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Birth weight, GDM exposure and cardio-metabolic health.

**Exposure to gestational diabetes is a stronger predictor of dysmetabolic traits in children than size at birth**

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Abbreviations: AGA, appropriate for gestational age; ANOVA, analysis of variance; BMI, body mass index; BW, birth weight; DEXA, dual-energy X-ray absorptiometry; DNBC, Danish National Birth Cohort; CI, confidence interval; GA, gestational age; GDM, gestational diabetes mellitus; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; IQR, interquartile range; LBW, low birth weight; LDL, low density lipoprotein; LGA, large for gestational age; MS, metabolic syndrome; SD, standard deviation; SGA, small for gestational age; T1D, type 1 diabetes; T2D, type 2 diabetes.

Context and Objective

Being born small or large for gestational age and intrauterine exposure to gestational diabetes (GDM) increase the risk of type 2 diabetes (T2D) in the offspring, however, the potential combined deleterious effects of size at birth and GDM exposure remains unknown. We aimed to examine the independent effect of size at birth as well as the influence of GDM exposure in utero on cardio-metabolic traits, body composition, and puberty status in children.

Design, Participants and Methods

This study is a longitudinal birth cohort study. We used clinical data from 490 offspring of mothers with GDM and 527 control offspring aged 9-16 years, born singleton at term from the Danish National Birth Cohort with available birth weight data.

Results

We found no evidence of a U-shaped association between size at birth (expressed as birth weight, sex and gestational age adjusted z-score) and cardio-metabolic traits. Body size in childhood and adolescence reflected size at birth, but was not reflected in any metabolic outcome. No synergistic adverse effect of being born small or large for gestational age and being exposed to GDM was shown. However, GDM was associated with an adverse metabolic profile and earlier onset of female puberty in childhood and adolescence independently of size at birth.

Conclusion

In childhood and adolescence, GDM is a stronger predictor of dysmetabolic traits than size at birth. The combination of being born small or large and being exposed to GDM does not exacerbate the metabolic profile in the offspring.

We examined the effect of size at birth and GDM exposure on metabolic traits, body composition, and puberty onset. We found that GDM is a stronger predictor of dysmetabolic traits than size at birth.

INTRODUCTION

Since the prevalence of type 2 diabetes (T2D) has increased dramatically in the last decade (1), early identification of individuals with an increased risk of T2D is of high importance. In 1991, Hales and Barker proposed that early fetal development, as expressed by reduced growth, was associated with an increased risk of developing T2D in adulthood (2). This association has subsequently been confirmed in a large number of studies during the last three decades (3,4), with increased abdominal fat distribution, increased fasting blood glucose, reduced muscle mass
(5,6), and hepatic insulin resistance (7) representing some of the adult metabolic abnormalities present in individuals born small at birth.

However, the shape of the association in the relationship between birth weight (BW) and T2D has been inconsistent across studies, including findings of both decreased (inverse association) (3,8), and increased risk of developing T2D with increased birth weight (3). In addition, a large meta-analysis of adult Caucasians reported that high birth weight (>4000g) was associated with an increased risk of developing T2D to the same extent as low birthweight (LBW) (<2500g) when compared to normal birth weight (2500-4000g), indicating a U-shaped association between BW and later risk of T2D (9).

The increased risk of metabolic disease at the high end of the BW spectrum has been suggested to be strongly influenced by the exposure to gestational diabetes mellitus (GDM) in fetal life (3,10). Previous studies have reported that offspring of women with GDM are more likely to be overall and centrally obese, and to exhibit insulin resistance and glucose intolerance in early adulthood (10–13). Recently, we showed that adolescent offspring of women with GDM have an adverse metabolic and body compositional profile compared to offspring born to control mothers (14). Additionally, earlier onset of puberty has been linked to an increased risk of cardiovascular and metabolic disease later in life (15), and both size at birth (16) and fetal exposure to GDM (14,17) are thought to affect pubertal maturation. As such, the effect of being born of a mother with GDM seems similar to those effects seen among individuals born with a low or high BW.

However, to the best our knowledge only few studies have investigated the potential joint association of BW and GDM on childhood health in a general population (18–20). Two longitudinal studies showed a combined deleterious effect of being born large for gestational age (LGA) and being exposed to GDM on the development of metabolic syndrome (MS) (18) and obesity (19) in childhood. A prospective cohort study reported an increased risk of overweight in adolescence with increasing BW, and further reported an adverse effect of exposure to GDM. However, the effect of GDM in the latter study was mainly explained by BW, indicating that BW is a more essential risk marker for being overweight in adolescence compared to GDM exposure in utero. Therefore, the evidence of a potential combined adverse effect of being born small or large and exposed to GDM in utero seems conflicting. Previous studies addressing this are limited by excluding children born small for gestational age (SGA) (18,19), including self-reported measures on anthropometrics (20), by not exploring the potential effect on puberty onset, and by lack of advanced measures of body composition (19,20).

In this study, we aimed to examine the associations between size at birth and GDM exposure in fetal life on cardio-metabolic traits, body composition, and puberty status in 9-16-year-old Danish children.

METHODS

Study population and design:
The present study is based on clinical data from a sub-cohort of children nested within the Danish National Birth Cohort (DNBC) (21). In brief, a total of 608 offspring of mothers with GDM and 626 randomly selected control offspring from the DNBC, attended a clinical examination. Data collection was conducted nationwide in Denmark, and included measures of anthropometry, body composition, puberty status, and cardio-metabolic traits from boys and girls aged 9-16 years. The study design and method have previously been described in detail (14).
We included offspring born at term (between 38\textsuperscript{th}-42\textsuperscript{nd} gestational week) with available information on gestational age (GA) and BW (n=1063). No twins or triplets (33 twins, 3 triplets) were included and the analyses were confined to the first child enrolled for each woman to avoid correlated observations between siblings (35 siblings excluded). Offspring with type 1 diabetes (T1D) (n=3) and offspring born to mothers with T1D (n=8) were excluded. Our final study sample included 1017 offspring. The study was performed according to the declaration of Helsinki and the protocol was approved by Regional Scientific Ethics Committee of the municipalities of Copenhagen and Frederiksberg (H-4-2001-045 and H-4-2013-129). Consent from both parents was given prior to the child’s participation in the study.

**Exposure assessment:**

**Birth measures**

Information on BW and date of birth were extracted from the Danish Medical Birth Registry. GA at birth was determined by one or more of the following: (i) the expected due date, (ii) the mother’s last menstrual period, or (iii) information derived from the Danish Medical Birth Registry (22).

BW-z-score was calculated as (BW-normal BW for GA)/SD for normal BW. Subjects were classified into one of three categories of BW for GA: SGA (< 10\textsuperscript{th} percentile), appropriate for gestational age (AGA) (10-90\textsuperscript{th} percentile), or large for gestational age (LGA) (> the 90\textsuperscript{th} percentile). Sex- and GA-specific BW standard curves produced from full-term singleton births from the entire DNBC population were used as reference for normal BW to compute the variables for birth size.

**GDM exposure and diagnosis**

Identification of suspected GDM exposure in utero was based on previously described GDM diagnosis criteria (23). Briefly, women were classified as having a GDM diagnosis if in index pregnancy they had a documented GDM diagnosis recorded in the Danish National Patient Registry (International Classification of Disease (ICD)-10 classifications 0244 and 0249) or a self-reported GDM diagnosis documented from the telephone interview conducted at 30 wk. of gestation or 6 mo. postpartum.

**Clinical outcome measures:**

Anthropometric measurements, resting blood pressure, fasting glucose, insulin, C-peptide, and lipid profiles were included. Sex and age specific z-scores for height, weight, and BMI were calculated according to Danish national reference curves based on the LMS method (24). Since no Danish reference curves for waist circumference (WC) exist, we calculated sex and age specific WC z-scores from British normal reference material ((WC\textsubscript{measured}-mean WC)/SD for WC) (25). Crude measures, as well as sex and age specific z-scores for weight, height, BMI and WC were used to characterize the anthropometric measurements of the offspring, whereas only z-scores for these variables were applied for the remaining analyses. Standard assays were applied for biomarker analysis as previously described (14). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was evaluated and calculated as \(((\text{Fasting plasma insulin (pmol/L)} × \text{fasting plasma glucose (mmol/L)})/22.5) × 0.144\). Puberty status was determined by Tanner score examination performed by educated staff. Breast development ≥B2 for girls and testicle volume ≥4 mL for boys was considered the primary marker of pubertal onset (26–28). Additionally, pubic hair stage ≥PH2 in girls and boys and genital stage ≥G2 in boys were considered secondary markers of onset of puberty. Information of body composition measured by DEXA scanning (Lunar, Scanex), were available for a sub-set of offspring (n=556).
Covariates:
Data on parental socio-occupational status (defined by the highest parental level of education and occupation: high proficiency, intermediate proficiency, skilled workers, unskilled workers, students, or unemployed), maternal parity (1st, 2nd or 3rd+ born), maternal smoking during pregnancy (non-smoker, or smoker), maternal height, and maternal pre-pregnancy BMI (<25, 25-29.9, or ≥ 30 kg/m2) were derived from pregnancy interviews at gestational weeks 12 and 30. Information on maternal weight gain during pregnancy was obtained from interviews at 6 mo. postpartum. All information was self-reported.

Statistical analysis
To address potential differences in background characteristics, anthropometrics, body composition and cardio-metabolic traits between SGA, AGA, and LGA offspring in GDM stratified analysis, one-way ANOVA was applied for normally distributed residuals, Kruskal-Wallis test for skewed residuals, and X²-test for categorical variables. Equality of variance between the BW groups was tested with Levens test, and in case of variance heterogeneity (p<0.05) the Welch test was applied instead of one-way ANOVA. Assumptions of normally distributed residuals were visually evaluated with histograms and QQ-plots. Data are presented as mean±standard deviation (SD) for normally distributed variables, median (interquartile range [IQR]) for log-transformed or skewed variables, and as n (percentages) for categorical variables.

The shape of association between BW z-score and continuous outcome variables were visualized by scatterplots and tested by linear regression analyses by adding the explanatory variable as a linear and squared term (to allow for a U-shaped relation). Since no U-shaped associations were found (p>0.05), linear regression analyses were applied to examine the impact of size at birth on childhood anthropometrics, metabolism, and body composition, and the effect size was given in changes pr. increase in BW z-score. Because the effect of birth weight may be sex-dependent (29), the effect modification by sex was examined. No significant interaction with sex was observed (p>0.05), and as such, we included sex as a potential confounding variable in our models. Subsequently, for variables that were statistically significant in our simple regression model, we applied multiple regression analysis with adjustment for confounding variables in three different steps. Model 1 was adjusted for age and sex. Model 2 was further adjusted for: maternal pre-pregnancy BMI, maternal pregnancy weight gain, paternal social-occupational status, maternal smoking during pregnancy, and maternal parity, while maternal height was additionally adjusted for in model 3 as a way to account for genetic influence on offspring body size. For all regression models, data were stratified by GDM exposure and variables were log-transformed when needed to meet the assumptions of the model.

To examine a potential additive adverse effect of GDM exposure in utero on the association between being born SGA or LGA compared to AGA on anthropometry, cardio-metabolic traits and body composition we applied an additive 2-way ANOVA analysis with correction for multiple comparisons (Tukey-Kramer test) while adding BW groups and GDM exposure as the explanatory variables. We calculated β-coefficient and 95% CI to estimate mean differences for normally distributed residuals or % differences for log-transformed data. Additionally, interaction analysis was conducted, to test potential interaction between BW groups and GDM exposure in relation to metabolic profile in childhood.

Onset of puberty was analyzed with logistic regression models and we calculated odds ratios (OR) and 95% CIs while adjusting for offspring age and BMI in two subsequent models.

SAS version 9.4 (SAS Institute, 127 Cary, North Carolina, USA) was used for all statistical analyses. Two-tailed p-value ≤0.05 was considered statistically significant.
RESULTS

Parental, birth, and childhood characteristics for control and GDM offspring born SGA, AGA, or LGA

The median BW ranged from 2900-4500g in control offspring and from 2816-4415g in the GDM offspring (table 1). Maternal weight gain during pregnancy was significantly different across BW groups with the largest weight gain found among mothers who gave birth to a LGA offspring (control; \( p=0.02 \), GDM; \( p<0.001 \)). The proportion of LGA births increased with maternal age and parity in offspring exposed to GDM in utero (\( p=0.05 \) and \( p=0.0004 \)). The proportion of mothers who smoked during pregnancy was almost twice as high in offspring born SGA compared with offspring born AGA or LGA among the control group (\( p=0.03 \)).

Among control offspring, BW groups were positively associated with z-scores for weight, height, BMI, and waist, as well as hip circumferences in childhood. In contrast, in children exposed to GDM in utero, childhood z-score for weight and height were the only measures of anthropometry which were significantly related to size at birth. No associations between size at birth and cardio-metabolic health or body composition in childhood were seen in either GDM or control offspring (table 2).

Shape of association and impact of size at birth

We found no U-shaped association between BW z-score and anthropometrics, cardio-metabolic traits, or any measures of body composition in offspring of control or GDM mothers (\( p>0.05 \), data not shown).

Z-scores for weight, height, BMI, and waist, as well as hip measures were positively associated with BW z-score independently of offspring sex and age in both control and GDM offspring (\( p<0.05 \), table 3 and supplemental table A (model 1) (30)). Furthermore, in GDM offspring, the BW z-score was positively associated with HDL cholesterol and inversely associated with systolic blood pressure after adjustment for offspring sex and age (\( p<0.05 \), supplemental table A (model 1)) (30).

When further adjusting for parental socio-occupational status and lifestyle factors, an increase in BW z-score was associated with an increase in weight and height z-score of 0.2 (95% CI: 0.1, 0.3, \( p<.0001 \)) and a 0.8cm (95% CI: 0.1-1.4cm, \( p=0.02 \)) increased hip circumference in control offspring. Similar estimates were observed for GDM offspring in relation to weight z-score (0.2; 95% CI: 0.1, 0.3, \( p=0.0003 \)), height z-score (0.2; 95% CI: 0.1, 0.3, \( p<.0001 \)), and hip circumference (0.9cm, 95% CI 0.1-1.8, \( p=0.05 \)), and was furthermore associated with an increase in waist z-score of 0.2 ((95% CI: 0.00, 0.32, \( p=0.05 \)) and a reduction in systolic blood pressure of 1% (95% CI: -2 to 0%, \( p=0.02 \)) pr. increase in BW z-score (supplemental table A (model 2)) (30). Weight and height z-scores for GDM and control offspring, and systolic blood pressure and hip circumference (for GDM offspring only) stayed statistically significantly associated to BW z-score after further adjustment for maternal height (supplemental table A (model 3)) (30). However, size at birth was not statistically significantly related to the remaining cardio-metabolic outcomes or measures of body composition by DEXA in offspring born of control or GDM mothers (table 3).

Potential combined effect of BW and GDM exposure on cardio-metabolic health in childhood

Examining the BW GDM association on cardio-metabolic health in a 2-way ANOVA analysis showed that size at birth was statistically significantly associated with size in childhood (for weight z-score and height z-score in SGA offspring and for weight z-score, BMI z-score, waist z-score and hip circumferences in LGA offspring) independently of GDM exposure status (table
However, size at birth was not statistically significantly associated with any cardio-metabolic or body compositional measure (table 4). GDM exposure within the same BW categories was statistically significantly associated with adverse anthropometric, metabolic and body compositional outcomes (table 4). We found no interaction between BW groups and GDM status in any of the outcomes (p>0.05, data not shown).

**Onset of puberty**

The proportion of offspring who had reached puberty did not differ significantly across BW groups in offspring exposed or not exposed to GDM (table 5). In age and BMI adjusted analyses, the size at birth was not statistically significantly associated with puberty onset among males or females. However, among female offspring exposed to GDM, the odds of having reached puberty, assessed by breast development, was more than 2 times greater compared to control offspring, independently of size at birth (p=0.04, table 6).

**DISCUSSION**

In the present study, we showed that being born small or large for gestational age was associated with anthropometric measurements including height, weight, BMI, waist, and hip circumference in childhood, whereas no association with measures of cardio-metabolic outcomes, body composition assessed by DEXA, or pubertal maturation was seen. Our results do not support a U-shaped association between BW and adverse cardio-metabolic traits in children at this young age. Most interestingly, we found no adverse synergistic effect between being born small or large for GA and being exposed to GDM. In contrast, our results indicate that independently of size at birth, GDM exposure in utero is a strong predictor of a disadvantageous body composition, adverse cardio-metabolic traits, and earlier onset of female puberty in childhood and adolescence.

**Impact of BW and shape of association**

In accordance with previous studies, we showed that individuals born small were shorter and lighter at the time of follow-up. Individuals born LGA were heavier, with a higher BMI and larger waist- and hip circumference compared to children born AGA (table 4).

An adverse fat distribution, in particular increased abdominal obesity, is a strong predictor of T2D (31), and waist circumference, which is related to intra-abdominal fat mass, has been shown to be a valid measure of trunk fat mass in children aged 3-19 years (32). Our data showed a significantly increased waist circumference in children born LGA independently of exposure to GDM (table 4). These findings were confirmed in our linear regression models, with an increase in waist circumferences z-score pr. increase in BW z-score in both exposed and unexposed children (table 3 and supplemental table A (30)). However, after adjusting for maternal confounders, the association was attenuated and no longer statistically significant (supplemental table A) (30).

It has previously been shown in a twin study with discordance for T2D that a non-genetic association exists between LBW and glucose intolerance and insulin resistance (33). Furthermore, we and others have documented several metabolic defects in young adult SGA individuals including decreased fat-free mass, increased abdominal obesity and dysmetabolic traits (5–7). Interestingly, such metabolic differences between SGA and AGA individuals were not apparent in our study population. The use of more advanced methods to study glucose metabolism in the aforementioned studies could account for some of the discrepancies. Additionally, the aforementioned studies in young adults showed that the differences between
AGA and SGA are particularly apparent when SGA individuals are subjected to metabolic challenges, e.g. overfeeding, 36-hour fasting, or bedrest (7,34,35). In the studies of discordant twins the association between LBW and insulin resistance was only seen among elderly twins, suggesting an age-dependent effect (36). Finally, we did not see any evidence of a U-shaped relationship between BW and T2D risk as previously reported (9). In contrast to these studies, we looked at early markers of T2D in young healthy individuals. It is plausible that detrimental metabolic effects of low or high BW could be masked during puberty or could be more evident later in adolescence or adulthood.

No combined adverse health effect of size at birth and intrauterine GDM exposure

Both individuals born SGA and offspring exposed to GDM in utero are at increased long-term risk of developing T2D due to an adverse fetal environment. Data from Danish follow-up studies strongly suggest that in utero exposure to diabetes may place the offspring at an increased risk for T2D that exceeds the magnitude recognized for people born SGA (4,37,38). In line with this, the results of the present study demonstrated a significant adverse effect of intrauterine exposure to GDM unrelated to the offspring’s size at birth on body composition and metabolic traits. This indicates that in utero GDM exposure has a stronger impact on offspring health in childhood compared to the effect of BW, and that the combination of being born SGA or LGA and exposed to GDM does not seem to exacerbate the adverse metabolic effect of being exposed to GDM per se. Results from a longitudinal prospective study of 4-7 year-old children born either AGA or LGA of GDM or control mothers, showed an increased prevalence of obesity at age 7 among children born LGA of GDM mothers compared to the other study groups (19). However, these differences were no longer evident at age 9 (18) in accordance with our results. At 11 years of age, LGA offspring had a 3.6-fold significantly greater cumulative risk of developing MS compared to children born AGA. This effect of BW only applied to offspring exposed to GDM in fetal life (18). A potential adverse synergistic effect of BW and GDM exposure on childhood metabolic health in SGA infants was not examined in the aforementioned study.

Our analyses, stratified by GDM exposure with and without adjustment for confounders, indicated a modest (1%) yet significant adverse effect of being born at the low end of the BW spectrum (BW z-score) and being exposed to GDM on systolic blood pressure (table 3 and supplemental table A (30)). A study investigating 14,881 offspring at the age of 9-14 years reported a protective effect of being born small on offspring adiposity with a 30% increased risk of being overweight (BMI > 95th percentile) for each 1kg increment of BW. The study further showed that GDM exposure increased the risk of being overweight in childhood significantly (20). They did not detect any interaction between GDM and BW, similar to our results. However, they found that the association between GDM exposure and later risk of being overweight was no longer statistically significant after adjustment for BW, suggesting that BW, rather than GDM exposure, is a superior risk marker of overweight in childhood (20). However, the use of self-reported measures of BW, GA, and anthropometrics in the aforementioned study potentially increased the risk of recall and reporting bias.

Puberty onset

We found that the likelihood of having reached puberty based on Tanner stages for breast development, which is considered the gold standard for evaluating puberty onset among girls (28), was increased among girls exposed to GDM, irrespective of their size at birth, age, and current BMI (table 6). The association between GDM exposure and earlier onset of pubertal development shown in our study was consistent with previous findings (14,17), and our results indicate that exposure to GDM in utero is a more important predictor for earlier onset of puberty.
among females compared to size at birth. However, this was not the case for boys where testicular size was used to define the beginning of puberty, nor when other secondary sexual characteristics, which do not necessarily represent puberty onset, were applied.

**Strength and limitations**

To the best of our knowledge, this study is the largest of its kind to use objective measures of cardio-metabolic outcomes, body composition, and puberty status to examine the impact of BW and GDM exposure. Recently, a validation study on self-assessed Tanner staging in boys and girls nested within the DNBC showed a substantial proportion of misclassification of Tanner stages (39), which emphasizes the value of using clinical data of pubertal development. By including both SGA and LGA individuals of GDM and control mothers, we were able to study the effect of size at birth and GDM exposure in relation to childhood cardio-metabolic health based on objective data of BW obtained from reliable records.

However, the use of BW as a proxy for an adverse fetal environment has been debated since BW is an unspecific marker of fetal growth. Indeed, the association between BW and risk of T2D may be confounded by a variety of factors including genetic and non-genetic factors (4). Furthermore, a large variability in body composition for a given BW category has been shown (40), and fetal and infant body composition and growth might play an important role in the association between BW and childhood health. A limitation of our study was the lack of information on body composition at birth and of genetic factors which might have affected BW and later risk of T2D. However, we were able to control for several other covariates which have been associated with BW and/or risk of T2D, including parental socio-occupational status (41,42), maternal smoking during pregnancy (41), parity (8,41) and maternal obesity (pre-pregnancy BMI and pregnancy weight gain) (18,19,43).

**CONCLUSION**

We demonstrated that body size in childhood and adolescence reflected size at birth, but potential deleterious metabolic effects of being born small or large were not apparent at the age of 9-16. Furthermore, the combination of being born small or large for GA and being exposed to GDM *in utero* did not increase the risk of adverse health outcome in childhood or adolescence. However, we confirmed that exposure to GDM is a risk factor for detrimental cardio-metabolic outcomes, disadvantageous body composition and earlier onset of female puberty independently of the offspring’s size at birth. From a health and prevention perspective, exposure to GDM may be a more important risk factor for the cardio-metabolic health of children and adolescents compared to their size at birth.

**ACKNOWLEDGMENTS**

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AUTHOR CONTRIBUTIONS

S.F.O., and L.G.G. are the guarantors of this work, had full access to the data, and takes responsibility for the integrity and contents of the data and the accuracy of the data analyses. F.B.K., AC.B.T., L.H., and L.G.G. collected the clinical data together with the GDM/DNBC study team. The research question for this study was designed in cooperation between F.B.K, AC.B.T, and L.G.G.

F.B.K. was responsible for analyzing the data and drafting the manuscript. AC.B.T., L.H., and L.G.G. assisted in interpretation of the results. All authors reviewed the manuscript and approved the final version.

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Disclosure Summary:
The authors declare no conflict of interest relevant to this paper.

REFERENCES

10. Mccance DR, Pettitt DJ, Hanson RL, Jacobsson LTH, Knowler WC, Bennett PH, Bmj S, Medical B, Apr N, Mccance DR, Pettitt DJ, Hanson RL, Jacobsson LTH, Knowler WC. Birth Weight And Non-Insulin Dependent Diabetes : Thrifty Genotype , Thrifty Phenotype , Or
Surviving Small Baby Genotype? Published by: BMJ Stable URL: http://www.jstor.org/stable/29723183 REFERENCES Linked references are available on JSTOR for this article. 2018;308(6934):942-945.


Table 1. Parental and birth characteristics of children born SGA, AGA, or LGA

<table>
<thead>
<tr>
<th>Birth characteristics</th>
<th>Control offspring</th>
<th>GDM offspring</th>
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<tbody>
<tr>
<td>Number of subjects, n (%)</td>
<td>SGA</td>
<td>AGA</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>2900</td>
<td>3600</td>
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<tr>
<td>Birthweight z-score</td>
<td>-1.7 (0.5)</td>
<td>-0.1 (1.0)</td>
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<tr>
<td>Sex (male), n (%)</td>
<td>29 (56)</td>
<td>219 (50)</td>
</tr>
<tr>
<td>Gestational age at birth, days</td>
<td>283 ±12</td>
<td>281 ±11</td>
</tr>
</tbody>
</table>

Table 2. Anthropometry, metabolic markers and body composition of offspring born SGA, AGA or LGA of mothers with GDM and control offspring

<table>
<thead>
<tr>
<th>Anthropicometric characteristics, N</th>
<th>Control offspring</th>
<th>GDM offspring</th>
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<tr>
<td></td>
<td>SGA</td>
<td>AGA</td>
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</tbody>
</table>

Data are presented as mean ± SD for normally distributed variables, median (IQR) for log transformed or skewed variables and n (%) for categorical variables.

P-values denote overall differences between BW categories stratified by GDM exposure; calculated using 1-way ANOVA (or Welch Test) for continues parametric variables or Kruskal-Wallis test for nonparametric continues variables, and Chi-square test for categorical variables (Fischers exact test).

Abbreviations: BMI: body mass index, SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age.
Data are presented as mean ± SD for normally distributed variables and median (IQR) for log transformed variables. P-values denote overall differences between BW categories stratified by GDM exposure; calculated using 1-way ANOVA (or Welch Test for nonparametric continues variables or Kruskal-Wallis test for nonparametric continues variables).

Systolic- and diastolic blood pressure were adjusted for current offspring height.


Sex- and GA-specific BW standard curves produced from full-term singleton births offspring from the entire DNBC population were used as reference for normal BW to categorize subjects into AGA (<10th percentile), AGA (10-90th percentile), or LGA (>90th percentile) BW groups.

Table 3. Influence of BW z-score on measures of childhood anthropometrics, metabolism, and body composition.

<table>
<thead>
<tr>
<th>Offspring characteristic</th>
<th>Control offspring</th>
<th>GDM offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unadjusted</strong></td>
<td><strong>β (95% CI)</strong></td>
<td><strong>p</strong></td>
</tr>
<tr>
<td><strong>Anthropometric variables, N</strong></td>
<td>523-526</td>
<td></td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.2 (0.2, 0.3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Height z-score</td>
<td>0.3 (0.2, 0.4)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.1 (0.02, 0.22)</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist z-score</td>
<td>0.2 (0.1, 0.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>1.3 (0.5, 2.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.0 (0.0, 0.0)</td>
<td>0.91</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>0% (~1 to 1%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>-0.02 (~0.58, 0.54)</td>
<td>0.94</td>
</tr>
</tbody>
</table>
The effect of exposure to GDM within BW groups.

P-values are obtained by simple linear regression. Estimates are presented as mean changes pr. increase in BW z-score, for normally distributes residuals, or as % changes (95% CI) when data are log transformed.

Systolic- and diastolic blood pressure were adjusted for current offspring height.

Abbreviations: BMI: body mass index, HOMA-IR: homeostatic model assessment Insulin resistance.

Table 4. The effect of size at birth for gestational age and GDM exposure in fetal life on offspring anthropometric, cardio-metabolic and body compositional outcomes in 9-16 years children

<table>
<thead>
<tr>
<th>Metabolic characteristics, N</th>
<th>SGA vs. AGA</th>
<th>LGA vs. AGA</th>
<th>GDM exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring outcome</td>
<td>mean difference or % difference (95% CI)</td>
<td>mean difference or % difference (95% CI)</td>
<td>mean difference or % difference (95% CI)</td>
</tr>
<tr>
<td>Birth characteristics</td>
<td>p</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>-812 (-906, -718)</td>
<td>&lt;.0001</td>
<td>876 (803, 949)</td>
</tr>
<tr>
<td>Gestational age at birth, days</td>
<td>0 (-2, 2)</td>
<td>1.00</td>
<td>0 (-2, 1)</td>
</tr>
<tr>
<td>Anthropometric characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight z-score</td>
<td>-0.3 (-0.6, -0.01)</td>
<td>0.04</td>
<td>0.4 (0.2, 0.7)</td>
</tr>
<tr>
<td>Height z-score</td>
<td>-0.6 (-0.9, -0.3)</td>
<td>&lt;.0001</td>
<td>0.04 (0.2, 0.7)</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-0.1 (-0.4, 0.3)</td>
<td>0.93</td>
<td>0.3 (0.04, 0.5)</td>
</tr>
<tr>
<td>Waist z-score</td>
<td>-0.2 (-0.7, 0.2)</td>
<td>0.54</td>
<td>0.3 (0.001, 0.7)</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>-2.4 (-5.24)</td>
<td>0.08</td>
<td>2.1 (0.1, 4.1)</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.01 (-0.01, 0.02)</td>
<td>0.52</td>
<td>0.00 (-0.01, 0.01)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg*</td>
<td>1% (-4.3% to 5%)</td>
<td>0.43</td>
<td>-1% (-8 to 1%)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>0.7 (-1.0, 2.4)</td>
<td>0.61</td>
<td>0.0 (-1.3, 1.3)</td>
</tr>
<tr>
<td>Metabolic characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>0.0 (-0.2, 0.1)</td>
<td>0.92</td>
<td>0.0 (-0.2, 0.1)</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l*</td>
<td>4% (-9 to 20%)</td>
<td>0.77</td>
<td>-3% (-13 to 8%)</td>
</tr>
<tr>
<td>Fasting C-peptide, pmol/l*</td>
<td>2% (-8 to 14%)</td>
<td>0.89</td>
<td>-2% (-10 to 6%)</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>3% (-12 to 20%)</td>
<td>0.90</td>
<td>-5% (-16 to 7%)</td>
</tr>
<tr>
<td>Triglycerides, mmol/l*</td>
<td>1% (-10 to 13%)</td>
<td>0.98</td>
<td>-7% (-15 to 2%)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>-0.09 (-0.20, 0.02)</td>
<td>0.15</td>
<td>0.04 (-0.04, 0.13)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>0.11 (-0.08, 0.30)</td>
<td>0.37</td>
<td>-0.02 (-0.17, 0.12)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>0.06 (-0.15, 0.27)</td>
<td>0.79</td>
<td>0.02 (-0.15, 0.18)</td>
</tr>
<tr>
<td>Body composition measured by DXA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat, %</td>
<td>1.12 (-1.45, 3.70)</td>
<td>0.56</td>
<td>-0.50 (-2.98, 1.97)</td>
</tr>
<tr>
<td>Total lean mass, %</td>
<td>-1.12 (-3.69, 1.46)</td>
<td>0.56</td>
<td>0.50 (-1.97, 2.98)</td>
</tr>
<tr>
<td>Android fat, %*</td>
<td>11% (-7 to 32%)</td>
<td>0.32</td>
<td>0% (-15 to 19%)</td>
</tr>
<tr>
<td>Gynoid fat, %</td>
<td>1.10 (-1.70, 3.90)</td>
<td>0.63</td>
<td>-0.15 (-2.84, 2.55)</td>
</tr>
</tbody>
</table>

Estimates and p-values are obtained by 2-way ANOVA with correction for multiple comparisons (Tukey-Kramer).

P-values are given as mean differences for normally distributed variables and % differences* (95% CI) for log transformed variables.

P*: p-values and estimates represent the additive effect of exposure to GDM within BW groups.

Systolic- and diastolic blood pressure were adjusted for current offspring height.

Table 5. Puberty characteristics of offspring born SGA, AGA or LGA of mothers with or without GDM

<table>
<thead>
<tr>
<th>Puberty status</th>
<th>Control offspring</th>
<th>GDM offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA</td>
<td>AGA</td>
</tr>
<tr>
<td>Girls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast stage, n yes ≥ B2 (%)</td>
<td>16 (89)</td>
<td>160 (84)</td>
</tr>
<tr>
<td>Pubic hair, n yes ≥ PH2 (%)</td>
<td>14 (74)</td>
<td>134 (77)</td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis size, n yes ≥ 4mL (%)</td>
<td>17 (81)</td>
<td>112 (86)</td>
</tr>
<tr>
<td>Pubic hair, n yes ≥ PH2 (%)</td>
<td>10 (45)</td>
<td>83 (56)</td>
</tr>
<tr>
<td>Genital stage, n yes ≥ G2 (%)</td>
<td>13 (61)</td>
<td>110 (76)</td>
</tr>
</tbody>
</table>

P-values denote differences across BW categories stratified by GDM exposure, calculated using Chi-square test (when expected counts were less than 5 Fischer’s exact test was applied) and given as n (%).

Table 6. Age and BMI adjusted estimates for onset of puberty

<table>
<thead>
<tr>
<th>Puberty status</th>
<th>N</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast stage ≥ B2</td>
<td>435</td>
<td>4.73 (0.89, 25.28)</td>
<td>0.12</td>
<td>1.41 (0.50, 3.94)</td>
<td>0.51</td>
<td>2.17 (1.03, 4.60)</td>
<td>0.04</td>
</tr>
<tr>
<td>Public hair ≥ PH2</td>
<td>406</td>
<td>1.08 (0.36, 3.25)</td>
<td>0.90</td>
<td>1.01 (0.44, 2.33)</td>
<td>0.95</td>
<td>1.49 (0.77, 2.86)</td>
<td>0.23</td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis size ≥ 4mL</td>
<td>323</td>
<td>1.67 (0.58, 4.79)</td>
<td>0.90</td>
<td>3.19 (1.25, 8.12)</td>
<td>0.08</td>
<td>0.63 (0.32, 1.26)</td>
<td>0.19</td>
</tr>
<tr>
<td>Public hair ≥ PH2</td>
<td>356</td>
<td>0.68 (0.29, 1.58)</td>
<td>0.32</td>
<td>1.11 (0.55, 2.25)</td>
<td>0.45</td>
<td>1.16 (0.67, 2.00)</td>
<td>0.60</td>
</tr>
<tr>
<td>Genital stage ≥ G2</td>
<td>345</td>
<td>0.93 (0.40, 2.16)</td>
<td>0.42</td>
<td>1.76 (0.80, 3.85)</td>
<td>0.16</td>
<td>1.48 (0.82, 2.68)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Age and BMI adjusted odds ratio (95% CI) for onset of puberty comparing offspring born SGA or LGA with AGA offspring born of GDM or control mothers. Breast development among girls and testicular size among boys was considered the primary outcome in defining puberty onset.

Abbreviations: OR: Odds Ratio, SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age.