower doses. Indeed, acesulfame-k exposure influences taste-bud development in the early post-natal period in mice[87]. Acesulfame-k is known to enter breastmilk in humans[33], and while it is tempting to speculate that this may represent a mechanism for the effects of NNS in childhood, it should be noted that breastfeeding for more than 6 months attenuates the impact of in utero sweetener exposure in infancy[75]. It is possible that other anti-

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obesity bioactive components may counteract these effects in the longer term. Further, maternal intake of NNS was not detailed or adjusted for during the lactation period, therefore it is unknown whether the NNS consumption pattern present during pregnancy persisted into the lactation period. One paper examined infant outcomes in lactating women who consumed NNS as diet beverages and while there was no association with infant BMI outcomes, there was a 2·78-fold increased risk of vomiting further highlighting the potential influence of NNS on the developing gastrointestinal system[88].

Metabolic effects

The impact of NNS during pregnancy on metabolic outcomes in rodent models is also relatively limited. For the purposes of this review, only adult offspring from NNS-fed mothers (during pregnancy and/or lactation only) were examined. Several studies examined the impact of NNS exposure during pregnancy on adipose tissue function in both mothers and offspring. Plows *et al.*[37] demonstrated that exposure of pregnancy C57/BL6 mice to acesulfame-k significantly increased glucose intolerance to the same extent as fructose, this was accompanied by increased adipocyte size and reduced expression of the insulin receptor and PPAR gamma in adipose tissue indicating evidence of adipose tissue insulin resistance and reduced adipose tissue expansion capacity. Reduced foetal weight and glucose concentrations were observed in both male and female foetuses from both NNS and fructose-exposed groups. The effects of in utero acesulfame-k and fructose exposure also extended to adult offspring. Female but not male offspring exhibited increased glucose intolerance accompanied by increased adipocyte size and reduced wound healing capacity in both males and females[60,71]. Azad *et al.*[78] also examined the impact of NNS during pregnancy on offspring adipose tissue outcomes. In utero exposure to sucralose, aspartame or sucrose in C57BL/6 mice increased male and female adipose tissue mass and adipocyte size (with the exception of female sucralose-exposed offspring)[78]. When they examined glucose tolerance via oral glucose tolerance test they found that only female sucrose exposed offspring had reduced glucose intolerance. However, male but not female offspring had increased glucose concentrations in response to an insulin tolerance test in sucrose, aspartame and sucralose offspring. Additional in vitro analysis in 3T3-L1 cells demonstrated that sucralose alters expression of pro-adipogenic and lipid metabolism genes during adipocyte differentiation[78].

Park *et al.*[89] examined the impact of exposure to aspartame and rebaudioside A (a bioactive component of stevia) during pregnancy and lactation. They found rebaudioside A increased lean mass and decreased fat mass in dams with aspartame having no effect on body composition. While there was no difference in glucose concentrations between groups, insulin concentrations were significantly increased in the aspartame but not rebaudioside A dams. In the offspring, there were no differences in weight but the aspartame and rebaudioside A groups had significantly increased fat mass, insulin concentrations and glucose intolerance compared to unexposed offspring, but only in males. To determine the mechanisms behind these effects, the hypothalamic melanocortin circuits of the brain were examined. They found decreased immunoreactive fibres density in proopiomelanocortin and agouti-related protein (AgRP) in male offspring of both aspartame and rebaudioside A groups suggesting a permanent rewiring of hypothalamic melanocortin circuits in the brain[89].

Nettleton *et al.* examined the consequences of adding aspartame and stevia to an obesogenic diet in Sprague-Dawley rats[61]. They demonstrated that by weaning, rats with in utero exposure to stevia and aspartame had increased body mass, however, this effect disappeared by adulthood suggesting that the early life effects of NNS on body weight may be overtaken by a high-fat exposure in later life. Despite no difference in body weight during adulthood, these offspring had increased glucose intolerance and fasting insulin concentrations. Similar to human studies outlined in previous sections of this review[75,79] the effects in relation to body weight were only observed in male offspring. However, this study did not incorporate a lean stevia or aspartame group making it difficult to disentangle the effects of maternal high-fat diet and NNS.

Microbiome effects

The microbiome has been identified as a potential regulator of multiple physiological functions including metabolic health[90]. There have been reports from human and animal studies that excessive NNS consumption (≥5 servings per week) is associated with alterations in the microbiome. Depletion of *Akkermansia muciniphilia* has been the most common microbiome alteration described in response to NNS consumption and has been linked to glucose intolerance in animal models[27,38,91] and in human studies[27,91]. However, studies have shown that inter-person variation in gut microbiome may influence this response[27]. Indeed, this extends to maternal consumption, with work in a Canadian birth cohort study by Laforest-Lafontane demonstrating a link between microbiome-derived metabolites and increased BMI in 1-year-old infants from mothers with high consumption of NNS during pregnancy[76].

These links have also been shown in animal studies with exposure to aspartame and stevia. Nettleton *et al.* demonstrate that while NNS exposure on an obese background does not impact alpha or beta diversity in rodent dams or offspring at 18 weeks, microbiome transfer from offspring exposure in utero to NNS to germ-free mice increased body weight and reduced glucose tolerance. Further, dams exposed to aspartame have increased concentrations of short-chain fatty acids[61]. A follow up study[92] further examined the microbiome of these offspring using a metagenomic reconstruction of cecal short-chain fatty acid metabolism with physiological measures such as body weight, fat mass and liver weight. They demonstrated that maternal consumption of stevia and aspartame altered microbial metabolism in the offspring which may be linked to increases in body weight. The design of this study (Wang *et al.*) raises several

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questions about the applicability to the human situation. There are no NNS-only controls making it difficult to determine the effects of the sweeteners in the absence of a high-fat diet. Further, the obesogenic diet is also high in sugar, so this study essentially examines the impact of sugar and NNS in combination, whether or not this is relevant to a human dietary pattern is not clear.

Olivier-Van Stichelen *et al.* demonstrated changes in pre-weaned C57/BL6 mouse pups in response to maternal exposure to a mixture of acesulfame-k and sucralose[38]. They demonstrated dose-dependent changes in blood and faecal metabolites that were associated with nutrient processing and detoxification pathways, particularly pathways involved in amino acid metabolism. They also found that pup microbiome was dramatically different to that of the dams with significant increases in firmicute bacteria and depletion of Verrucomicrobia. These alterations have previously been identified in humans and rodents with obesity and metabolic dysfunction[93,94]. However, this study only examined doses at and double the ADI and it is unclear whether these results were derived from male or female offspring.

The effects of sucralose consumption during pregnancy and microbiome changes have also been examined in a C57/BL6 mouse model in two linked studies[95,96]. This group show that maternal sucralose impairs intestinal development and disrupts barrier function at 3 weeks of age. They found that this was due to reduction of butyrate-producing bacteria and intestinal inflammation. Offspring were also examined at 12 weeks of age and displayed further signs of microbial dysbiosis which was accompanied by increased hepatic steatosis and altered fatty acid biosynthesis and metabolism in response to 4 weeks high-fat diet exposure. A follow up study found that the number of Paneth cells in the intestine was reduced and expression of genes related to gastrointestinal health such as cryptdins and lysozyme were reduced in the offspring in response to maternal sucralose consumption. Despite significant evidence of sex-specific effects in relation to maternal NNS and offspring outcomes, there was no mention of whether male or female (or both) offspring were used for this analysis.

Conclusion

NNS consumption during pregnancy remains a conflicted topic. When interpreting the evidence on this topic there are a number of factors which need to be considered. All of the studies examining the impact of maternal NNS exposure on offspring weight/BMI outcomes are from observation studies. While there are clear associations in each of the cohorts studied (albeit not in every individual study), correlation does not necessarily indicate causation and additional biological, demographic and lifestyle factors not included in the statistical models may influence the overall result. Our understanding of the environmental causes of overweight/obesity has expanded over the last decade with factors such as eating environments and behaviours as well as psychosocial factors influencing weight gain during childhood, none of these factors are included in these studies. It is also clear that high NNS consumers often share demographic characteristics such as being younger, having lower education status, higher BMI but lower overall energy intakes. It is therefore possible that NNS consumption is associated with specific dietary patterns which may influence maternal and offspring health.

There is also the issue of reverse causality, where individuals who are at increased risk of pregnancy complications may use NNS during pregnancy at a higher rate than those who are not deemed at risk for health complications. Indeed, in some of the studies examined, NNS was associated with increased pre-pregnancy BMI. Several considerations in statistical analysis were made to counteract this issue, including adjustment for BMI in statistical models, stratification based on weight and sensitivity analysis which limited the analysis to individuals of healthy weight or removing those at risk for complications. In some cases, these considerations reduced the association, but the underlying association was still statistically significant.

Considering the evidence available there is still cause for concern. Doses reported in each study are based on FFQ data and refer specifically to NNS beverage consumption which has a tendency to be underreported[97] and omits the many other commonly consumed sources of NNS in foodstuffs such as low-fat yoghurts, vitamin tablets and chewing gums. Indeed, a recent Irish study demonstrated that low-fat yoghurt was a source of NNS consumption in pregnant women[17]. Furthermore, FFQ data is difficult to break down the individual sweeteners consumed.

These studies were all carried out in cohorts who were recruited over a decade ago and with the introduction of sugar taxes and increased availability of NNS sweetened food products, habitual consumption of NNS is likely to be increased at a population level during this timeframe[16]. These studies are also all carried out in high-income countries with little or no evidence available from low/middle-income countries despite increases in consumption of NNS[98]. Analysis showed that individuals from lower SES situations are more likely to be lost to follow up, this limits the applicability of the findings in relation to some of the groups which are at high-risk and may ultimately have an influence on the overall findings. Evidence from cohort studies presented in this review suggests that where associations between high NNS consumption (≥5 servings per week) and increased BMI *z*-scores in infants/children can be negated by longer breastfeeding duration[75]. High-risk demographic groups such as younger mothers and those in socially deprived areas are less likely to breastfeed for longer durations, more likely to introduce solids earlier and are more likely to consume NNS[75,81]. This is particularly worrisome given the increased risk that may be associated with NNS sweetener exposure in utero and childhood overweight/obesity. However, the mechanisms which govern this association are not clear and more work to tease out how breastfeeding may protect against these potential effects is warranted.

It is apparent that there is a major lack of evidence in this area and further studies are required to make a definitive

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conclusion in relation to the impact of NNS consumption during pregnancy. Given that many NNS cross the placenta and are found in amniotic fluid and breastmilk, this demonstrates potential risk for developmental programming of offspring health. Animal studies have demonstrated that early life exposure can influence taste perception, alter the microbiome and create metabolic dysregulation. However, these studies need to be interpreted with caution as they often use doses at the limit of or exceeding the ADI. The evidence does not support complete cessation of use as many studies only associate risk with highest consumption of NNS (more than 5 servings a week). Therefore, limiting consumption and recommending water or milk, in addition to promotion and support of preventative approaches such as breastfeeding to 6 months and education around appropriate weaning age, may be beneficial to health, particularly in situations where the risk appears to be elevated. In addition, the majority of studies to date have focused on NNS beverages and consideration of the whole diet, including foods and other ingested products is warranted given the increase of NNS in our food chain which may contribute substantially to NNS levels[19]. Overall, there is a clear need to expand research in this area both in relation to mechanistic animal studies and human translational studies, particularly in high-risk groups.

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Competing interests

There are no conflicts of interest.

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