Accepted manuscript

Consumption of Artificial Sweeteners during Pregnancy and the risk of Overweight in the Offspring

Eva M. Gjørup, BSc. Med.[1,2,3], Bodil H. Bech, M.D., Ph.D.[4], Sofie Stampe, BSc. Med.[1,2,3], Thorhallur I. Halldorsson, Ph.D.[5,6], Anne A. Bjerregaard, Ph.D.[5,7], Sjurdur F. Olsen, M.D., D.M.Sc.[5,8,9], Per G. Ovesen, M.D., D.M.Sc.[1,2,3], Magnus Leth-Møller, M.D.[1,2,3]

[1]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; [2]Department of Gynecology and Obstetrics, Aarhus University Hospital, Aarhus, Denmark; [3]Steno Diabetes Center Aarhus, Aarhus University Hospital, Aarhus, Denmark; [4]Department of Public Health, Aarhus University, Aarhus, Denmark; [5]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; [6]Faculty of Food Science and Nutrition, University of Iceland; [7]Centre for Clinical Research and Prevention, Copenhagen University Hospital – Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark; [8]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, United States; [9]Department of Public Health, University of Copenhagen, Copenhagen, Denmark

Corresponding author: Eva Marie Gjørup; E-mail address: evagjoerup@clin.au.dk; Phone no. +45 30 13 54 32

Short title: Artificial Sweeteners & Offspring Overweight

Key words: Childhood Obesity; Epidemiology; Longitudinal Cohort Study; Artificial Sweeteners; Pregnancy; Maternal-Fetal Health; Fetal Exposure

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI

10.1017/S0007114525000455

The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

Abstract

Artificial sweeteners are used to reduce energy intake, but studies suggest that consumption during pregnancy may impact the offspring's risk of overweight. In this longitudinal cohort study, we aimed to examine the association between consumption of artificially sweetened or sugar-sweetened beverages during pregnancy and offspring overweight from birth to 18 years in the Danish National Birth Cohort (DNBC). 101,042 pregnancies were enrolled in the DNBC from 1996-2002. Follow-up was conducted throughout pregnancy, childhood, and adolescence. 72,821 women completed a Food Frequency Questionnaire during pregnancy reporting intake of beverages sweetened with artificial sweeteners or sugar. Offspring height and weight were obtained during childhood and adolescence. Multivariate logistic regression was performed to estimate the odds ratio (OR) for overweight concerning maternal beverage consumption. Analyses were adjusted for risk factors for childhood overweight, including maternal age, pre-pregnancy body mass index (BMI), physical activity and smoking in pregnancy, healthy eating index, paternal BMI, socioeconomic status, and duration of breastfeeding. We found increased odds of overweight in 7, 11, 14, and 18-year-old offspring, whose mothers reported drinking ≥1 artificially sweetened beverages daily during pregnancy compared to no consumption (18 years: adjusted OR 1.26 (95% confidence interval 1.12, 1.42)). We found decreased adjusted odds of overweight in 11 and 18-year-old offspring, whose mothers reported drinking ≥1 sugar-sweetened beverages daily during pregnancy compared to no consumption. We found that consumption of artificially sweetened beverages during pregnancy was associated with an increased risk of overweight in childhood and adolescence. Adjustment for risk factors for overweight and total energy intake did not explain the association. Further studies are warranted to establish the mechanism of the association.

List of abbreviations:

ASB: Artificially sweetened beverages

DNBC: Danish National Birth Cohort

GW: Gestational week

LGA: Large for gestational age

SD: Standard deviations

SSB: Sugar sweetened beverages

Introduction

The rate of obesity has increased dramatically throughout the last decades[1], posing a threat to public health due to the increased burden of associated comorbidities[2; 3]. To avoid excessive weight gain, many people try to lower energy intake, e.g. by substituting caloric sweeteners with artificial sweeteners. True artificial sweeteners are synthetic sweetening agents used in a wide range of “sugar-free” (diet) food products and beverages[4; 5; 6]. However, recent research suggests that artificial sweetener intake is associated with obesity[7; 8], and the World Health Organization now advises against the use of non-nutritive sweeteners (including both artificial sweeteners and natural sweeteners such as steviol glycosides) for weight loss purposes[9]. Some studies have shown impairment in insulin tolerance in aspartame-exposed rats independently of body fat composition[10] and increased glucose intolerance in both mice and humans exposed to artificial sweeteners, partly mediated by changes in the gut microbiome[11; 12].

Childhood obesity is associated with an increased risk of metabolic and cardiovascular disease in adulthood[13; 14]. Several parental and early-life factors have been linked to childhood obesity risk, including duration of breastfeeding[15], smoking in pregnancy[16], parental socioeconomic status[17], maternal pre-pregnancy body mass index (BMI)[18], and gestational weight gain[18]. Obesity rates among children and adolescents are high globally[19], but intervention strategies to limit weight gain in youth differ in effectiveness[20], underscoring the need for primary prevention strategies.

Although the World Health Organization does not recommend artificial sweetener consumption for weight management, pregnant women with obesity or diabetes are recommended to substitute sugar-sweetened beverages (SSB) with artificially sweetened beverages (ASB) to reduce weight gain during pregnancy in some countries[21]. However, ASB consumption in pregnancy has previously been associated with overweight in infancy, early childhood, and mid-childhood[22; 23; 24]. Yet, there is conflicting evidence in the field[25], as others have found no association[26].

Several hypotheses have been proposed to explain the association between ASB consumption and overweight in childhood, including metabolic programming, influence on sweet taste preference, and influence on the human microbiome and microbiome-dependent glycemic response[11; 27; 28]. It has been demonstrated that artificial sweeteners can cross the placenta into the fetal circulation, highlighting the possibility of prenatal exposure[29; 30]. However, the

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

underlying mechanism for the association has not yet been established[31]. Moreover, postnatal exposure to artificial sweeteners through breast milk has been established[32; 33].

To expand the knowledge on the safety of ASB consumption during pregnancy regarding offspring obesity, we aimed to examine the association between ASB and SSB consumption during pregnancy and offspring overweight from birth to 18 years of age. Additionally, we aimed to evaluate this association with SSB consumption and compare the two groups.

Methods

Study design and data collection

In this nationwide cohort study, we utilized data from the Danish National Birth Cohort (DNBC)[34] which prospectively enrolled 101,042 pregnant women from 1996-2002 to investigate health across generations. The women were followed throughout pregnancy and after birth. Data on the offspring was collected from birth until 18 years of age. The last data were collected in 2022.

The pregnant women were enrolled at their first antenatal visit with their general practitioner[34]. Pregnant women were asked to participate if they spoke Danish, and if they wished to carry their child to term, regardless of ethnic and cultural background. The women who agreed to participate were asked to participate in 4 interviewer-assisted phone interviews; two during pregnancy scheduled in gestational week (GW) 12 and GW 30, and two after pregnancy when the offspring was approximately 6 and 18 months old. In GW 25, the women completed a Food Frequency Questionnaire (FFQ)[35]. The FFQ has been validated for different variables, but not in totality[36; 37; 38]. Information on birth outcomes was extracted from the Danish Medical Birth Register. Written questionnaires were distributed to the families at ages 7 years (2005-2010) and 11 years (2010-2014), and to the offspring at ages 14 years (2013-2017) and 18 years (2016-2022). The timeline for the data collection is visualized in eFigure S1.

Population

We included all singleton pregnancies that resulted in a live birth in GW 34+0 or later (Figure 1). We included women who completed the FFQ with information on the consumption of ASB or SSB. We excluded women with diabetes, including pre-existing diabetes, newly detected manifest diabetes, and gestational diabetes. Information on the diagnosis of diabetes was obtained from the Danish National Patient Register and based on ICD-8 (1977-1993) and ICD-10 codes (1994 onward). We excluded offspring with missing data on weight and length/height at each age of follow-up, but the offspring could be included in analyses for subsequent ages.

Variables and data sources

Exposure

The exposures were defined as maternal consumption of ASB and SSB during pregnancy. In the FFQ completed in GW 25, the pregnant women were asked: “How many glasses/cups of the following beverages have you drunk within the last month?”. One glass/cup was defined as 250 mL. The mothers were asked to indicate the number of glasses consumed of sugar-sweetened and artificially sweetened (diet) carbonated and non-carbonated beverages. They could choose a number per month (None, 1, 2-3), per week (1-2, 3-4, 5-6), or per day (1, 2-3, 4-5, 6-7, 8 or more).

The consumption of ASB was calculated by adding the frequency of artificially sweetened carbonated and non-carbonated beverages. Similar calculations were made for SSB. Frequency of consumption of ASB and SSB was grouped into four exposure groups; never consumption; less than 1 beverage (250 mL) per week, 1-6 beverages per week, and 1 or more beverages per day.

Outcome

The primary outcome was large for gestational age (LGA) birthweight, and overweight at 5 and 12 months, and 7, 11, 14, and 18 years. Length- and weight measurements of the offspring in infancy (5 and 12 months) were done by the general practitioner at the 5- and 12-month health visits. The mothers reported these measurements during the last phone interview when the offspring was 18 months old. The measurements throughout childhood and adolescence were done by the parents or the offspring themselves and provided in the written questionnaires at ages 7, 11, 14, and 18 years. To avoid outliers, we excluded values of more than 5 standard deviations (SD) from the mean, in either direction (n = 489).

The offspring were defined as LGA if their birth weight was above 2 SD with the study population as internal reference material. Postnatal outcomes were based on offspring BMI reported at ages 5 and 12 months, and 7, 11, 14, and 18 years. The cutoff value for overweight was BMI +1.30 SD for boys and BMI +1.19 SD for girls[39]. BMI z-scores were calculated by estimating mean (with SD) BMI dependent on age and sex using linear regression. We then estimated z-scores by subtracting the mean predicted BMI from the individual measured BMI and dividing it by the SD.

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

Covariates

Several covariates, all potential determinants of childhood obesity, were included in the analyses to control potential confounding and minimize bias (eFigure S2). Maternal age was retrieved from the Danish Civil Registration System[40]. Maternal pre-pregnancy BMI and physical activity in early pregnancy were provided in the first phone interview (GW 12). The women were asked whether they did any physical activity during pregnancy (yes/no). Gestational weight gain (kg) was provided by the mothers in the third interview. If the women confirmed smoking during pregnancy in any of the first three phone interviews, they were counted as having smoked during pregnancy (yes/no).

Maternal healthy eating index (points/80) was calculated from the FFQ[41]. The diet was rated using a score of 0-80, with 80 being the healthiest. It should be noted, that SSB is included in the healthy eating index. The amount of each food item consumed was calculated by multiplying the frequency of consumption, as reported in the FFQ, with standard portion sizes. Total energy intake was then estimated by multiplying the amount consumed with the energy content of food as reported in the food composition tables[35]. Paternal BMI was reported in the 4th interview. Duration of breastfeeding was reported in the two post-partum phone interviews. Two variables were created: One describing breastfeeding more or less than four months used for the analysis at 5 months, and one describing breastfeeding more or less than six months used for the 12-month and subsequent analyses. The mother's occupational status defined her socioeconomic status. If unavailable, the father's occupational status was used instead. Socioeconomic status was grouped into categories: Unskilled, other work or receives public benefits; working class, craftsmen, short education or under education; and leaders or long/medium long education. The criteria for the categorization are described in further detail elsewhere[42].

Statistical analysis

For baseline characteristics, mean (SD) was calculated for continuous variables, and frequencies (%) for categorical variables. The associations between ASB or SSB consumption in pregnancy and offspring overweight were assessed using multiple logistic regression using "no consumption" as reference. To limit potential confounding, we adjusted for pre-pregnancy BMI, maternal age, maternal healthy eating index, physical activity in early pregnancy, smoking in pregnancy, combined socioeconomic status, paternal BMI, and breastfeeding duration. To evaluate associations between ASB consumption during pregnancy and birthweight and BMI z-scores as continuous measures, we used multiple linear regression to estimate the mean difference and 95% CI. All analyses were performed as complete case analyses.

A few sub-analyses were performed; One analysis exchanged maternal healthy eating index with total energy intake, another analysis included additional adjustment for gestational weight gain. In addition, we designed an isocaloric substitution model including the same covariates as the main analysis, with total energy intake replacing healthy eating index, and with mutual adjustment of the exposures, with ASB adjusted for SSB and SSB adjusted for ASB. All analyses were performed as complete case analyses.

All data analysis was performed using R for Windows (version 4.3.1 (2023) (R Foundation for Statistical Computing, Vienna, Austria).

Results

101,042 pregnancies were enrolled in the DNBC. 90,060 children born to 83,833 women were eligible to participate in the study. 66,668 women had information on ASB from the FFQ and 66,568 women had information on SSB from the FFQ (Figure 1). Pregnant women consuming ≥1 ASB/day were younger, had higher BMI, more often smoked, and had a lower socioeconomic status compared to pregnant women with no ASB consumption (Table 1). There was no difference in healthy eating index, offspring sex, birth weight, or gestational age at birth. Physical activity in early pregnancy was highest in the <1 ASB/week group. Women with high ASB intake tended to have shorter breastfeeding duration compared to the never group.

The associations between maternal ASB or SSB consumption and childhood overweight and LGA at birth are shown in Table 2 and Figure 2. At birth and in infancy (5 and 12 months), we found no association between daily ASB consumption and LGA or overweight after adjustment. At 7, 11, and 18 years, we found higher odds of overweight in offspring, whose mothers consumed 1-6 ASB/week or ≥1 ASB/day compared to no consumption. The associations attenuated but remained after adjustment (18 years in the ≥1 ASB/day group: adjusted OR 1.26 (95%CI 1.12, 1.42)). At 14 years, we found higher odds for overweight in the ≥1 ASB/day group compared to never consumption, but not in the 1-6 ASB/week group. Daily maternal SSB consumption in pregnancy entailed lower odds for overweight at 11 years and 18 years compared to no consumption in both crude and adjusted analyses.

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

Evaluating the difference in z-score at birth or BMI z-score dependent on maternal beverage consumption is shown, regarding ASB, the largest difference in BMI z-score was seen among the 18-year-olds whose mothers consumed ≥1 ASB/day compared to no consumption (adjusted OR 0.07 (95%CI 0.03, 0.10)) (eTable S1). For SSB, the largest difference in BMI z-score was seen among the 18-year-olds whose mothers consumed ≥1 SSB/day compared to no consumption (BMI z-score -0.12 (95%CI -0.16, -0.07)) (eTable S1).

While we did not adjust for gestational weight gain in the primary analysis, doing so minimally attenuated the associations, and the associations persisted at ages 7, 11, and 18 years (eTable S3 and eTable S4). Similar results were seen when adjusting for Total Energy Intake, where minimal changes were seen for ASB (eTable S5) and SSB – however, for SSB, the attenuation was more profound (eTable S6). When performing a substitution analysis substituting SSB with ASB and vice versa (see eTable S5 and S6), moderate attenuations were seen for SSB.

Discussion

Our results indicate that daily ASB consumption compared to no ASB consumption during pregnancy is associated with a higher risk of overweight during childhood and adolescence, but not at birth or in infancy. We found a higher risk of being overweight at 7, 11, and 18 years among offspring, whose mothers consumed 1-6 ASB/week or ≥1 ASB/day during pregnancy. Additionally, we found that consumption of ≥1 SSB/day during pregnancy was associated with a lower risk of overweight in the offspring at ages 11 and 18 years compared to no consumption. These results remained after adjustments for known risk factors for childhood obesity and overweight as well as in substitution models. Previous studies have found a higher risk of overweight during infancy in children exposed to the largest amounts of artificial sweeteners[22; 24]. The incongruence with our results could be due to different populations and adjustment models. Notably, the observed effect sizes for these associations were modest, and reservations should be kept before basing clinical advice on these results.

We adjusted for a variety of covariates that are potential determinants of childhood obesity. We sought to account for lifestyle variables by adjusting for overall diet quality (healthy eating index), smoking in pregnancy, physical activity, duration of breastfeeding, and socioeconomic status. However, acknowledging that it is an inherent challenge to capture lifestyle factors in an observational study, residual confounding may persist. Our study focused on prenatal and early-life factors, and future investigations may benefit from

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

considering ASB consumption during childhood and adolescence as a potential risk factor. Gestational weight gain was not included in the primary analysis, as we considered it a potential mediator of the association between ASB and childhood overweight as a previous study showed an association between ASB consumption in pregnancy and higher gestational weight gain[43].

Additionally, we found that daily consumption of SSB was associated with a trend towards a lower overweight risk in the offspring. Since the main difference between ASB and SSB is the sweetening agent, these findings may indicate that artificial sweeteners, rather than other components of the beverage, account for the association found for ASB. Still, differences between those choosing ASB over SSB remain, hence residual confounding cannot be excluded. Additionally, we found a trend towards a decreased risk of overweight among children, whose mothers reported daily consumption of SSB, seemingly suggesting a protective effect on childhood overweight. It should be noted, as shown in Table 1, that the group reporting daily consumption of ASB and the group reporting daily consumption of SSB differed on several baseline values, which could affect the results. For instance, women who reported daily consumption of SSB tended to have a higher SES, had a lower pre-pregnancy BMI, and tended to breastfeed for a longer time compared to women who reported daily consumption of ASB. This indicates that the reference groups are differently composed, which could potentially introduce bias. This should be considered when interpreting results.

When comparing low-calorie beverages and calorie-dense beverages it is likely to observe confounding due to differences in energy consumption. Indeed, as portrayed in Table 1, total energy intake in kcal was higher in the daily SSB-consumption group than in the daily ASB-consumption group. In a sub-analysis adjusting for Total Energy Intake (kcal) instead of Healthy Eating Index, the results for ASB were minimally attenuated, while for SSB, some regression towards the null was observed (eTable S5 and eTable S6). To further investigate if substitution of ASB or SSB with other drinks would affect the results, we constructed an isocaloric substitution model. In this model, replacing ASB with SSB was associated with lower weight gain, which is consistent with findings from unadjusted analyses; that consumption of ASB is associated with a higher risk of overweight.

The observed association between maternal ASB consumption and offspring overweight manifested in mid-childhood, while we saw no discernible correlation during infancy. There is a substantial time gap between the measurements at 12 months and 7 years along with a

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

large difference in overweight risk. Keeping in mind that rapid weight gain from 2-6 years of age increases the risk of manifest obesity[44], longitudinal analyses are called for to fully comprehend the development of overweight in this age period concerning ASB consumption. In infancy, we saw a limited influence of ASB on overweight risk, which could indicate that changes appear when the offspring starts choosing their own food to a larger extent. Furthermore, we found the highest OR for overweight among 18-year-olds exposed to ≥1 ASB/day during pregnancy. One possibility could be that dietary habits and lifestyle behavior become increasingly independent of parental behaviors with increasing age[45] while height has mostly stabilized at age 18[46; 47].

We found increasing BMI Z-score with increasing consumption of ASB, suggestive of a dose-dependent association between artificial sweetener consumption and risk for overweight in the offspring. One ASB or SSB was defined as 250 mL, corresponding to one regular glass of e.g. artificially sweetened soda. Danish Health Authorities recommend that adults consume no more than 500 mL of soda or non-carbonated sweetened beverages weekly[48]. According to our data, this recommendation was followed by 36% of the women regarding SSB and 71% of the women regarding ASB (Table 1). Our results highlight the importance of limiting consumption of SSB and ASB, and the low adherence to recommendations is concerning.

As previously described, some artificial sweeteners are transferred to the child via breast milk, enabling postnatal exposure. When the women report consuming artificial sweeteners during pregnancy, we speculate that these patterns continue postpartum, meaning that the children are further exposed to artificial sweeteners in infancy. Early exposure to artificial sweeteners showed contradictory evidence on metabolic effects in a systematic review[49], however, no reports on infant artificial sweetener exposure were included. If the children, who were exposed to artificial sweeteners in utero continue exposure postnatally, this could account for some of the association observed in our results. Studies on the consequences of infant exposure to artificial sweeteners are warranted to investigate this possible association.

Despite the robust sample size and extended follow-up, limitations inherent in observational studies, such as self-reported data and recall bias, remain in this study. Limitations include the reliance on self-reported beverage consumption, which holds uncertainty and makes it impossible to investigate specific artificial sweeteners. Furthermore, the questionnaire did not include other sources of artificial sweeteners, including table-top sweeteners. Additionally, there have been substantial changes in artificial sweetener usage patterns since the data collection (1996-2003) with an increase in consumption[50], which could impact the generalizability of our findings today. There is evidence of some selection bias in the Danish National Birth Cohort, and despite inclusive inclusion criteria, some segments of the population may be underrepresented[51].

Adjusting for a variety of confounders eliminated many potential participants from this study. Thus, the adjusted results are based on fewer participants than those available for the unadjusted analyses, resulting in less precise adjusted estimates. An influence by confounding factors cannot be discounted despite adjustment due to the uncontrolled environment intrinsic in observational studies. Characteristics for the participants at each follow-up are shown in eTable S2. When interpreting the results, it is important to consider that due to the extensive sample size, even minor associations may achieve statistical significance. Thus, the clinical implications of these associations may be modest.

Presently, women with diabetes and/or overweight are recommended to substitute sugar-sweetened beverages with artificially sweetened alternatives during pregnancy[21]. While this may be motivated by goals of limiting gestational weight gain[52; 53], this study suggests adverse long-term effects, as there is an observed tendency towards an increased risk of overweight in the offspring if these recommendations are met. We excluded women with diabetes in this analysis, but existing evidence shows an increased OR of overweight among children born to women who had gestational diabetes, who were exposed to artificial sweeteners during pregnancy[23].

In conclusion, we found in adjusted analyses that daily consumption of ASB during pregnancy increased the odds of overweight in mid-childhood and adolescence but not in infancy. Furthermore, we found a trend suggesting that daily consumption of SSB during pregnancy was associated with a decreased risk of overweight in adolescence. Longitudinal evidence for the offspring at ages 2-6 years is warranted to better understand the association.

Acknowledgements

Financial support

The work presented in this article is supported by Novo Nordisk Foundation grant NNF21SA0069371, A.P. Møller og Hustru Chastine Mc-Kinney Møllers Fond til almene Formaal grant L-2022-00216, and Kong Christian den Tiendes Fond grant 51/2023. The Novo Nordisk Foundation, A.P. Møller og Hustru Chastine Mc-Kinney Møllers Fond til almene Formaal, and Kong Christian den Tiendes Fond had no role in the design, analysis or writing of this article.

The Danish National Birth Cohort was established with a significant grant from the Danish National Research Foundation. Additional support was obtained from the Danish Regional Committees, the Pharmacy Foundation, the Egmont Foundation, the March of Dimes Birth Defects Foundation, the Health Foundation and other minor grants. The DNBC Biobank has been supported by the Novo Nordisk Foundation and the Lundbeck Foundation.

Follow-up of mothers and children have been supported by the Danish Medical Research Council (SSVF 0646, 271-08-0839/06-066023, O602-01042B, 0602-02738B), the Lundbeck Foundation (195/04, R100-A9193), The Innovation Fund Denmark 0603-00294B (09-067124), the Nordea Foundation (02-2013-2014), Aarhus Ideas (AU R9-A959-13-S804), University of Copenhagen Strategic Grant (IFSV 2012), and the Danish Council for Independent Research (DFF – 4183-00594 and DFF - 4183-00152).

March of Dimes Birth Defects Foundation (6-FY-96-0240, 6-FY97-0553, 6-FY97-0521, 6-FY00-407), the European Union (QLK1-2000-00083), the Danish Medical Research Foundation (9601842 and 22-03-0536), the Health Foundation (11/263-96), and the Heart Foundation (96-2-4-83-22450) supported the collection, development, and elaboration of the dietary data.

Declaration of interests

The authors declare none.

Author contributions statement

Eva Gjørup; Conceptualization, methodology, validation, formal analysis, writing - original draft, writing - review & editing, visualization, project administration, funding acquisition. **Bodil Hammer Bech**; Conceptualization, methodology, writing - review & editing, supervision. **Sofie Stampe**; Conceptualization, methodology, writing - original draft, writing - review & editing, supervision. **Thorhallur Halldorsson**; Methodology, resources, writing - review & editing. **Anne Ahrendt Bjerregaard**; Methodology, resources, writing - review & editing. **Sjurdur Olsen**; Methodology, writing - review & editing. **Per Ovesen**; Conceptualization, methodology, writing - review & editing, supervision, project administration, funding acquisition. **Magnus Leth-Møller**; Conceptualization, methodology, validation, formal analysis, writing - review & editing, supervision, project administration.

References

1. (2017) Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet* **390**, 2627-2642.

2. Powell-Wiley TM, Poirier P, Burke LE *et al.* (2021) Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation* **143**, e984-e1010.

3. Maggio CA, Pi-Sunyer FX (2003) Obesity and type 2 diabetes. *Endocrinol Metab Clin North Am* **32**, 805-822, viii.

4. Ng SW, Slining MM, Popkin BM (2012) Use of caloric and noncaloric sweeteners in US consumer packaged foods, 2005-2009. *J Acad Nutr Diet* **112**, 1828-1834.e1821-1826.

5. Godshall MA The expanding world of nutritive and non-nutritive sweeteners. *Sugar Journal* **69**, 12–20.

6. Chattopadhyay S, Raychaudhuri U, Chakraborty R (2014) Artificial sweeteners - a review. *J Food Sci Technol* **51**, 611-621.

7. Sylvetsky AC, Jin Y, Clark EJ *et al.* (2017) Consumption of Low-Calorie Sweeteners among Children and Adults in the United States. *J Acad Nutr Diet* **117**, 441-448.e442.

8. Pearlman M, Obert J, Casey L (2017) The Association Between Artificial Sweeteners and Obesity. *Curr Gastroenterol Rep* **19**, 64.

9. Organization GWH (2023) Use of non-sugar sweeteners: WHO guideline.

10. Palmnäs MS, Cowan TE, Bomhof MR *et al.* (2014) Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. *PLoS One* **9**, e109841.

11. Suez J, Cohen Y, Valdés-Mas R *et al.* (2022) Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell*.

12. Suez J, Korem T, Zeevi D *et al.* (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* **514**, 181-186.

13. Weihrauch-Blüher S, Schwarz P, Klusmann JH (2019) Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. *Metabolism* **92**, 147-152.

14. Robertson J, Schaufelberger M, Lindgren M *et al.* (2019) Higher Body Mass Index in Adolescence Predicts Cardiomyopathy Risk in Midlife. *Circulation* **140**, 117-125.

15. Qiao J, Dai LJ, Zhang Q *et al.* (2020) A Meta-Analysis of the Association Between Breastfeeding and Early Childhood Obesity. *J Pediatr Nurs* **53**, 57-66.

16. Rayfield S, Plugge E (2017) Systematic review and meta-analysis of the association between maternal smoking in pregnancy and childhood overweight and obesity. *J Epidemiol Community Health* **71**, 162-173.

17. Sares-Jäske L, Grönqvist A, Mäki P *et al.* (2022) Family socioeconomic status and childhood adiposity in Europe - A scoping review. *Prev Med* **160**, 107095.

18. Voerman E, Santos S, Patro Golab B *et al.* (2019) Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLoS Med* **16**, e1002744.

19. Di Cesare M, Sorić M, Bovet P *et al.* (2019) The epidemiological burden of obesity in childhood: a worldwide epidemic requiring urgent action. *BMC Med* **17**, 212.

20. Smith JD, Fu E, Kobayashi MA (2020) Prevention and Management of Childhood Obesity and Its Psychological and Health Comorbidities. *Annu Rev Clin Psychol* **16**, 351-378.

21. (2019) Treatment Gestational Diabetes, https://www.nhs.uk/conditions/gestational-diabetes/treatment/. https://www.nhs.uk/conditions/gestational-diabetes/treatment/ (accessed 07/01/2022

22. Plows JF, Aris IM, Rifas-Shiman SL *et al.* (2022) Associations of maternal non-nutritive sweetener intake during pregnancy with offspring body mass index and body fat from birth to adolescence. *Int J Obes (Lond)* **46**, 186-193.

23. Zhu Y, Olsen SF, Mendola P *et al.* (2017) Maternal consumption of artificially sweetened beverages during pregnancy, and offspring growth through 7 years of age: a prospective cohort study. *Int J Epidemiol* **46**, 1499-1508.

24. Azad MB, Sharma AK, de Souza RJ *et al.* (2016) Association Between Artificially Sweetened Beverage Consumption During Pregnancy and Infant Body Mass Index. *JAMA Pediatr* **170**, 662-670.

25. Li G, Wang R, Zhang C *et al.* (2022) Consumption of Non-Nutritive Sweetener during Pregnancy and Weight Gain in Offspring: Evidence from Human Studies. *Nutrients* **14**.

26. Gillman MW, Rifas-Shiman SL, Fernandez-Barres S *et al.* (2017) Beverage Intake During Pregnancy and Childhood Adiposity. *Pediatrics* **140**.

27. von Poser Toigo E, Huffell AP, Mota CS *et al.* (2015) Metabolic and feeding behavior alterations provoked by prenatal exposure to aspartame. *Appetite* **87**, 168-174.

28. Sylvetsky AC, Conway EM, Malhotra S *et al.* (2017) Development of Sweet Taste Perception: Implications for Artificial Sweetener Use. *Endocr Dev* **32**, 87-99.

29. Halasa BC, Sylvetsky AC, Conway EM *et al.* (2021) Non-Nutritive Sweeteners in Human Amniotic Fluid and Cord Blood: Evidence of Transplacental Fetal Exposure. *American Journal of Perinatology*.

30. Leth-Møller M, Duvald CS, Stampe S *et al.* (2023) Transplacental Transport of Artificial Sweeteners. *Nutrients* **15**, 2063.

31. Archibald AJ, Dolinsky VW, Azad MB (2018) Early-Life Exposure to Non-Nutritive Sweeteners and the Developmental Origins of Childhood Obesity: Global Evidence from Human and Rodent Studies. *Nutrients* **10**.

32. Stampe S, Leth-Møller M, Greibe E *et al.* (2022) Artificial Sweeteners in Breast Milk: A Clinical Investigation with a Kinetic Perspective. *Nutrients* **14**, 2635.

33. Sylvetsky AC, Gardner AL, Bauman V *et al.* (2015) Nonnutritive Sweeteners in Breast Milk. *J Toxicol Environ Health A* **78**, 1029-1032.

34. Olsen J, Melbye M, Olsen SF *et al.* (2001) The Danish National Birth Cohort - its background, structure and aim. *Scandinavian Journal of Public Health* **29**, 300-307.

35. Olsen SF, Mikkelsen TB, Knudsen VK *et al.* (2007) Data collected on maternal dietary exposures in the Danish National Birth Cohort. *Paediatr Perinat Epidemiol* **21**, 76-86.

36. Mikkelsen TB, Olsen SF, Rasmussen SE *et al.* (2007) Relative validity of fruit and vegetable intake estimated by the food frequency questionnaire used in the Danish National Birth Cohort. *Scand J Public Health* **35**, 172-179.

37. Mikkelsen TB, Osler M, Olsen SF (2006) Validity of protein, retinol, folic acid and n-3 fatty acid intakes estimated from the food-frequency questionnaire used in the Danish National Birth Cohort. *Public Health Nutr* **9**, 771-778.

38. Madsen MTB, Bjerregaard AA, Furtado JD *et al.* (2019) Comparisons of Estimated Intakes and Plasma Concentrations of Selected Fatty Acids in Pregnancy. *Nutrients* **11**.

39. Cole TJ, Bellizzi MC, Flegal KM *et al.* (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *Bmj* **320**, 1240-1243.

40. Pedersen CB (2011) The Danish Civil Registration System. *Scand J Public Health* **39**, 22-25.

41. Bjerregaard AA, Halldorsson TI, Tetens I *et al.* (2019) Mother's dietary quality during pregnancy and offspring's dietary quality in adolescence: Follow-up from a national birth cohort study of 19,582 mother-offspring pairs. *PLoS Med* **16**, e1002911.

42. Bech BH, Nohr EA, Vaeth M *et al.* (2005) Coffee and fetal death: a cohort study with prospective data. *Am J Epidemiol* **162**, 983-990.

43. Renault KM, Carlsen EM, Nørgaard K *et al.* (2015) Intake of Sweets, Snacks and Soft Drinks Predicts Weight Gain in Obese Pregnant Women: Detailed Analysis of the Results of a Randomised Controlled Trial. *PLoS One* **10**, e0133041.

44. Geserick M, Vogel M, Gausche R *et al.* (2018) Acceleration of BMI in Early Childhood and Risk of Sustained Obesity. *N Engl J Med* **379**, 1303-1312.

45. McKeown A, Nelson R (2018) Independent decision making of adolescents regarding food choice. *International Journal of Consumer Studies* **42**, 469-477.

46. de Oliveira MH, Araújo J, Ramos E *et al.* (2023) MULT: New height references and their efficiency in multi-ethnic populations. *Am J Hum Biol* **35**, e23859.

47. Tinggaard J, Aksglaede L, Sørensen K *et al.* (2014) The 2014 Danish references from birth to 20 years for height, weight and body mass index. *Acta Paediatr* **103**, 214-224.

48. Authority DH (2024) Anbefalinger om kost.

49. Reid AE, Chauhan BF, Rabbani R *et al.* (2016) Early Exposure to Nonnutritive Sweeteners and Long-term Metabolic Health: A Systematic Review. *Pediatrics* **137**, e20153603.

50. Sylvetsky AC, Figueroa J, Rother KI *et al.* (2019) Trends in Low-Calorie Sweetener Consumption Among Pregnant Women in the United States. *Curr Dev Nutr* **3**, nzz004.

51. Jacobsen TN, Nohr EA, Frydenberg M (2010) Selection by socioeconomic factors into the Danish National Birth Cohort. *Eur J Epidemiol* **25**, 349-355.

52. Rasmussen KM, Catalano PM, Yaktine AL (2009) New guidelines for weight gain during pregnancy: what obstetrician/gynecologists should know. *Curr Opin Obstet Gynecol* **21**, 521-526.

53. Johansson K, Bodnar LM, Stephansson O *et al.* (2024) Safety of low weight gain or weight loss in pregnancies with class 1, 2, and 3 obesity: a population-based cohort study. *Lancet*.

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

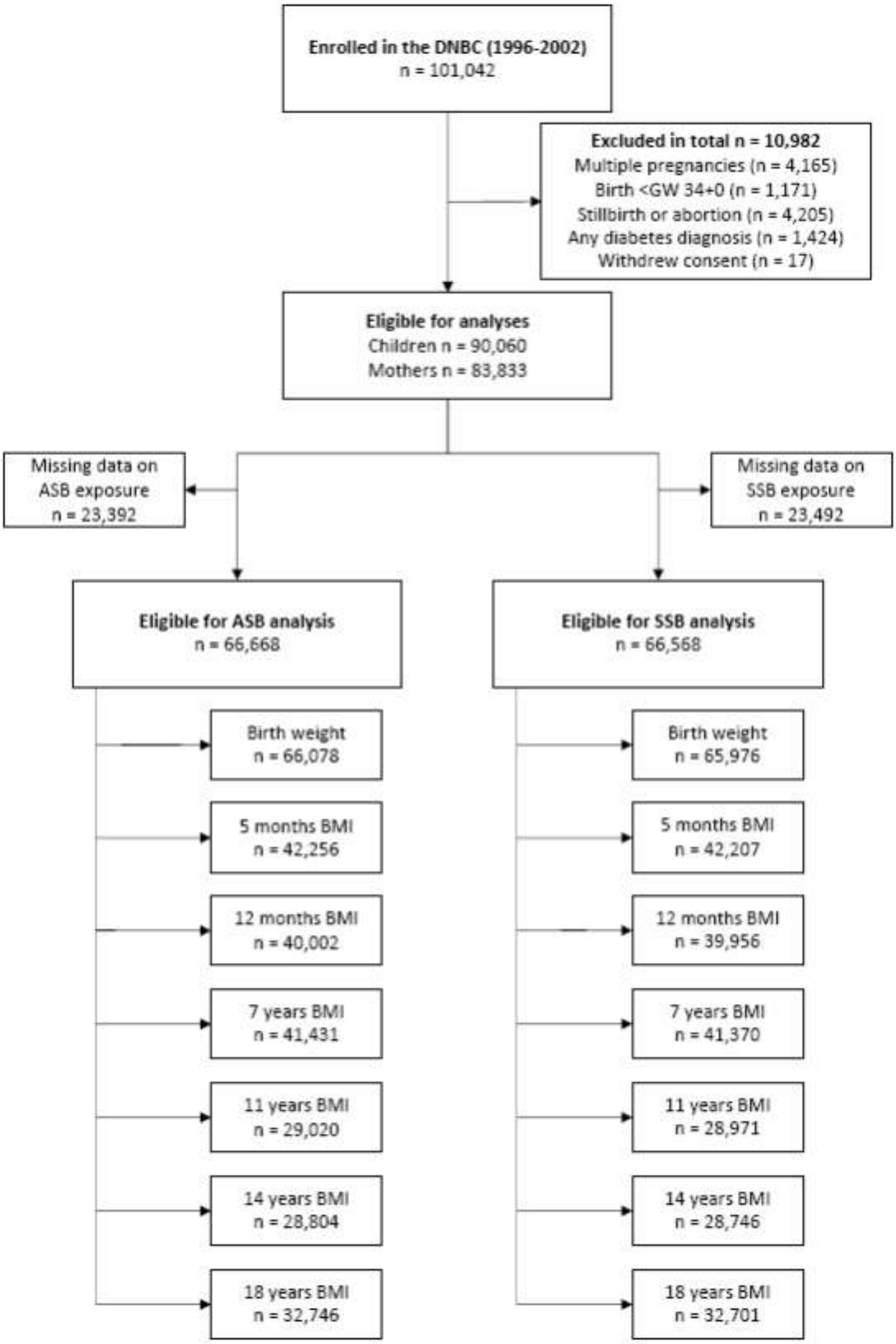

Figure 1: Flow chart for the inclusion process.

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

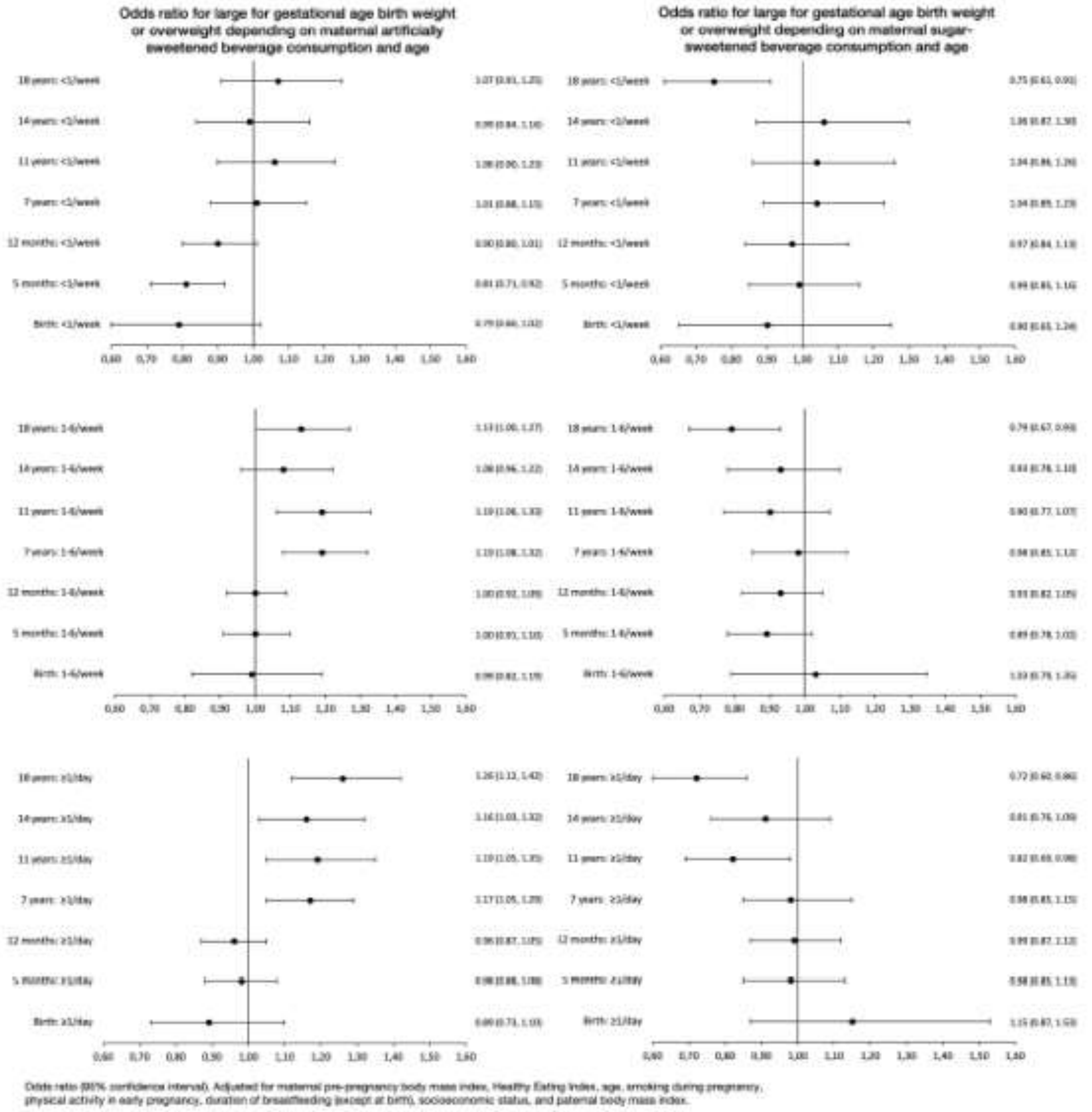

Figure 2: Forest plots summarizing adjusted odds ratios for large for gestational weight birth weight or overweight depending on maternal artificially sweetened beverage consumption and age. The adjustment model included maternal pre-pregnancy body mass index, Healthy Eating Index, age, smoking during pregnancy, physical activity in early pregnancy, duration of breastfeeding (except at birth), socioeconomic status, and paternal body mass index.

Table 1: Characteristics for all live born, singleton offspring born after gestational week 34 to mothers without diabetes in the Danish National Birth Cohort

	Total		Maternal ASB intake					Maternal SSB intake				
Characteristic	**N**	**N = 90,293**[1]	**N**	**never,** N = 34,910[1]	**<1/ week,** N = 6,990[1]	**1-6/ week,** N = 13,636[1]	**≥1/ day,** N = 11,313[1]	**N**	**never,** N = 5,875[1]	**<1/ week,** N = 8,285[1]	**1-6/ week,** N = 29,143[1]	**≥1/ day,** N = 23,443[1]
Maternal Age	90,293	30.4 (4.3)	66,849	30.8 (4.3)	30.2 (4.2)	29.9 (4.1)	29.7 (4.1)	66,746	30.8 (4.4)	30.8 (4.4)	30.4 (4.2)	30.1 (4.2)
Pre-pregnancy BMI	83,195	23.5 (4.3)	63,079	22.9 (3.8)	23.4 (4.0)	24.0 (4.3)	24.6 (5.0)	62,975	24.2 (5.0)	23.5 (4.2)	23.5 (4.2)	23.3 (4.0)
Healthy Eating Index, points/80	68,955	23 (7)	66,692	23 (7)	23 (7)	23 (7)	22 (7)	66,647	28 (7)	26 (7)	23 (6)	20 (6)
Total Energy Intake, kcal/day	66,590	2,471 (683)	64,334	2,510 (675)	2,390 (669)	2,406 (637)	2,507 (713)	64,249	2,246 (645)	2,278 (603)	2,407 (610)	2,689 (696)
Smoking in pregnancy	88,457	23,306 (26%)	66,612	8,418 (24%)	1,497 (22%)	3,273 (24%)	3,095 (27%)	66,502	1,429 (24%)	1,706 (21%)	6,673 (23%)	6,393 (27%)
Socioeconomic status	84,249		63,839					63,736				

Unskilled, other work or receives public benefits		3,331 (4.0%)		1,060 (3.2%)	216 (3.2%)	456 (3.5%)	491 (4.5%)		218 (3.9%)	281 (3.6%)	853 (3.1%)	841 (3.8%)
Working class, craftsmen, short education or under education		24,449 (29%)		8,371 (25%)	1,724 (26%)	3,899 (30%)	3,855 (36%)		1,683 (30%)	2,118 (27%)	7,409 (27%)	6,559 (29%)
Leaders or long/medium long education		56,469 (67%)		23,833 (72%)	4,740 (71%)	8,686 (67%)	6,508 (60%)		3,700 (66%)	5,512 (70%)	19,585 (70%)	14,977 (67%)
Maternal SSB intake	67,032		65,712									
< 500mL/week		23,833 (36%)		9,250 (27%)	3,009 (44%)	5,633 (42%)	5,559 (51%)					
500-1500 mL/week		18,335 (27%)		9,826 (28%)	1,832 (27%)	4,183 (31%)	2,210 (20%)					
≥ 1500 mL/week		24,864 (37%)		15,450 (45%)	2,036 (30%)	3,510 (26%)	3,214 (29%)					
Maternal ASB	67,00							65,590				

intake	5										
< 500mL/week		47,644 (71%)							2,537 (44%)	5,549 (68%)	20,812 (72%)	17,916 (78%)
500-1500 mL/week		7,585 (11%)							930 (16%)	983 (12%)	3,747 (13%)	1,731 (7.6%)
≥ 1500 mL/week		11,776 (18%)							2,345 (40%)	1,670 (20%)	4,169 (15%)	3,201 (14%)
Paternal BMI	62,047	25.2 (3.2)	48,661	24.97 (3.18)	25.04 (2.98)	25.27 (3.12)	25.53 (3.38)	48,601	25.30 (3.34)	25.08 (3.37)	25.11 (3.11)	25.13 (3.17)
Sex, Boy	90,293	46,232 (51%)	66,849	17,784 (51%)	3,589 (51%)	7,111 (52%)	5,753 (51%)	66,746	2,964 (50%)	4,243 (51%)	14,985 (51%)	11,987 (51%)
Birth Weight, g	89,897	3,602 (537)	66,566	3,604 (532)	3,609 (529)	3,613 (539)	3,605 (538)	66,466	3,599 (533)	3,599 (523)	3,610 (529)	3,608 (542)
Gestational age, weeks	90,288	40.06 (1.48)	66,848	40.07 (1.47)	40.09 (1.48)	40.07 (1.48)	40.04 (1.50)	66,745	40.05 (1.50)	40.07 (1.48)	40.08 (1.46)	40.06 (1.49)
Physical activity in early pregnancy	84,537	31,133 (37%)	64,033	12,513 (37%)	2,793 (42%)	5,190 (40%)	4,081 (37%)	63,930	2,377 (42%)	3,318 (42%)	10,937 (39%)	7,886 (35%)
Breastfeeding	65,13		50,88					50,820				

duration	2		8									
≤6 months		26,433 (41%)		9,571 (36%)	2,007 (38%)	4,486 (43%)	4,162 (49%)		1,950 (44%)	2,431 (38%)	8,634 (39%)	7,173 (40%)
>6 months		38,699 (59%)		17,024 (64%)	3,340 (62%)	5,937 (57%)	4,361 (51%)		2,445 (56%)	3,937 (62%)	13,652 (61%)	10,598 (60%)
Timing of introduction of solid foods, months	66,126	4.44 (0.70)	51,211	4.48 (0.70)	4.45 (0.68)	4.41 (0.69)	4.34 (0.67)	51,164	4.42 (0.71)	4.47 (0.71)	4.45 (0.69)	4.42 (0.69)

[1]Mean (SD); n (%). ASB = artificially sweetened beverages. SSB = sugar-sweetened beverages. BMI = body mass Index.

Table 2: Odds ratio for large for gestational age birth weight or overweight at different ages dependent on maternal beverage consumption during pregnancy.

		ASB consumption				SSB consumption			
Age	**Consumption frequency**	**N**	**Unadjusted**	**N**	**Adjusted**[2]	**N**	**Unadjusted**	**N**	**Adjusted**[2]
			OR (95%CI)[1]		**OR (95%CI)**[1]		**OR (95%CI)**[1]		**OR (95%CI)**[1]
LGA at birth		66,078		45,690		65,976		45,656	
	never		—		—		—		—
	<1/week		0.92 (0.73, 1.13)		0.79 (0.60, 1.02)		0.86 (0.65, 1.13)		0.90 (0.65, 1.24)
	1-6/week		1.12 (0.96, 1.31)		0.99 (0.82, 1.19)		0.94 (0.76, 1.18)		1.03 (0.79, 1.35)
	≥1/day		1.08 (0.91, 1.28)		0.89 (0.73, 1.10)		1.01 (0.81, 1.28)		1.15 (0.87, 1.53)
5 months		42,256		32,049		42,207		32,020	
	never		—		—		—		—
	<1/week		0.85 (0.77, 0.95)		0.81 (0.71, 0.92)		0.96 (0.84, 1.09)		0.99 (0.85, 1.16)
	1-6/week		1.05 (0.97, 1.14)		1.00 (0.91, 1.10)		0.86 (0.77, 0.97)		0.89 (0.78, 1.02)

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

Accepted manuscript

		ASB consumption				SSB consumption			
Age	**Consumption frequency**	**N**	**Unadjusted**	**N**	**Adjusted**[2]	**N**	**Unadjusted**	**N**	**Adjusted**[2]
			OR (95%CI)[1]		**OR (95%CI)**[1]		**OR (95%CI)**[1]		**OR (95%CI)**[1]
	≥1/day		1.05 (0.97, 1.15)		0.98 (0.88, 1.08)		0.92 (0.83, 1.04)		0.98 (0.85, 1.13)
12 months		39,361		35,988		39,320		35,969	
	never		—		—		—		—
	<1/week		0.94 (0.84, 1.05)		0.90 (0.80, 1.01)		0.94 (0.82, 1.07)		0.97 (0.84, 1.13)
	1-6/week		1.08 (1.00, 1.18)		1.00 (0.92, 1.09)		0.89 (0.79, 1.00)		0.93 (0.82, 1.05)
	≥1/day		1.07 (0.98, 1.17)		0.96 (0.87, 1.05)		0.90 (0.81, 1.02)		0.99 (0.87, 1.12)
7 years		41,431		30,646		41,370		30,611	
	never		—		—		—		—
	<1/week		1.10 (0.98, 1.22)		1.01 (0.88, 1.15)		0.84 (0.73, 0.96)		1.04 (0.89, 1.23)
	1-6/week		1.34 (1.23, 1.45)		1.19 (1.08, 1.32)		0.79 (0.70, 0.88)		0.98 (0.85, 1.12)
	≥1/day		1.53 (1.41,		1.17 (1.05,		0.79 (0.71,		0.98 (0.85,

https://doi.org/10.1017/S0007114525000455 Published online by Cambridge University Press

		ASB consumption				SSB consumption			
Age	Consumption frequency	N	Unadjusted	N	Adjusted[2]	N	Unadjusted	N	Adjusted[2]
			OR (95%CI)[1]		OR (95%CI)[1]		OR (95%CI)[1]		OR (95%CI)[1]
			1.67)		1.29)		0.89)		1.15)
11 years		29,020		21,909		28,971		21,866	
	never		—		—		—		—
	<1/week		1.10 (0.97, 1.25)		1.06 (0.90, 1.23)		0.85 (0.73, 1.00)		1.04 (0.86, 1.26)
	1-6/week		1.38 (1.26, 1.51)		1.19 (1.06, 1.33)		0.77 (0.68, 0.88)		0.90 (0.77, 1.07)
	≥1/day		1.69 (1.54, 1.87)		1.19 (1.05, 1.35)		0.77 (0.68, 0.89)		0.82 (0.69, 0.98)
14 years		28,804		21,594		28,746		21,550	
	never		—		—		—		—
	<1/week		1.04 (0.92, 1.19)		0.99 (0.84, 1.16)		0.84 (0.72, 0.99)		1.06 (0.87, 1.30)
	1-6/week		1.34 (1.22, 1.48)		1.08 (0.96, 1.22)		0.78 (0.69, 0.90)		0.93 (0.78, 1.10)
	≥1/day		1.73 (1.57, 1.91)		1.16 (1.03, 1.32)		0.80 (0.70, 0.92)		0.91 (0.76, 1.09)

		ASB consumption				SSB consumption			
Age	**Consumption frequency**	**N**	**Unadjusted**	**N**	**Adjusted[2]**	**N**	**Unadjusted**	**N**	**Adjusted[2]**
			OR (95%CI)[1]		**OR (95%CI)[1]**		**OR (95%CI)[1]**		**OR (95%CI)[1]**
18 years		32,746		23,852		32,701		23,807	
	never		—		—		—		—
	<1/week		1.09 (0.95, 1.23)		1.07 (0.91, 1.25)		0.65 (0.56, 0.76)		0.75 (0.61, 0.91)
	1-6/week		1.41 (1.28, 1.55)		1.13 (1.00, 1.27)		0.68 (0.60, 0.77)		0.79 (0.67, 0.93)
	≥1/day		1.86 (1.69, 2.05)		1.26 (1.12, 1.42)		0.69 (0.60, 0.78)		0.72 (0.60, 0.86)

[1]OR = Odds Ratio (CI = Confidence Interval). [2]Adjusted for maternal pre-pregnancy body mass index, Healthy Eating Index, age, smoking during pregnancy, physical activity in early pregnancy, duration of breastfeeding (except at birth), socioeconomic status, and paternal body mass index. ASB = artificially sweetened beverages. SSB = sugar-sweetened beverages. LGA = large for gestational age.