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Association of Placental Epigenetic Age Acceleration with Birthweight and Postnatal Growth in the First 2 Years of Life in the GLOWING Cohort (n=153)

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By

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B.S.
Cornell University
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Abstract

Association of Placental Epigenetic Age Acceleration with Birthweight and Postnatal Growth in the First 2 Years of Life in the GLOWING Cohort (n=153)

By Angela Shen

Background: Epigenetic age acceleration has been linked to several adverse health outcomes in adults; however, only a few studies have examined its effects in newborns. Over the past decade, novel epigenetic clocks derived from placental tissue have proven to be highly accurate estimators of gestational age. To understand how patterns of newborn growth and development are influenced by gestational epigenetic age acceleration (GAA), we used a placenta epigenetic clock to investigate the association between GAA and newborn birthweight, as well as postnatal growth trajectories through the first two years of life.

Methods: We utilized data from 153 mother-infant pairs enrolled in the Glowing Life Optimizing Wellness (GLOWING) Study conducted in central Arkansas. Gestational Epigenetic age was estimated using Lee's control placental clock (CPC). Birthweight and placental tissue were collected upon delivery. Postnatal growth trajectories were assessed through repeated measurements of weight until 2 years of age. Weight gain was modeled using SITAR and patterns were described using two model generated parameters. We regressed birthweight z-scores and SITAR *size* and *intensity* parameters on GAA, controlling for potential confounders. Sex-specific effects were also explored.

Results: Infants subjected to higher placental GAA had higher birthweights, yet concurrently lower *size* and *intensity* parameter values, thus signifying slower rates and lower amounts of postnatal weight gain. We found little evidence for sex-specific differential effects.

Conclusions: Our analyses revealed associations between GAA and birthweight and infant growth trajectories consistent with previous research. Our finding suggest higher placental GAA may be an indicator of heathier infants, but further research in larger, more diverse cohorts are needed to confirm observed associations and characterize sex-specific effects.

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1. INTRODUCTION

The Placenta

The placenta performs many functions imperative for proper fetal growth and development. As the interface between mother and fetus, the placenta facilitates maternal-fetal nutrient transport and supports processes including endocrine regulation, toxin and waste removal, and thermal regulation[1]. Furthermore, the placenta also produces critical hormones which maintain pregnancy[2] and serves as a physical barrier to prevent the transfer of pathogens from mother to fetus[3]. Due to the importance of the placenta, there has been a growing interest in placental research, with most researchers focused on elucidating placental physiology to develop tools that can detect pregnancy or post pregnancy complications, and identify potential health outcomes for the infant early on. Among of the most intriguing areas of investigation has been the exploration of placental development and intrauterine environment, and its influence on fetal growth and birthweight.

Birthweight and Postnatal Growth Patterns

Birthweight is among the most relevant indicators towards assessing infant, child, and even adult health. Low or aberrant birthweight is associated with increased infant morbidity, disabilities including cerebral palsy, and various behavioral and cognitive impairments[4-6]. The complex and multifactorial nature of birthweight makes understanding the full pathophysiology of abnormal birthweight difficult; however, research suggests that compromised placental function and poor maternal nutrition are major risk factors of low birthweight[7,8]. Additionally, emerging evidence suggests that placental aging may also play a role in influencing birthweight[9,10]. This, however, has yet to be fully explored.

Postnatal growth, specifically postnatal weight gain, is another indicator predictive of future health outcomes. Excessive growth, or rapid weight gain from infancy through mid-childhood, is consistently associated with later life obesity and increased cardiometabolic risk factors [11-14]. Moreover, patterns of postnatal growth are also strongly associated with birthweight for gestational

age, with lower birthweight newborns exhibiting "catch-up" growth (CUG) and gaining weight rapidly during the first 2 years of life [15,16]. Research indicates that newborns of lower birthweights are more likely to experience rapid weight gain during infancy and have higher fat mass and total fat percentage at age five.[17] Thus, low birthweight for gestational age may predispose newborns to an accelerated postnatal growth trajectory and an increased risk of an adverse cardiometabolic profile in the future.

Ageing

Ageing is a unidirectional phenomenon inevitably experienced by all cells, tissues, and organs, including the placenta, which grows rapidly upon implantation and is eventually discarded upon delivery of the fetus. As gestational time increases, placental cells accumulate characteristics of ageing marked by telomere shortening, cellular senescence, and mitochondrial dysfunction [18,19].

Cellular senescence is a key biological process underlying aging and the development of age-related tissue dysfunction[19]. During cellular replication, a parent cell divides into two identical daughter cells, requiring the unwinding and splitting of the two DNA strands in the parent cell to form two new daughter strands. Telomeres, which are cap-like nucleotide repeats located at the ends of chromosomes, serve as bioprotective mechanisms to ensure that important genetic material is completely copied during replication[19,20]. It is widely observed that telomeres tend to shorten after every replication cycle, and their progressive shortening eventually leads to cellular senescence and/or apoptosis[20].

Telomere length has been extensively studied as a reliable predictor of aging and biological age, and was previously considered the gold standard in investigations of age-related diseases. However, a growing body of evidence now suggests that its predictive ability may not be as strong as once purported[21-23]. While there is an association between decreasing telomere length with increasing age, evidence of substantial interindividual variation suggests that telomere length serves only as a

rough estimate of aging rate[22,23]. These limitations emphasize the need for a more robust biomarker for predicting age.

Epigenetic Age as a Surrogate Measure of Biological Age

DNA methylation (DNAm), the covalent addition of a methyl group to the fifth carbon atom of a cytosine ring, is now among the most promising biomarkers for predicting biological age. Within the last decade, several DNA methylation-based estimators of biological age, also known as "epigenetic clocks", have been developed. These clocks yield highly accurate and precise estimates of chronological age, otherwise known as "DNA methylation age" (DNAm age), across a variety of tissues and at different stages of life [24-27].

Horvath et al. developed the first and most well-known epigenetic clock in 2013[24], which was designed as a robust pan-tissue age predictor based on DNAm profiles at 353 CpG sites. Since then, researchers have developed many other tissue-specific and pan-tissue clocks.

Epigenetic clocks have also been used to assess the rate of biological aging by exploring deviations of epigenetic (biological) age from chronological age. The difference between an individual's epigenetic and chronological age is known as epigenetic age acceleration[28]. Epigenetic age acceleration within various tissues has been associated with several age-related diseases and conditions, such HIV and Huntington's Disease[29,30]. Age acceleration has also been linked to cancer and cardiovascular disease in adults, physical and cognitive fitness, and been shown to be influenced by a broad range of environmental exposures[28,31,32].

To date, the health impacts of age acceleration have mainly been studied in adults, with little investigation into the potential implications and effects of accelerated aging during the gestational period on children's health. This gap in research can be attributed in part to the only recent development of epigenetic clocks specifically designed to estimate gestational age. Now, multiple gestational clocks have been constructed using a wide range of CpG sites, across a variety of tissues,

and over the entire pediatric age spectrum. With the emergence of these clocks, there is an opportunity to explore associations between biological age, prenatal exposures, and children's health.

Current Research in Gestational Epigenetic Age Acceleration

Gestational age can be accurately estimated using any of four newly developed epigenetic gestational age clocks, two of which use DNAm profiles of CpG sites in newborn cord blood (Knight and Bohlin)[33,34], and two of which use DNAm profiles in placental tissue (Mayne and Lee)[35,36]. Studies of epigenetic gestational age acceleration have primarily used cord-blood-based gestational age clocks.

Recent studies applying the Knight clock algorithm to investigate prenatal environment and fetal aging have shown epigenetic age deceleration to be linked to maternal history of depression and birth outcomes such as lowered birthweight, length, and an increased need for respiratory interventions [37-39]. These findings are consistent with that of another study utilizing the Bohlin algorithm, where epigenetic age acceleration was demonstrated to be linked to higher birthweight, length, and head circumference [40]. Together, these findings posit epigenetic age deceleration may be associated with a lower developmental maturity at birth.

However, these findings have not been consistent across the few studies using placental-tissue-based clocks. One study using the Mayne clock algorithm reported sex differences in the associations between placental epigenetic aging with fetal growth, where increased placental epigenetic age acceleration was associated with increased odds for lower birthweight among males, but higher birthweight among females [9]. Presently, no comparable studies have been performed with the placental clock presented by Lee. The inconsistent findings across different gestational epigenetic clocks, along with the high tissue-specificity of epigenetic age acceleration, emphasizes the need for additional research to investigate the effects of age acceleration specifically within the placenta. The placenta is a critical growth-organ and a known driver of pre-and-postnatal growth and development;

hence, age acceleration within the placenta may have significant impacts on short- and long-term child and adult health.

Research Gaps

Although birthweight has been studied as an outcome of placental epigenetic age and as an exposure in studies of postnatal development, no studies have bridged the gap between placental epigenetic age, birthweight, and patterns of infant weight gain. We aimed to fill this present knowledge gap by examining i) the presently unclear associations between placental epigenetic age acceleration and birthweight and ii) the potential links between epigenetic age acceleration and newborn postnatal weight gain. Our findings will provide crucial context for future investigations that seek to examine the effects of prenatal environmental exposures on placental age acceleration, particularly for exposures that are believed to impact fetal metabolic activity and postnatal growth.

2. METHODS

Subjects

Second parity women who were of normal weight (NW, BMI 18.5-24.9 kg/m2, N=74) and overweight/obese (OW, BMI 25-35 kg/m2, N=79), along with their term infants were enrolled in a longitudinal study (*GLOWING* study, ClinicalTrials.gov ID: NCT01131117) aimed to explore associations between maternal programming of offspring growth, metabolism, and adiposity in *utero.* All mothers were recruited from 2011 to 2014 in central Arkansas and enrolled within the first 10 weeks of gestation. Inclusion criteria included all second parity mothers ≥21 years old, BMI of 18.5–35kg/m^2 at enrollment, who had singleton pregnancies conceived without assisted fertility treatments. Enrollees were excluded if they smoke, take medication known to influence fetal growth (e.g., glucocorticoids, insulin, and thyroid hormones), have existing medical conditions (e.g., chronic renal failure, hypertension, malignancies, seizure disorder, lupus, serious psychiatric disorders), sexually

transmitted diseases, abuse alcohol, tobacco, and drugs, to limit the role of these effect modifiers on the outcome of interest. Additionally, mothers with pregnancy or birth complications (gestational diabetes mellitus, preeclampsia, eclampsia, etc.) and children with serious medical conditions were also excluded. Only healthy, term (≥37 weeks gestation) infants were eligible for postnatal visits.

Institutional Review Board (IRB) Approval

The study protocol was approved by the Institutional Review Board at the University of Arkansas for Medical Sciences (UAMS) and written informed consent was obtained and signed from all participants prior to the study.

Anthropometry

Maternal gestational weight gain (GWG) was calculated by taking the difference between the subject's weight at first study visit (4-10 weeks gestation) and their weight at the final prenatal visit (36 weeks gestation). Infant birthweight was collected at birth using a tared scale (SECA). To evaluate postnatal growth, newborns were longitudinally followed-up at 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24 months (up to 11 visits) during the postnatal period. At each visit, weight was assessed using a tared scale (SECA).

Self-Reported Variables

Upon enrollment, participants were asked to self-report race, ethnicity, age, and date of last menstrual period. Marital status, education, and income were self-reported during the first study visit. At 0.5 months postpartum, mothers reported their infant's race, sex, and their mode of delivery.

Gestational Age

Gestational age at birth was calculated from the date of their last menstrual period, or if unknown, estimated from information obtained by early ultrasound.

Placental Sample Collection

Placental samples were collected following a highly rigorous and systematized workflow. Within 30 minutes after delivery, all placentas were collected and processed within 2 hours. Placental

anthropometrics of size, shape, and weight were recorded following severance of the placenta from the umbilical cord and fetal membranes. Placental tissues were collected from the villous core, with maternal and fetal sides separately collected at 6 random sites (~1 sq. in) and washed thrice to remove maternal blood. Samples were then flash-frozen in preparation for DNA isolation. To ensure comprehensive representation of the highly heterogenous placenta, ~1g of the pooled tissue from the six collection sites were pulverized in liquid nitrogen prior to DNA isolation.

Placental DNA Isolation

Genomic DNA from placental samples were isolated using TRI reagent and PureLink genomic DNA isolation methods. Qualitative and quantitative analysis of nucleic acids were conducted to ensure the collection of high-quality DNA.

Genome-wide Methylation Profiling (GWMP)

DNA samples (~500 ng) were randomized across 96-well plates and sent to the Emory Integrated Genomics Core for genome-wide methylation profiling (GWMP). GWMP was conducted using Illumina Infinium MethylationEPIC (EPIC) BeadChip, which includes approximately 860,000 CpG sites across the genome (Illumina, San Diego, CA). Principle component analysis (PCA) was used to assess sources of variability and potential batch effects. The methylation status of individual CpG loci was calculated as the ratio of fluorescent signals from 0 (indicating no methylation) to 1 (indicating complete methylation). Samples and probes with poor detection were excluded, and data were normalized with functional normalization and beta-mixture quantile normalization[41,42].

Placental Cellular Heterogeneity

The *planet* package in RStudio was used to perform placental cellular deconvolution to estimate proportions of Trophoblasts, Hofbauer cells, Endothelial cells, Stromal cells, Nucleated RBCs, and Syncytiotrophoblasts.

Epigenetic Age Estimation Methods

Mayne et al. and Lee et al. have developed epigenetic clocks to estimate gestational age from DNAm profiles in placental tissue. The Mayne placental clock predicts gestational age through 62 CpG probes from DNAm data measured on the 450k Illumina platform. This clock has been shown to accurately predict gestational age, with a high correlation between chronological and epigenetic gestational age ($r=0.95$, $p<2.2E-16$) [36]. Following the Mayne placental clock, Lee et al. developed three more placental epigenetic clocks using an extensive collection of publicly available DNAm datasets to yield more accurate estimations of gestational age. These clocks include the Robust Placental Clock (RPC), the Control Placental Clock (CPC), and the Refined Robust Placental Clock (rRPC), which are tailored to measure gestational age in pregnancies unaffected by new-onset obstetric complications, normal pregnancies, and uncomplicated term pregnancies (GA >36), respectively[35].

For this analysis, placental gestational age was estimated using all three clocks, but we focused on the CPC, which combines placental DNAm beta values from 546 CpG sites selected by an elastic net regression model [35]. The CPC was chosen because the datasets used in its construction are most comparable to the placentas and pregnancies observed in the GLOWING cohort.

Placental epigenetic age acceleration was derived by regressing predicted epigenetic gestational age on chronological gestational age and extracting the residuals. Positive residuals represent epigenetic age acceleration, while negative residuals indicate epigenetic age deceleration.

Statistical Analyses

Linear mixed effects (LME) modelling is a well-established method for analyzing longitudinal growth data; thus, LME models were initially used to explore weight gain over time. However, we ultimately used the SuperImposition by Translation and Rotation (SITAR) model, a shape invariant mixed effects model developed by Cole et al., to model, analyze, and describe postnatal growth trajectories. The SITAR model estimates a single curve representing the population-average weight gain trajectory, which is modified (translated) by the inclusion of random effects to

match the observed weight gain curves for each individual[43]. In fitting the curve, each individual's deviation from the average trajectory is expressed through random effects estimates for three parameters*: size*, *tempo*, and *intensity*. SITAR *size* refers to differences in mean weight and is reflected in an up-down shift of the mean curve. SITAR *tempo* refers to differences in the timing of weight gain and is reflected in a left-right shift of the curve. Lastly, SITAR *intensity* refers to differences in the velocity or speed of weight gain and is reflected in counterclockwise-clockwise rotation of the curve.

Prior to fitting the model, weight values from every visit were natural log transformed to meet the normality assumptions of the SITAR method. The SITAR package in RStudio was used to model growth trajectories for all children with at least six follow up visits[44]. We used the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), to determine the number of degrees of freedom to include in the model, and found that 8 degrees of freedom was appropriate for our data. We included weight measurements from up to 11 postnatal visits occuring between 0.5 to 24 months of age. The model successfully estimated two of the three parameters—*size* and *intensity*— for downstream analyses.

To assess the relationship between birthweight, weight gain and epigenetic age acceleration, birthweight z-scores, as well as postnatal *size* and *intensity* parameters obtained from SITAR, were regressed on gestational age acceleration. We used gestational age acceleration as the dependent variable in all our models and included several covariates to adjust for potential confounding. The covariates included maternal age at birth, maternal education level, pre-pregnancy BMI, gestational weight gain, placental cell types, and infant sex. These covariates were selected based on prior hypothesized associations with gestational age acceleration and their potential impact on growth trajectories and placental function. Maternal educational attainment was categorized into three levels (high, medium, or low) and was included as an indicator of socioeconomic status. Cell type proportions were adjusted based on their association with age acceleration. Last, we performed

surrogate variable analysis with the*sva* package in RStudio to identify the major sources of variation in our data which may be reflective of residual unmeasured confounding. We identified three surrogate variables that were included in our models as sensitivity analyses.

3. RESULTS

Study Population

Demographics and clinical characteristics for mother-infant dyads are summarized as means and standard errors or counts and percentages and reported in *Table 1*. Among the 153 dyads included in the study, on average, mothers were 30.53 ± 3.49 years of age at childbirth. 86.3% (n=132) of mothers self-identified as White, 10.5% (n=16) self-identified as African American, and the remaining (n=5) self-identified as either Asian, more than one race, or were unknown/not reported. 88.9% (n=136) of mothers reported being married. The average gestational age at delivery was 39.31 ± 0.85 weeks. The average pre-pregnancy BMI was 25.91 ± 4.35 kg/m^2 and average maternal gestational weight gain was 11.64 ± 4.28 kg. Nearly all mothers reported having completed high school and two-thirds (67.3%) obtained college degrees.

The average infant birthweight was 3.53 ± 0.44 kg and 58.2% (n=89) of infants were male. Infant weight gain was measured from 0.5 to 24 months over a maximum of 11 postnatal visits. All infants attended at least 6 follow-up visits. The medians, quartiles, and counts of missing data for each postnatal visit are presented in *Table 2.*

Epigenetic Age and Chronological Age

We utilized the Control Placental Clock (CPC) to predict gestational age in our cohort. The average predicted gestational age of newborns was (38.80 ± 0.80 weeks), while the average reported gestational age at time of delivery was (39.31 ± 0.85 weeks). To evaluate the correlation between epigenetic gestational age and reported gestational age, we computed the Pearson's correlation coefficient. Our analysis revealed a strong positive correlation between predicted epigenetic age and

reported gestational ages, as indicated by a correlation coefficient value of 0.645 (p-value < 2.2e-16). To calculate gestational age acceleration, we regressed predicted epigenetic age on reported gestational age, and extracted the residuals.

Growth Trajectory Modeling

Infant weight gain trajectories were modeled using SITAR and described in terms of parameters of *size* and *intensity* (*Figure 1*). SITAR *size* is expressed as a percent for the mean weight of the child, relative to the average weight for all children included in the model. Differences in the *size* parameter are reflected in an up-down shift of the child's weight gain curve. *Size* in this analysis ranged between (-0.208 to 0.245). SITAR *intensity* is expressed as a percentage deviation from the mean weight gain velocity of all children included in the model. Differences in the *intensity* parameter are reflected as a rotation of the child's weight growth curve. *Intensity* in this analysis ranged from (-0.146 to 0.137).

Associations Between Acceleration with Birthweight, Size, and Intensity

Birthweight z-scores and SITAR *size* and *intensity* were independently regressed on placental gestational age acceleration (*Table 3 & Figure 2*). The results indicate that on average, infants with higher GAA had slightly higher birthweights. However, p-values were large, and the confidence intervals crossed the null. Unadjusted models showed that infants with higher GAA had modest decreases in *size*, but confidence intervals were also wide and crossed the null. On the other hand, in unadjusted analyses of *intensity*, we observed that infants with higher GAA had lower *intensity* values, or a lower weight gain velocity.

We then adjusted our models for potential confounders by controlling for maternal age at birth, maternal education level, pre-pregnancy BMI, gestational weight gain, and infant sex. These adjustments did not alter the associations we observed for birthweight, *size*, or *intensity*.

Next, we examined whether adjustments for specific placental cell type proportions were necessary. We regressed gestational age acceleration on placental cell types and found that only trophoblasts and syncytiotrophoblasts were associated with age acceleration (p-value < 0.05). Thus, we included these cell types in our models. While these adjustments resulted in only minor deviations in the parameter estimates, the confidence interval for *intensity* now crossed the null.

Finally, to account for residual confounding or potential batch effects, we estimated and adjusted for three surrogate variables. This did not significantly alter our previous associations between GAA and birthweight, *size*, and *intensity*.

Overall, our findings consistently showed that higher gestational age acceleration was associated with decreased SITAR *intensity* across all adjustment models (*Figure 2C*). However, relationships between GAA and birthweight and SITAR *size* were less clear, with all confidence intervals crossing the null. Despite this, our effect estimates consistently indicated that higher GAA increased birthweight and decreased SITAR *size (Figure 2A &2B).*

Secondary Analyses

Given the accumulating evidence for sex-specific differences in infant growth and development trajectories[45,46], we performed exploratory secondary analyses to investigate whether there are sex differences in the associations between placental age acceleration and patterns of postnatal weight gain. Birthweight z-scores, SITAR *sex,* and *intensity* were regressed on gestational age acceleration after being stratified by infant sex (*Table 4*). Sex-stratified analyses were adjusted for the same variables as the main analyses, now excluding sex as a covariate.

Our results did not indicate major differences between GAA and birthweight and SITAR *intensity* by sex. However, among females, birthweight effect estimates were consistently slightly larger, and SITAR *intensity* estimates were always slightly smaller than male estimates (*Figure 3A & 3C*). We observed consistent differences between GAA and SITAR *size* by sex, with higher GAA

increasing SITAR *size* in females but decreasing SITAR *size* in males (*Figure 3B*). Despite this, all confidence intervals crossed the null.

In all, we likely were underpowered to detect significant sex-specific effects between GAA and birthweight, SITAR *intensity*, and *size*. Notwithstanding, the divergent estimates of SITAR *size* between males and females indicate the potential for such effects, and thus, warrant further research.

4. DISCUSSION

Previous Research

In this study, we investigated the relationship between placental age acceleration and newborn birthweight and postnatal growth trajectories in the first two years of life. Using SITAR, we derived parameters of *size* and i*ntensity,* which characterize each child's mean weight gain and rate of weight gain, respectively. Our findings revealed that infants subjected to higher placental age acceleration had higher birthweights, yet concurrently lower *size* and *intensity* values, signifying slower rates and lower amounts of postnatal weight gain. We also conducted sex-stratified analyses and found little evidence for sex-specific effects between placental age acceleration and birthweight and postnatal weight gain.

Our findings on the association between GAA and birthweight are consistent with previous investigations. Specifically, these studies showed that neonates experiencing GAA tend to be within the highest birthweight percentiles in their respective cohorts[47,48]. Although these studies used cord blood DNAm profiles to estimate epigenetic gestational age, the results align with our present findings and together, corroborate the existing hypothesis that higher GAA may serve as an indicator of developmental maturity.

Very few studies have investigated gestational age acceleration and measures of growth beyond birth. To our knowledge, this is the first study to use a placenta-specific epigenetic clock to examine the association GAA with measures of weight beyond birth and across early childhood.

Nonetheless, our results are largely mirrored by the findings of another study which examined the ARIES subsample of the Avon Longitudinal Study of Parents and Children (ALSPAC) from birth until 10 years of age. GAA, derived from cord blood DNAm profiles, was associated with higher birthweight, but this association began to weaken at 9 months of age, before reversing, with each additional week of increased GAA resulting in a 0.6 kg reduction in weight at the 10-year mark[49]. Our findings suggest this reversal occurs during early childhood and is observable as early as 24 months. Together, our findings indicate a discrepancy between GAA on birthweight and postnatal growth, with children experiencing high GAA having larger birthweights, but a slower rate of growth during early childhood.

Previous research has suggested that several key postnatal factors, specifically postnatal weight gain, can predict future cardiometabolic risk[50,51]. Investigations have shown that slower postnatal growth rates are linked to a reduced risk of developing cardiovascular and cardiometabolic diseases later in life, while accelerated postnatal growth rates are considered a risk factor for such health problems[11-14]. However, emerging evidence suggests a more complicated relationship between growth trajectories and health, which is highly population dependent. For instance, faster postnatal growth in infants born pre-term appears to be associated with lower morbidity and more optimal neurocognitive development[52]. Since our cohort did not include preterm infants, our findings most closely align with GAA likely being a positive indicator of infant health.

These emerging observations about GAA are particularly intriguing given that they appear to contradict findings in adult populations, where age acceleration is linked to a range of adverse health outcomes, making it a marker of poorer health[28-32]. Such observations provide evidence of a more nuanced relationship between age acceleration and health outcomes in children, where the risks associated with epigenetic aging depend on the life stage. GAA at birth may be a marker of healthier infants, but continued age acceleration through adolescence and adulthood can lead to the development of negative health outcomes.

Strengths and Limitations

Our study has several notable strengths. First, the large number of follow-up measurements of weight, covering the critical first two years of life. Compared to the ARIES study, which only collected weight measures from health visit records and had scheduled follow-up visits from age 7 onwards, our study had 11 scheduled follow-up visits over two years. In scheduling set follow-up visits, all children were measured for anthropometrics using consistent protocol and machinery thereby reducing the possibility of measurement error. In all, the high density of follow-up visits and precise anthropometric evaluations cements our study as among the most robust assessments of GAA and patterns of postnatal weight gain.

In addition to data collection methods, we have taken steps to strengthen the internal validity of our investigation. The exclusionary factors and variables we have adjusted for adequately account for potential confounding effects and eliminate potential sources of bias, giving us confidence in our results. Specifically, we excluded mothers who smoked during or before pregnancy, as smoking has been shown to have a substantial impact on the placental methylome and birthweight[53,54].

The Lee et al. Control Placental Clock was also highly suited in predicting gestational age in our cohort, as construction of the CPC incorporated datasets comparable to the placentas and pregnancies in our sample. We observed a strong correlation (r=0.645) between predicted epigenetic age and reported gestational age.

The limited generalizability of our findings is an important consideration when interpreting our results. Our study population of predominately white, non-Hispanic, relatively healthy, second-parity mothers who gave birth to full-term infants, is not a representative sample, and suggests we must exercise caution when interpreting our findings in the contexts of other populations. Furthermore, our recruitment of patients from a single location may hinder our ability to extend our conclusions to populations in diverse locations with varying sociodemographic characteristics, particularly given the known differences in age acceleration and the drivers of birthweight and

postnatal growth between racial and ethnic groups[55-57]. However, differences in GAA by race and/or ethnicity have yet to be investigated, and should explored within large, diverse cohorts.

Although we explored sex-specific effects, our study may have been underpowered to evaluate and detect sex differences, which is a common issue in studies with small sex strata and can lead to distorted conclusions. Therefore, although we did not observe significant sex-specific effects in our analysis, we should not assume such effects do not exist. Future research is necessary to elucidate sex differences between GAA and infant birthweight and postnatal growth.

5. CONCLUSION

In our study, we have demonstrated that placental age acceleration is associated with newborn birthweight and postnatal growth trajectories through the first two years of life. Our findings indicate that infants subjected to higher placental age acceleration had higher birthweights, yet concurrently lower *size* and *intensity* values, signifying slower rates and lower amounts of postnatal weight gain. This provides supporting evidence that GAA may be a marker of healthier infants with positive developmental outcomes, but caution should be exercised in interpreting our findings in the contexts of different populations. Further research is needed to confirm our observed associations and to better characterize the existence of sex-specific effects between PAA, birthweight, and patterns of postnatal growth.

6. REFRENCES

1. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. *Philos Trans R Soc Lond B Biol Sci.* 2015;370(1663):20140066.
2. Napso T, Yong HEJ, Lopez-Tello J, Sferruzzi-Perri AN. The Role of Placental Hormones in Mediating Maternal Adaptations to Support Pregnancy and Lactation. *Front Physiol.* 2018;9:1091.
3. Delorme-Axford E, Sadovsky Y, Coyne CB. The Placenta as a Barrier to Viral Infections. *Annual Review of Virology.* 2014;1(1):133-146.
4. Schieve LA, Tian LH, Rankin K, et al. Population impact of preterm birth and low birth weight on developmental disabilities in US children. *Ann Epidemiol.* 2016;26(4):267-274.
5. Johnson EO, Breslau N. Increased risk of learning disabilities in low birth weight boys at age 11 years. *Biol Psychiatry.* 2000;47(6):490-500.
6. Lie KK, Grøholt EK, Eskild A. Association of cerebral palsy with Apgar score in low and normal birthweight infants: population based cohort study. *Bmj.* 2010;341:c4990.
7. K CA, Basel PL, Singh S. Low birth weight and its associated risk factors: Health facility-based case-control study. *PLoS One.* 2020;15(6):e0234907.
8. Ramakrishnan U. Nutrition and low birth weight: from research to practice. *The American Journal of Clinical Nutrition.* 2004;79(1):17-21.
9. Tekola-Ayele F, Workalemahu T, Gorfu G, et al. Sex differences in the associations of placental epigenetic aging with fetal growth. *Aging (Albany NY).* 2019;11(15):5412-5432.
10. Paules C, Dantas AP, Miranda J, et al. Premature placental aging in term small-for-gestational-age and growth-restricted fetuses. *Ultrasound Obstet Gynecol.* 2019;53(5):615-622.
11. Arisaka O, Ichikawa G, Koyama S, Sairenchi T. Childhood obesity: rapid weight gain in early childhood and subsequent cardiometabolic risk. *Clin Pediatr Endocrinol.* 2020;29(4):135-142.
12. Belsky DW, Moffitt TE, Houts R, et al. Polygenic risk, rapid childhood growth, and the development of obesity: evidence from a 4-decade longitudinal study. *Arch Pediatr Adolesc Med.* 2012;166(6):515-521.
13. Nummela SR, Salo P, Pahkala K, et al. Weight gain in infancy and markers of cardiometabolic health in young adulthood. *Acta Paediatrica.* 2022;111(8):1603-1611.
14. Woo J. Infant Growth and Long-term Cardiometabolic Health: a Review of Recent Findings. *Current Nutrition Reports.* 2019;8.
15. Jain V, Singhal A. Catch up growth in low birth weight infants: striking a healthy balance. *Rev Endocr Metab Disord.* 2012;13(2):141-147.
16. Singhal A. Long-Term Adverse Effects of Early Growth Acceleration or Catch-Up Growth. *Annals of Nutrition and Metabolism.* 2017;70(3):236-240.
17. Ong KKL, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ.* 2000;320(7240):967-971.
18. Sultana Z, Maiti K, Aitken J, Morris J, Dedman L, Smith R. Oxidative stress, placental ageing-related pathologies and adverse pregnancy outcomes. *Am J Reprod Immunol.* 2017;77(5).

19. Manna S, McCarthy C, McCarthy FP. Placental Ageing in Adverse Pregnancy Outcomes: Telomere Shortening, Cell Senescence, and Mitochondrial Dysfunction. *Oxid Med Cell Longev.* 2019;2019:3095383.
20. Zhu Y, Liu X, Ding X, Wang F, Geng X. Telomere and its role in the aging pathways: telomere shortening, cell senescence and mitochondria dysfunction. *Biogerontology.* 2019;20(1):1-16.
21. Lohman T, Bains G, Berk L, Lohman E. Predictors of Biological Age: The Implications for Wellness and Aging Research. *Gerontology and Geriatric Medicine.* 2021;7:23337214211046419.
22. Vaiserman A, Krasnienkov D. Telomere Length as a Marker of Biological Age: State-of-the-Art, Open Issues, and Future Perspectives. *Frontiers in Genetics.* 2021;11.
23. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci.* 2011;66(2):202-213.
24. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol.* 2013;14(10):R115.
25. Raj K, Horvath S. Current perspectives on the cellular and molecular features of epigenetic ageing. *Exp Biol Med (Maywood).* 2020;245(17):1532-1542.
26. Jylhava J, Pedersen NL, Hagg S. Biological Age Predictors. *EBioMedicine.* 2017;21:29-36.
27. Simpson DJ, Chandra T. Epigenetic age prediction. *Aging Cell.* 2021;20(9):e13452.
28. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet.* 2018;19(6):371-384.
29. Horvath S, Langfelder P, Kwak S, et al. Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. *Aging (Albany NY).* 2016;8(7):1485-1512.
30. Horvath S, Lin DTS, Kobor MS, et al. HIV, pathology and epigenetic age acceleration in different human tissues. *Geroscience.* 2022;44(3):1609-1620.
31. Dhingra R, Nwanaji-Enwerem JC, Samet M, Ward-Caviness CK. DNA Methylation Age-Environmental Influences, Health Impacts, and Its Role in Environmental Epidemiology. *Curr Environ Health Rep.* 2018;5(3):317-327.
32. Perna L, Zhang Y, Mons U, Holleczek B, Saum KU, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. *Clin Epigenetics.* 2016;8:64.
33. Knight AK, Craig JM, Theda C, et al. An epigenetic clock for gestational age at birth based on blood methylation data. *Genome Biology.* 2016;17(1):206.
34. Bohlin J, Håberg SE, Magnus P, et al. Prediction of gestational age based on genome-wide differentially methylated regions. *Genome Biology.* 2016;17(1):207.
35. Lee Y, Choufani S, Weksberg R, et al. Placental epigenetic clocks: estimating gestational age using placental DNA methylation levels. *Aging (Albany NY).* 2019;11(12):4238-4253.
36. Mayne BT, Leemaqz SY, Smith AK, Breen J, Roberts CT, Bianco-Miotto T. Accelerated placental aging in early onset preeclampsia pregnancies identified by DNA methylation. *Epigenomics.* 2017;9(3):279-289.
37. Suarez A, Lahti J, Czamara D, et al. The Epigenetic Clock at Birth: Associations With Maternal Antenatal Depression and Child Psychiatric Problems. *J Am Acad Child Adolesc Psychiatry.* 2018;57(5):321-328.e322.

38. Girchenko P, Lahti J, Czamara D, et al. Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clinical Epigenetics.* 2017;9(1):49.
39. Knight AK, Smith AK, Conneely KN, et al. Relationship between Epigenetic Maturity and Respiratory Morbidity in Preterm Infants. *J Pediatr.* 2018;198:168-173.e162.
40. Bright HD, Howe LD, Khouja JN, Simpkin AJ, Suderman M, O'Keeffe LM. Epigenetic gestational age and trajectories of weight and height during childhood: a prospective cohort study. *Clin Epigenetics.* 2019;11(1):194.
41. Teschendorff AE, Marabita F, Lechner M, et al. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. *Bioinformatics.* 2013;29(2):189-196.
42. Fortin J-P, Labbe A, Lemire M, et al. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biology.* 2014;15(11):503.
43. Cole TJ, Donaldson MDC, Ben-Shlomo Y. SITAR—a useful instrument for growth curve analysis. *International Journal of Epidemiology.* 2010;39(6):1558-1566.
44. Cole TJ: sitar: Super Imposition by Translation and Rotation growth curve analysis. R package version 1.2.0. 2021. https://cran.r-project.org/web/packages/sitar/index.html.
.
45. Chou FS, Yeh HW. Sex differences in postnatal weight gain trajectories of extremely preterm newborns. *J Perinatol.* 2021;41(8):1835-1844.
46. Broere-Brown ZA, Baan E, Schalekamp-Timmermans S, Verburg BO, Jaddoe VWV, Steegers EAP. Sex-specific differences in fetal and infant growth patterns: a prospective population-based cohort study. *Biology of Sex Differences.* 2016;7(1):65.
47. Girchenko P, Lahti J, Czamara D, et al. Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestational age at birth. *Clin Epigenetics.* 2017;9:49.
48. Khouja JN, Simpkin AJ, O'Keeffe LM, et al. Epigenetic gestational age acceleration: a prospective cohort study investigating associations with familial, sociodemographic and birth characteristics. *Clinical Epigenetics.* 2018;10(1):86.
49. Bright HD, Howe LD, Khouja JN, Simpkin AJ, Suderman M, O'Keeffe LM. Epigenetic gestational age and trajectories of weight and height during childhood: a prospective cohort study. *Clinical Epigenetics.* 2019;11(1):194.
50. Joyce BT, Gao T, Zheng Y, et al. Epigenetic Age Acceleration Reflects Long-Term Cardiovascular Health. *Circulation Research.* 2021;129(8):770-781.
51. Pottinger TD, Khan SS, Zheng Y, et al. Association of cardiovascular health and epigenetic age acceleration. *Clinical Epigenetics.* 2021;13(1):42.
52. Singhal A. Long-Term Adverse Effects of Early Growth Acceleration or Catch-Up Growth. *Ann Nutr Metab.* 2017;70(3):236-240.
53. Everson TM, Vives-Usano M, Seyve E, et al. Placental DNA methylation signatures of maternal smoking during pregnancy and potential impacts on fetal growth. *Nature Communications.* 2021;12(1):5095.
54. Kataoka MC, Carvalheira APP, Ferrari AP, Malta MB, de Barros Leite Carvalhaes MA, de Lima Parada CMG. Smoking during pregnancy and harm reduction in birth weight: a cross-sectional study. *BMC Pregnancy and Childbirth.* 2018;18(1):67.

55. Levine ME, Crimmins EM. Evidence of accelerated aging among African Americans and its implications for mortality. *Soc Sci Med.* 2014;118:27-32.
56. Crimmins EM, Thyagarajan B, Levine ME, Weir DR, Faul J. Associations of Age, Sex, Race/Ethnicity, and Education With 13 Epigenetic Clocks in a Nationally Representative U.S. Sample: The Health and Retirement Study. *The Journals of Gerontology: Series A.* 2021;76(6):1117-1123.
57. Lee SM, Sie L, Liu J, Profit J, Main E, Lee HC. Racial and ethnic disparities in postnatal growth among very low birth weight infants in California. *Journal of Perinatology.* 2023;43(3):371-377.

7. TABLES & FIGURES

Table 1: Maternal and Infant Characteristics (N=153)

	N (%) or mean (SD)
Maternal Characteristics	
Race (%)	
African American	16 (10.5)
Other	5 (3.3)
White	132 (86.3)
Pre-pregnancy BMI (kg/m^2)	25.91 (4.35)
Gestational Weight Gain (kg)	11.64 (4.28)
Education Level (%)	
College Graduate	58 (37.9)
Graduate Training or Degree	45 (29.4)
High School Graduate, GED, Associate, Partial College, or Specialized Training	50 (31.4)
Age at Birth	30.53 (3.49)
Marital Status (%)	
Cohabitating	13 (8.5)
Married	136 (88.9)
Single or Divorced	4 (2.6)
Placental Cell Composition	
Hofbauer Cells	0.01 (0.01)
Stromal Cells	0.11 (0.02)
Endothelial Cells	0.10 (0.02)
nRBC	0.01 (0.01)
Syncytiotrophoblast	0.6 (0.05)
Trophoblasts	0.14 (0.04)
Infant Characteristics	
Infant Sex (%)	
Female	64 (41.8)
Male	89 (58.2)
Birthweight (kg)	3.53 (0.44)
Gestational Age at Birth (weeks)	39.31 (0.85)

Table 2: Summary Statistics of Weight (kg) at Each Visit

Visit (month)	Q1	Median	Q3	Missed Visits
0.5	3.473	3.708	3.990	3
1	4.030	4.362	4.649	3
2	4.992	5.385	5.782	2
3	5.650	6.055	6.580	4
4	6.265	6.680	7.280	8
5	6.750	7.270	7.780	4
6	7.190	7.720	8.344	5
9	8.315	8.880	9.580	4
12	9.185	9.865	10.55	8
18	10.53	11.30	12.24	14
24	11.42	12.52	13.50	20

Q1:25th percentile weight
Q3:75th percentile weight

Figure 1: SITAR GLOWING Growth (Weight Gain) Curves

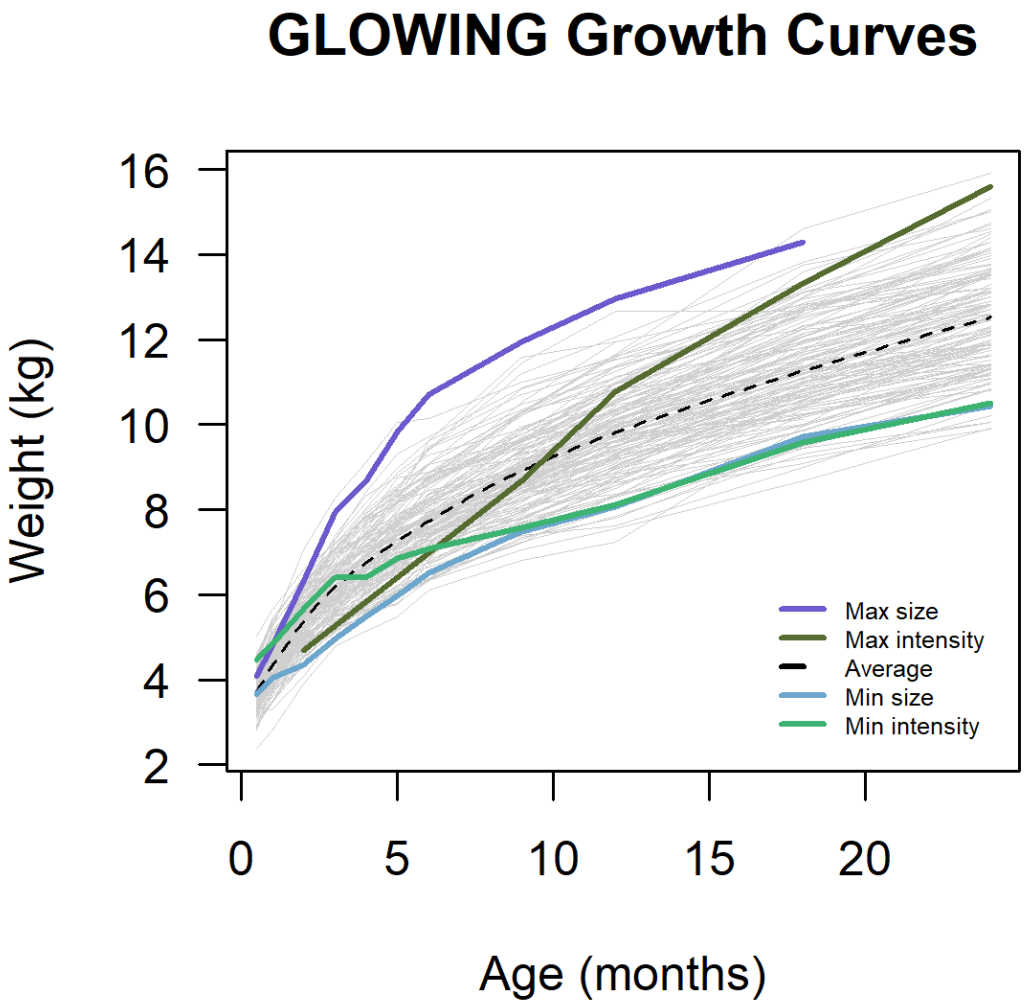
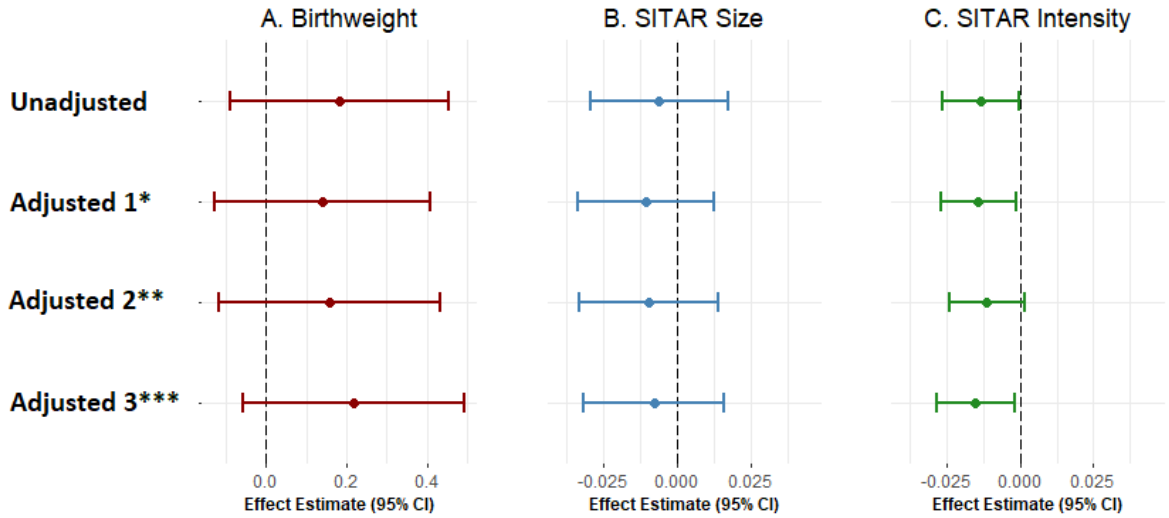


Table 3: Parameter estimates from linear regression models to examine the associations between growth characteristics and gestational age acceleration.

Model	***Birthweight***	***Size***	***Intensity***
Unadjusted	0.1827 (-0.0895, 0.4550)	-0.0059 (-0.0291, 0.0173)	-0.0133 (-0.0261, -0.0005)
Adjusted 1*	0.1392 (-0.1291, 0.4074)	-0.0106 (-0.0336, 0.0124)	-0.0139 (-0.0266, -0.0013)
Adjusted 2**	0.1572 (-0.1186, 0.4330)	-0.0093 (-0.0329, 0.0143)	-0.0110 (-0.0238, 0.0018)
Adjusted 3***	0.2180 (-0.0590, 0.4949)	-0.0078 (-0.0318, 0.0161)	-0.0150 (-0.0282, -0.0018)

* adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, infant sex
** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, infant sex, cell types
*** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, infant sex, 3 surrogate variables

Figure 2: Forest Plots for Birthweight, SITAR Size, and SITAR Intensity



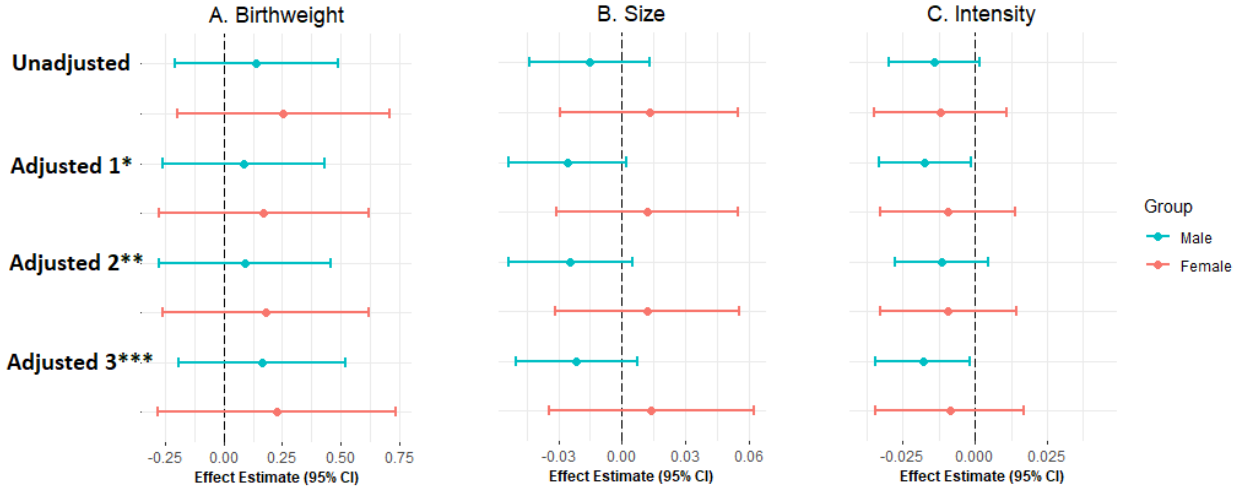
* adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, infant sex
** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, infant sex, cell types
*** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, infant sex, 3 surrogate variables

Table 4: Sex stratified parameter estimates from linear regression models to examine the associations between growth characteristics and gestational age acceleration

	Birthweight		***Size***		***Intensity***	
Model	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Unadjusted	0.1390 (-0.2109, 0.4889)	0.2555 (-0.1998, 0.7108)	-0.0153 (-0.0434, 0.0129)	0.0129 (-0.0289, 0.0547)	-0.0142 (-0.0300, 0.0017)	-0.0117 (-0.0344, 0.0110)
Adjusted 1*	0.0851 (-0.2634, 0.4335)	0.1705 (-0.2774, 0.6184)	-0.0256 (-0.0535, 0.0022)	0.0119 (-0.0309, 0.0546)	-0.0172 (-0.0330, -0.0014)	-0.0091 (-0.0322. 0.0140)
Adjusted 2**	0.0906 (-0.2784, 0.4596)	0.1782 (-0.2628, 0.6193)	-0.0243 (-0.0535, 0.0049)	0.0122 (-0.0312, 0.0556)	-0.0114 (-0.0276, 0.0049)	-0.0088 (-0.0322, 0.0146)
Adjusted 3**	0.1651 (-0.1908, 0.5211)	0.2272 (-0.2802, 0.7346)	-0.0215 (-0.0503, 0.0074)	0.0138 (-0.0345, 0.0620)	-0.0179 (-0.0343, -0.0015)	-0.0084 (-0.0339, 0.0171)

* adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education
** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, cell types
*** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, 3 surrogate variables

Figure 3: Forest Plots for Birthweight, SITAR Size and SITAR Intensity Stratified by Sex



* adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education
** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, cell types
*** adjusted for maternal age, pre-pregnancy BMI, gestational weight gain, maternal education, 3 surrogate variables