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Placental Aging: Association Between the Placental Epigenetic Clock and Maternal Smoking During Pregnancy

By

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B.A.
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2018

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An abstract of
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2020

Abstract

Placental Aging: Association Between the Placental Epigenetic Clock and Maternal Smoking During Pregnancy

By Alexandra C Skoczek

Gestational age is the current standard used by physicians to predict the due date of an infant and customize patient care. Understanding age at birth is necessary, as premature birth is a factor contributing to adverse respiratory outcomes, decreased neurological development, and increased mortality. Epigenetic age, based on DNA methylation at specific genomic regions, has been used as a marker of biological aging in adult populations, and an accelerated epigenetic age (compared to chronologic age) has been linked to morbidity and mortality. These biomarkers, though, have not been applied as extensively in pediatric or neonatal research. Recently a novel method for estimating epigenetic gestational age from placental DNA methylation levels, a placental epigenetic clock, has been developed.

Our objective was to investigate the relationship between maternal smoking and acceleration of the placental epigenetic clock as well as the association between DNA methylation acceleration and birthweight. Pregnant women were recruited and enrolled as a part of The Behavior and Mood in Babies and Mothers (BAM BAM) study, and this analysis included 89 participants (mothers/infant pair), 35 of which did not smoke at all during pregnancy and 54 which smoked at some point during pregnancy.

The difference between actual gestational age and placental epigenetic age was found to be smaller in participants who did not smoke at any time during pregnancy compared to participants who did smoke during pregnancy. An increasing epigenetic age acceleration was also associated with decreased birthweight; for each 1 week of epigenetic acceleration, birthweight was decreased by 113g ($p=0.012$). These results suggest smoking during pregnancy induces a reduced biological aging of the placenta, which is associated with birthweight, and indicates a need for additional analyses to understand the functional impact of altered placental aging.

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Introduction

Low birthweight is defined as a child being born with a birthweight of less than 2,500 g or 5.5 lbs regardless of gestational age. UNICEF-WHO estimates that 20.5 million babies are born with low birthweight every year making reducing low birthweight a top global health priority. Compared to children born with normal birth weight children with low birth weight have been shown to experience stunted growth, lower IQ, increased morbidity within 28 days of birth, obesity, heart disease, diabetes, and other non-communicable disease,[1]. Previous studies have shown that there is an increased risk of low birthweight due to premature birth (identified by birth before 37 weeks gestation) and/or abnormal growth in the womb[2]. In order to decrease the prevalence of low birthweight understanding reasons behind premature birth is of the utmost importance.

One factor leading to low birthweight that has been previously established is gestational age[3]. Physicians use gestational age to predict the delivery date and customize patient care. Ideal gestational length is 40 weeks with suboptimal birth outcomes being noted between 37 and 39 weeks gestation[4]. The WHO categorizes moderate to late preterm birth as 32 to <37 weeks gestation, very preterm as 28 to <32 weeks gestation, and extremely preterm birth as <28 weeks gestation[5]. Preterm

[1] World Health Organization. (2019, May 16). Too many babies born too small. Retrieved from https://www.who.int/news-room/detail/16-05-2019-too-many-babies-are-born-too-small
[2] World Health Organization; UNICEF;. (2019). Low Birthweith Estimates. Retrieved from World Health Organization: https://www.who.int/nutrition/publications/UNICEF-WHO-lowbirthweight-estimates-2019/en/
[3] ibid
[4] World Health Organization. (2019, May 16). Too many babies born too small. Retrieved from https://www.who.int/news-room/detail/16-05-2019-too-many-babies-are-born-too-small
[5] Organization World Health. (2012). Born too soon: The global action report on preterm birth.

birth is a leading cause for adverse respiratory outcomes, decreased neurological development, and increased mortality[6],[7]. Low gestational age at birth also presents long term hazards such as increased mortality in young adulthood. A study done by Crump et al. found this relationship to be caused by congenital anomalies, cardiovascular disorders, endocrine disorders, and respiratory disorders[8]. The development of a more accurate measurement of the age and health of a developing fetus carries immense healthcare implications, and while gestational age has proven to be a useful method in estimating a fetus's developmental age, studies have pursued more precise tools through the use of genetic and molecular estimators[9].

Placental gene alterations are one route to provide a more accurate estimate of gestational age. During pregnancy the placenta undergoes immense changes to its function, reflected by its gene expression profile. There are approximately 12000 genes expressed in the placenta, and throughout the first to the third trimester over 7000 of these genes change their expression[10]. Gene expression can be regulated through epigenetic mechanisms, including the hypomethylation or hypermethylation of the regulatory regions of those genes. This represents the

[6] Glass, H. C., Costarino, A. T., Stayer, S. A., Brett, C., Cladis, F., & Davis, P. J. (2015). Outcomes for Extremely Premature Infants. Anesth Analg, 120(6), 1337-51. doi:10.1213/ANE.0000000000000705

[7] Son, M., & Miller, E. S. (2017). Predicting preterm birth: Cervical length and fetal fibronectin. Seminars in perinatology, 445-451. doi:10.1053/j.semperi.2017.08.002

[8] Crump, C., Sundquist, K., Sundquist, J., & Winkleby, M. A. (2011). Gestational Age at Birth and Mortality in Young Adulthood. Jama, 306(11), 1233-1240.

[9] Organization World Health. (2012). Born too soon: The global action report on preterm birth.

[10] Sitras, V., Fenton, C., Paulssen, R., Vartun, A., & Acharya, G. (2012). Differences in gene expression between first and third trimester human placenta: a microarray study. PloS one, 7(3). doi:e33294

removal or the addition of a methyl group (-CH3) to DNA[11],[12],[13]. The methyl group binds directly to the cytosine in a cytosine-phosphate-guanine (CpG) site on DNA[14],[15],[16]. Recent research indicates there is a unique ink between DNA methylation and age, suggesting DNA methylation (DNAm) may present a novel approach to assessing gestational age [17].

Recent research has advanced the knowledge of DNA methylation and its relationship to aging in different tissues, leading to the creation of an epigenetic clock for different tissues[18]. This epigenetic clock allows for comparison of the genetic age of a tissue and the actual age of a tissue. To establish the epigenetic clock for different tissues Horvath et al. used 82 different DNA methylation data sets and 51 different tissues and cell types. In all, 353 different CpGs were identified using a penalized regression model of CpGs that could predict chronological age with high accuracy; the weighted average of DNA methylation

[11] Johnson, A. A., Akman, K., Calimport, S. R., Wuttke, D., Stolzing, A., & de Magalhaes, J. (2012). The Role of DNA Methylation in Aging, Rejuvenation, and Age-Related Disease. REJUVENATION RESEARCH, 15(5), 483-94.

[12] Horvath, S. (2013). DNA methylation age of human tissues and cell types. Genome Biology, 14(10).

[13] Girchenko, P., Lahti, J., Czamara, D., Knight, A. K., Jones, M. J., Suarez, A., . . . Raikkonen, K. (2017). Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestation age at birth . Clinical Epigenetic , 9(49). doi:10.1186

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[18] Horvath, S. (2013). DNA methylation age of human tissues and cell types. Genome Biology, 14(10).

present at these 353 CpGs forms the epigenetic clock[19]. Once the DNAm age has been derived the average age of acceleration can be calculated. The average age of acceleration is the difference between the actual age and the DNAm age[20]. Accelerated gestational aged based on blood-based DNAm age has been shown to be associated with negative health outcomes for both mother and fetus, including pre-eclampsia, low birth weight, low birth length, and lower 1 minute Apgar score[21].

Previous research has shown DNAm changes may be caused by normal aging, diet, inflammation, and a variety of exposures[22],[23]. Carcinogens, such as tobacco, have been well studied and shown to cause genetic alterations such as the methylation of CpGs[24]. A large meta-analysis was conducted by Joubert et al analyzing 450,000 CpG sites across 13 different cohorts. The meta-analysis uncovered 6,073 statistically significant CpGs across cohorts for newborns who were exposed to maternal smoking[25]. In addition to changes to DNAm maternal smoking has adverse health outcomes for the fetus and newborns such as low birth

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[19] Ibid.

[20] Ibid.

[21] Girchenko, P., Lahti, J., Czamara, D., Knight, A. K., Jones, M. J., Suarez, A., . . . Raikkonen, K. (2017). Associations between maternal risk factors of adverse pregnancy and birth outcomes and the offspring epigenetic clock of gestation age at birth . Clinical Epigenetic , 9(49). doi:10.1186

[22]Christensen, B. C., Houseman, E. A., Marsit, C. J., Zheng, S., Wrensch, M. R., Wiemels, J. L., . . . Kelsey, K. T. (2009). Aging and Environmental Exposures Alter Tissue-Specific DNA Methylation Dependent upon CpG Island Context. Plos Genetics, 5(8).

[23] Jones, M. J., Goodman, S. J., & Kobor, M. S. (2015). DNA methylation and healthy human aging. Aging Cell, 14(6), 924-32. doi:10.1111/acel.12349

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weight, sudden infant death syndrome, oral clefts, and other adverse illnesses[26]. Therefore, the causes of and subsequent changes in DNAm may provide an important window into determining the health of both mother and fetus.

Placental epigenetics are a novel approach to understanding the overall development of the fetus in utero. Thus far, no studies have begun to compare the placental epigenetic clock, or DNAm age, to the gestational age, nor have any analyzed how the epigenetic clock can mediate the effects of exposures on outcomes. In my analysis, the placenta epigenetic clock variable (DNAm age) and age acceleration difference is calculated for 89 subjects. Maternal smoking during pregnancy is analyzed as an environmental factor related to age acceleration differences. Age acceleration difference is then analyzed for its association with infant birthweight.

Methods

Participants

In total, 89 participants (mothers and infants) were included in this analysis. Participants were part of The Behavior and Mood in Babies and Mothers (BAM BAM) study which recruited participants from health care offices, health centers, and through community postings in southern New England[27]. Eligibility criteria included an age >18 and <40, no current/prior involvement with child protective services prior to birth, had no illicit drug use besides marijuana, and no serious medical conditions or complications of

[26] US Department of Health and Human Services. (2014). The health consequences of smoking—50 years of progress: a report of the Surgeon General.

[27] Stroud, L. R., McCallum, M., & Salisbury, A. (2018, August). Impact of maternal prenatal smoking on fetal to infant neurobehavioral development. Development and Psychopathology, 30(3), 1087-1105. doi:10.1017/S0954579418000676

pregnancy. Infants born to those mothers with no congenital abnormalities were included in the study[28].

Study Design

Interview

Pregnant women enrolled in the BAM BAM study were interviewed 2-4 times throughout their 2nd and 3rd trimesters as well as interviewed at delivery. Pregnant women were interviewed regarding smoking status as well as alcohol use during pregnancy and 3 months prior to conception. Women were also interviewed about ethnicity, race, and socioeconomic status. To confirm smoking status saliva was collected to measure cotinine levels and starting in the third trimester maternal weight was also measured[29].

Placental DNA methylation data

Sample Collection

To assess DNA methylation levels, placental tissue samples were collected after delivery and stored in RNAlater solution (Life Technologies, Grand Island, NY) at 4° C. Placental tissue was kept in RNAlater solution for 72 hours, where they were later removed, frozen quickly using liquid nitrogen, pulverized, and stored at -80° C until analysis was conducted. QIAmp DNA Mini kit (Qiagen, Inc.) was used to extract placental genomic DNA. Quality and quantity of DNA was measured using ND-1000 Spectophotometer (Nanodrop, DE).

[28] Ibid.

[29] Stroud, L. S., Papandonatos, G. D., Salisbury, A. L., Phipps, M. G., Huestis, M. A., Niaura, R., . . . Lester, B. (2016). Epigenetic Regulation of Placental NR3C1: Mechanism Underlying Prenatal Programming of Infant Neurobehavior by Maternal Smoking? Chiled Development, 87(1), 49-60. doi:10.1111/cdev.12482

DNA Methylation Age

Epigenetic gestational age was estimated using the robust placenta clock [ref]. This method estimates gestational age based on placenta DNA methylation levels at previously identified CpGs. We also calculated two measures of age accelerations: the difference between epigenetic and chronological gestational age, and the residuals when epigenetic gestational age is regressed on chronological gestational age in a linear model.[30]

Data Analysis

To analyze the effects of smoking on DNAm age a linear model was used with smoking during pregnancy as the exposure and placental DNA age acceleration as the outcome. DNA age acceleration is accessed by the difference between the actual gestational age and the DNAm age producing the age acceleration difference. Infant weight as well as infant sex are controlled for as covariates in the model. To analyze the effects of placental DNA age acceleration on birthweight, a linear model was also conducted. To assess the difference between sample groups, bi-variate tests were conducted. Independent samples t-test were performed for continuous variables, and chi-square tests were utilized for categorical variables.

Hypothesis:

The hypothesis of the first analysis is as follows:

H_0: Smoking during pregnancy will have no effect on the epigenetic clock, viewed through placental DNA age acceleration.

[30] Lee, Y., Choufani, S., Weksberg, R., Wilson, S. L., Yuan, V., Burt, A., . . . Horvath, S. (2019). Placental epigenetic clocks: estimating gestational age using placental DNA methylation levels. Aging, 11(12).

H_A: Smoking during pregnancy will lead to changes in the epigenetic clock, viewed through placental DNA age acceleration.

The hypothesis of the second analysis is as follows:

H_0: Placental DNA age acceleration will have no effect on infant birth weight.

H_A: Placental DNA age acceleration will lead to changes in infant birth weight.

Results

Study Characteristics

In total, 89 participants (mothers/infant pair) were included in the final analysis. Of these, 35 mothers did not smoke at any point during pregnancy, confirmed through cotinine levels, and 54 smoked or quit smoking during pregnancy. The demographics of the maternal study population can be seen in table 1. Among the mothers who had no smoking at all during pregnancy and the mothers who smoked at some point during pregnancy there was no significant difference in maternal age (p= 0.8533, 25.6±5.33, 26.6±4.94), maternal ethnicity (p=1.0, Hispanic/Latinx [31.4%, 33.3%]), or mothers education level(p=.1873, high school degree or less[54.3%, 70.4%]).

Infant demographics are presented in table 2. For infants with mothers who did not smoke during pregnancy compared with mothers who smoked during pregnancy there was no significant difference in gestational age in weeks (p=.1845, 39.3±1.75, 39.8±.995) or infant weight at birth in grams (p=.7235, 3370±552, 3410±448).

Epigenetic Clock Normality Analysis

Shapiro-Wilk normality test for age acceleration difference showed that the age acceleration difference was indeed normally distributed (p=.446). The Q-Q plot comparing

the theoretical quantile values for age acceleration difference as well as the actual quantile values for age acceleration difference values can be seen in figure 1.

Epigenetic Clock Analysis

Significant differences in age acceleration were observed by smoking. The age acceleration difference was found to be smaller in participants who did not smoke at any time during pregnancy (-1.68, [-3.67 , 0.086]) compared to participants who did smoke during pregnancy (-2.11, [-4.10 , 0.072]; Figure 2). To create a construct to analyze smoking effects on the age acceleration difference a covariate-adjusted statistical linear model was created controlling for sex and infant birth weight. The results of the linear model indicate that maternal smoking status does have a significant impact on the age acceleration difference (R^2=.079, F=3.52, p=0.038; Table 3).

Birth Weight Analysis

A significant relationship was seen between the age acceleration difference and infant birth weight. Both male and female infant birth weight are normally distributed with no significant difference in birth weight by sex (Figure 4). The covariate-adjusted statistical linear model was created controlling for maternal age at birth as a significant covariate. The results of the linear model reveal placental DNA age acceleration does have an effect on infant birth weight (R^2=.158, F=9.255, p=0.012; Figure 5; Table 4). For each 1 unit increase in age acceleration, we observed a decrease in birthweight of greater than 100g (Table 4).

Discussion

In this study, we investigated the relationship between smoking and placental epigenetic age acceleration as well as placental epigenetic age acceleration and birthweight. A significant relationship was found between smoking and placental age

acceleration as well as a significant relationship between placental age acceleration and birthweight. A limitation of this study is the small sample size, which restricts our ability to formally assess a mediating relationship of DNAm age acceleration, on smoking's impact on birthweight. Future studies with a larger sample size would be necessary to analyze the mediating effect.

Previous studies have demonstrated that carcinogens, such as tobacco smoke, can have a significant relationship on DNA methylation[31],[32]. Our results are consistent in showing that maternal exposure to tobacco smoke, at any point during pregnancy, can alter the epigenetic age, which has been previously been shown to be caused by DNA methylation[33]. Our present study found that smoking led to a decrease in DNAm age. Studies have uncovered that an increase DNA methylation is associated with a decrease in age, or a longer tissue life, thus while our present study did not investigate the amount of DNA methylation, it can be speculated that smoking would lead to a increase in DNA methylation[34],[35]. Future investigative studies should be done examining the link between the amount of DNA methylation and specific genomic regions and DNAm age.

DNAm age acceleration was associated with a change in birthweight, specifically an increase in age acceleration was associated with a decrease in overall birthweight. While previous studies have found relationships between smoking and changes in

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[31] Christensen, B. C., Houseman, E. A., Marsit, C. J., Zheng, S., Wrensch, M. R., Wiemels, J. L., . . . Kelsey, K. T. (2009). Aging and Environmental Exposures Alter Tissue-Specific DNA Methylation Dependent upon CpG Island Context. Plos Genetics, 5(8).

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[35] Langton, A. K., Herrick, S. E., & Headon, D. J. (2008, May). An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. J Invest Dermatol , 128(5), 1311-8. doi:10.1038/sj.jid.5701178

birthweight, specifically the decrease in overall birthweight, very few studies have examined the relationship between DNA methylation and birthweight.[36] A 2010 study on placental methylation found an association between placental promoter methylation and lower birth weight based on gestational age, which is consistent with our current analysis.[37]

In summary, smoking during pregnancy will lead to changes in the epigenetic clock, viewed through placental DNA age acceleration. Placental DNA age acceleration will also lead to changes in infant birth weight. These findings show the importance of further investigation into the epigenetic clock of a variety of tissues, specifically the placenta, and this better understanding can lead to better health outcomes of infants.

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Table 1 Maternal Demographics

	No smoking at all during pregnancy (n=35)	Smoked during pregnancy (n=54)	Overall (n=89)
Mothers Age			
Mean (SD)	25.6 (5.33)	26.6 (4.95)	26.2 (5.10)
Median [Min, Max]	25.0 [18.0, 37.0]	26.5 [18.0, 37.0]	26.0 [18.0, 37.0]
Mothers Ethnicity			
Hispanic/Latinx	11 (31.4%)	18 (33.3%)	29 (32.6%)
Not Hispanic/Latinx	24 (68.6%)	36 (66.7%)	60 (67.4%)
Mothers Education Level			
High School Degree or less	19 (54.3%)	38 (70.4%)	57 (64.0%)
Partial college [min 1 year] or more	16 (45.7%)	16 (29.6%)	32 (36.0%)

Table 2 Infant Demographics

	No smoking at all during pregnancy (n=35)	Smoked during pregnancy (n=54)	Overall (n=89)
Infant Gestational Age Weeks			
Mean (SD)	39.3 (1.75)	39.8 (0.995)	39.6 (1.35)
Median [Min, Max]	39.6 [33.7, 41.1]	40.1 [37.7, 41.3]	39.9 [33.7, 41.3]
Infant Gestational Age			
Fullterm birth [Greater than 40 weeks GA]	15 (42.9%)	28 (51.9%)	43 (48.3%)
Late preterm [Between 32 and 37.9 weeks GA]	3 (8.6%)	2 (3.7%)	5 (5.6%)
Subobtimal birth [Between 38 and 39.9 weeks GA]	17 (48.6%)	24 (44.4%)	41 (46.1%)
Infant Weight (g)			
Mean (SD)	3370 (552)	3410 (448)	3390 (489)
Median [Min, Max]	3380 [1890, 4390]	3400 [2240, 4520]	3400 [1890, 4520]
Epigenetic Clock DNAm Age			
Mean (SD)	37.7 (1.56)	37.7 (1.03)	37.7 (1.26)
Median [Min, Max]	38.0 [33.2, 40.1]	37.6 [35.4, 40.2]	37.7 [33.2, 40.2]
Difference between GA and DNAmAge			
Mean (SD)	-1.61 (1.08)	-2.11 (1.06)	-1.91 (1.09)
Median [Min, Max]	-1.68 [-3.67, 0.864]	-2.07 [-4.10, 0.716]	-1.93 [-4.10, 0.864]

Figure 1 Q-Q Plot of Age Acceleration Difference

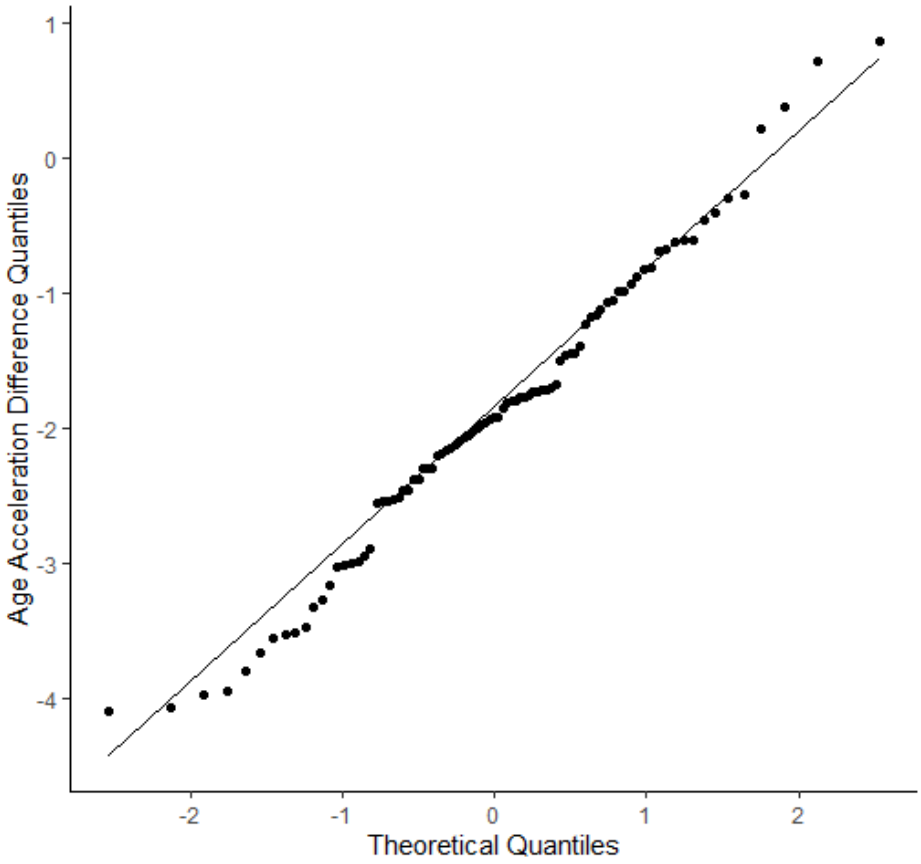

Figure 2 Box Plot of Age Acceleration Difference by Maternal Smoking Status

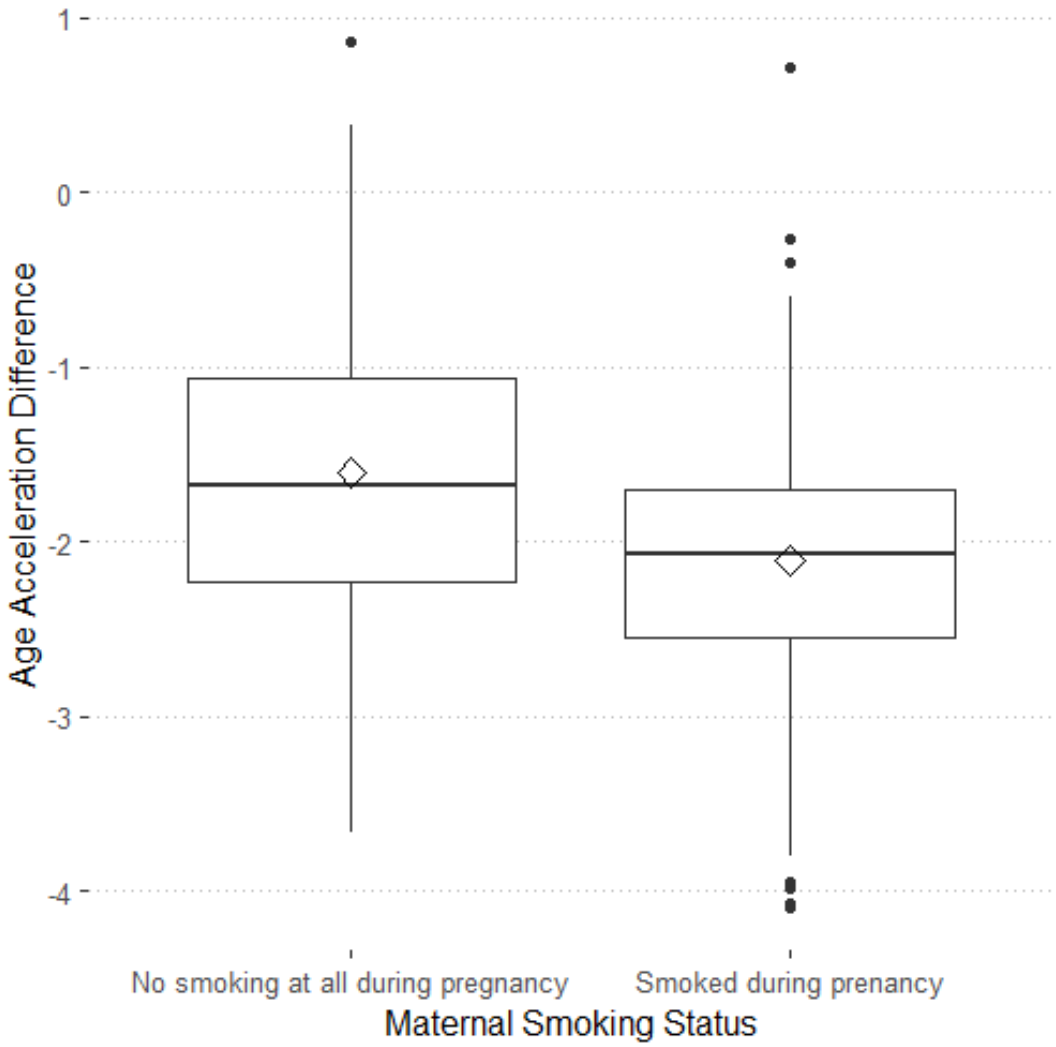

Table 3 Linear Model of Age Acceleration

	Age Acceleration Difference
Maternal Smoking Status	-.481*
	p = .038
Sex	-.298
	p = .196
Infant Weight	-0.000
	p = .104
Constant	-.173
	p = .828
Observations	89
Adjusted R^2	.079
Residual Std. Error	1.046 (df = 85)
F Statistic	3.519* (df = 3; 85)
Notes:	p<0.05

Figure 4 Histogram of Birth Weight by Sex

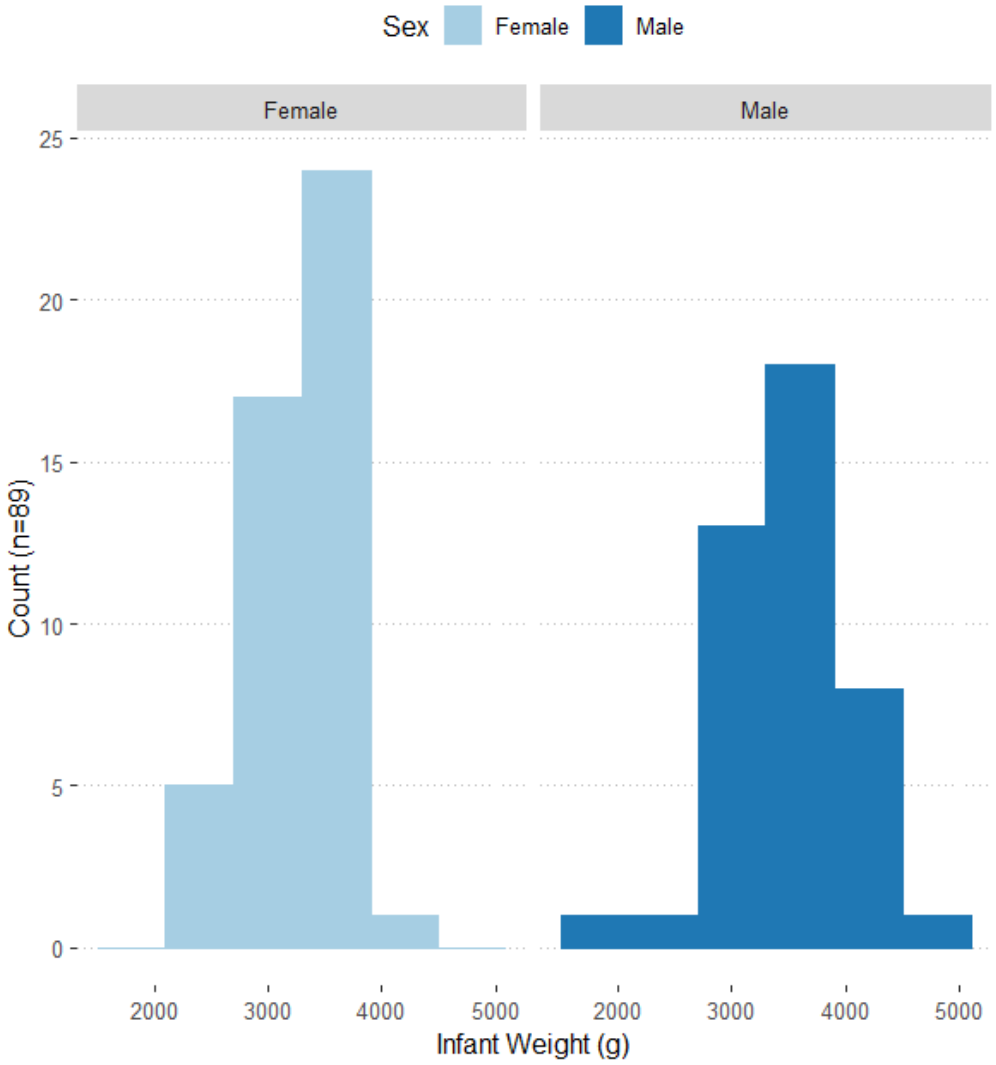

Figure 5 Infant Weight Linear Model

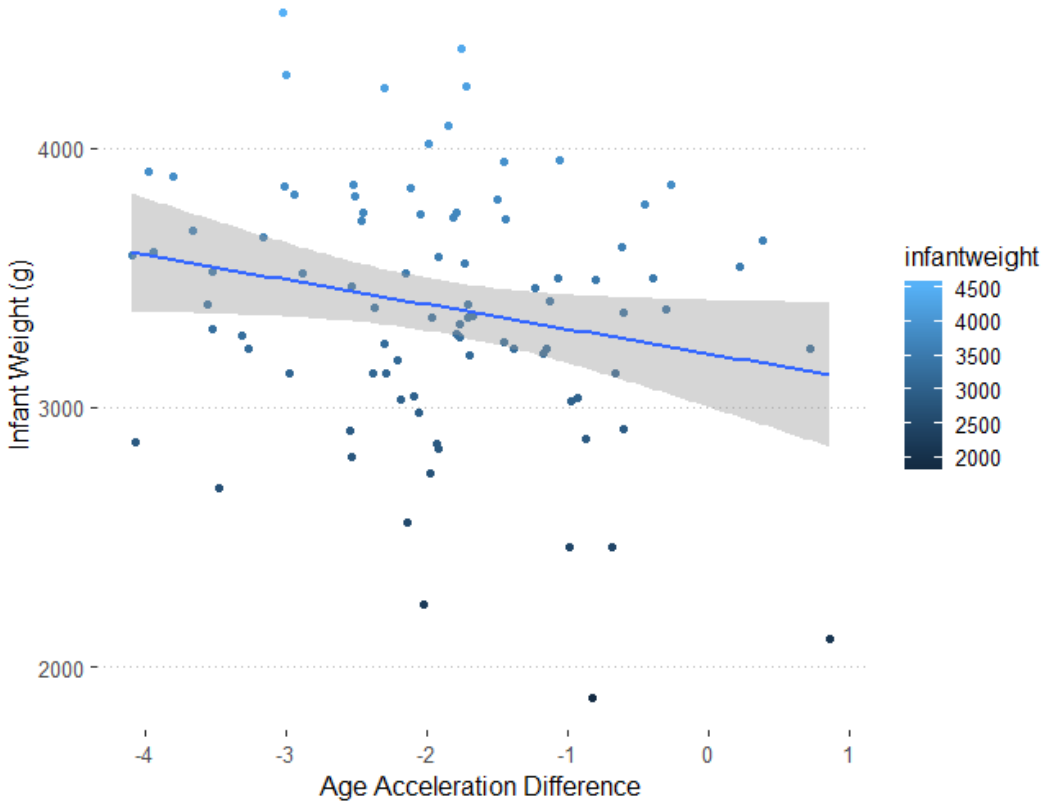

Table 4 Linear Model of Infant Birth Weight

	Infant Birth Weight
Age Acceleration Difference	-113.292*
	p = .012
Maternal Age	35.100***
	p = .001
Constant	2,253.616***
	p = 0.000
Observations	89
Adjusted R^2	.158
Residual Std. Error	448.468 (df = 86)
F Statistic	9.255*** (df = 2; 86)
Notes:	p<0.05