Intergenerational Transmission of Health Inequities: Early Life Socioeconomic Factors, Adult Cardiometabolic and Pregnancy Outcomes, and Potential Epigenetic Mechanisms in Young Adult Women.

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Abstract

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Epidemiology

Parental socioeconomic status (SES) experienced by a woman *in utero* may directly affect her adult health, independent of her life course experiences. Developmental programming of gene expression through DNA methylation may be involved. However, investigations using prevailing regression methods have been impeded by complex causal structures and unmeasured confounding. Using a U.S.-national, longitudinal cohort, we investigated the effect of mother's education on (1) cardiometabolic risk and (2) pregnancy outcomes among women averaging 30 years of age. Using an Israeli birth cohort, we investigated associations between parental education and father's occupational class on (3) DNA methylation at cardiometabolic genes in 32-year old women.

(1) Using marginal structural models estimated by inverse probability weighting, we found young adult women whose mothers had higher educational attainment (*e.g.* college versus high school) had 40% lower risk (Odds Ratio = 0.60, 95% Confidence Interval: 0.45, 0.80)

of metabolic syndrome, independent of childhood maltreatment, adolescent overweight, adult SES, and behavioral risk. Additionally, there was evidence that women with more highly educated mothers were taller, thinner, and had a smaller waist, lower resting pulse rate, lower levels of inflammatory markers, and better blood sugar control.

(2) Additionally, women born to more highly educated mothers who themselves bore children delivered newborns who were 90 grams heavier (95% CI: 20.8, 156.5), independent of childhood maltreatment, pre-pregnancy overweight, adult SES, and prenatal smoking. Moreover, results from (1) and (2) were robust to several sensitivity analyses including model alteration, data replacement, and quantitative bias analyses.

(3) Finally, we found that lower SES at birth measured by father's occupational class was associated with reduced methylation at the *ABCA1* cholesterol transporter gene and the *NR3C1* glucocorticoid receptor genes in 32-year old women, after adjusting for numerous parental and offspring characteristics. Similarly, fewer years of mother's education was associated with reduced *HSD11B2* glucocorticoid-inactivating enzyme gene methylation. However, such associations did not appear to mediate relationships between birth SES and young adult cardiometabolic risk.

Overall, there appears to be substantial evidence that early life SES is independently related to adult women's health and DNA methylation, however the mechanisms relating them require further elucidation.

Dedication

To Claire and our baby boy.

May we strive to leave the world a better place for all future generations.

Acknowledgements

Thanks are due to each of my committee members, Ali, Amelia, David, and Thomas for their mentorship and guidance. Special thanks is due to my chair Daniel, who reviewed each of my numerous drafts thoroughly, as well as provided strategic paper-writing and career advice over many meetings. Additionally, my work was supported by the NIH Reproductive, Perinatal, and Pediatric Epidemiology (RPPE) T-32 Training Grant and the University of Washington Global Woman, Adolescent, and Child Health (Global WACh) Integrated Health Seed Grant (2013-2014).

Finally, a special thanks goes to Dr. Stephen Bezruchka, whose tireless energy and passion inspired me to pursue this line of research. I'm truly lucky to have met such an irreplaceable friend, mentor, and role model.

What will you prescribe for the sick woman, doctor - you who have seen at a glance that the cause of her illness is general anemia, want of good food, lack of fresh air? Say, a good beefsteak every day? a little exercise in the country? a dry and well-ventilated bedroom? What irony! If she could have afforded it this would have been done long since without waiting for your advice.

~ Peter Kropotkin, *An Appeal to the Young* (1880)

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Introduction

Background and Significance

Health disparities between socioeconomic classes remains an intractable problem in the U.S. and globally. Although numerous risk factors across the life course have been identified, the causal mechanisms, and therefore the potential efficacy of interventions, are uncertain.[1,2] One segment of the life course that has recently garnered interest is early life including intrauterine and early post-natal life.[2-7] In women, low parental socioeconomic status (SES)[8,9] and childhood maltreatment[10] are associated with increased risk of obesity, cardiovascular disease, diabetes, hypertension and early mortality[11-17] as well as having low birth weight offspring.

Developmental Origins of Health and Disease (DOHaD) theory suggest stressors experienced in early life may adversely influence fetal programming and organ growth and development[15-17] and subsequent increased risk for adult cardiometabolic disease.[18-24] Among women of child-bearing age, this may also lead to poor response to the physical challenge of pregnancy and adverse birth outcomes,[33] reinforcing health disparities[27,28] and historical trauma[34] across generations. Persistent and widening socioeconomic disparities in cardiometabolic disease and low birth weight infants (< 2500 grams) in the U.S. and globally suggest understanding early life mechanisms and identifying related interventions may be necessary to solve these intractable public health challenges.[2,11-13]

However, empirical analysis of causal and mediating mechanisms explaining associations between early life SES and adult cardiometabolic risk (CMR) or pregnancy outcomes are lacking and methodologically challenging.[25] Importantly, the temporal nature of a woman's experiences across the life course, *in utero*, child, adolescent, and adult, should be explicitly incorporated into any analysis. Because life course mediators are sequentially dependent[1] and may be confounded by still other mediators, simple covariate adjustment models are often biased. Consequently, estimating the direct effect of a distant, early life exposure such

as SES on adult outcomes likely requires the application of nascent epidemiologic methods,[25,35] such as marginal structural models.

Additionally, current social theory suggests that the biological mechanism for persistent health disparities may be through epigenetic modifications of DNA following early life exposures to stress, poverty, or malnutrition.[26-30] 'Epigenetic' refers to any heritable pre- or post- transcriptional regulation of DNA function – from DNA methylation, histone modification, or micro RNAs -- which occur without changes to the underlying DNA sequence. DNA methylation is of particular interest, as it can have wide-ranging effects during fetal development and beyond.[14] Recent studies suggest that *in utero* exposure to stress hormones[31,32] may program fetuses for increased cortisol secretion and stress reactivity, insulin resistance, poor kidney and vascular development, and obesity, through alterations in the methylation profile of important cardiometabolic and stress-related genes. In turn, this may make such individuals more susceptible to hypertension, diabetes, and other cardiometabolic diseases later in life.[14]

While interest in DNA methylation is growing, it remains unclear how epigenetic mechanisms may be investigated in epidemiologic studies.[32] Challenges include the characterization of early life exposures and assessment of DNA methylation, including timing, tissues-specificity, and candidate sites. While some studies have associated low early life SES and adulthood differences in methylation, [30,43,44] substantial evidence is lacking supporting the theory that early life SES may be related specifically to adult cardiometabolic health through epigenetic mechanisms.

Project Description

In light of identified gaps in the literature, this dissertation seeks to clarify the causal relationship between early life socioeconomic status and adverse adult cardiometabolic risk and pregnancy outcomes among women, as well as the potential DNA methylation mechanisms thereof.

Using the National Longitudinal Study of Adolescent Health (Add Health) and the Jerusalem Perinatal Study Family Follow-Up (JPS-1), described subsequently, we investigated the following three specific aims, corresponding to the three numbered chapters of this dissertation. Briefly:

1. Using Add Health, we investigated whether there was evidence for a controlled direct effect of a woman's early life SES on her cardiometabolic risk in young adulthood (mean age = 30 years). (*Chapter 1*)
2. Using Add Health, we investigated whether there was evidence for a controlled direct effect of a woman's early life SES on the birth weight of her first, singleton child. (*Chapter 2*)
3. Using JPS-1, we investigated whether early life SES was associated with candidate cardiometabolic gene methylation in young adult women (mean age = 32 years). (*Chapter 3*)

Study Settings

The National Longitudinal Study of Adolescent Health (Add Health) is a school-based, nationally-representative, longitudinal study of United States adolescents enrolled in grades 7 through 12 in 1994-95 (N = 90,118). In-home interviews were conducted in 1994-5 (Wave I) with a subset of individuals and their parents, siblings, friends, and romantic partners to collect extensive information on demographics, health, attitudes, behaviors, and environment (N = 20,745). Individuals were followed for up to three additional waves of in-home interviews: Individuals were re-interviewed in 1996 (Wave II), anthropometric

measurements were collected in addition to re-interview in 2001-2 (Wave III), and capillary whole blood collection was also collected in 2008-9 (Wave IV). The final round (Wave IV) included 80.3% of eligible participants (N = 15,70). Additional characteristics and documentation of the study design and sampling frame can be found on the Add Health website (www.cpc.unc.edu/projects/addhealth/).

Individuals were eligible for specific aims 1 and 2 of this study if they were women, had a biological mother interviewed and participated in both Wave I and Wave IV interviews. For specific aim 2, women must have had at least one live birth during the course of the study. Consequently, 4,026 and 1,681 women were used for Aims 1 and 2, respectively.

The Jerusalem Perinatal Study (JPS), is a longitudinal study following all births to residents of Jerusalem in the years 1974 through 1976 (N = 17,003). The JPS Family Follow-Up (JPS-1) is an ancillary study conducted amongst a subset of JPS participants (mother-offspring dyads). Participants for JPS-1 were selected using a sampling scheme that oversampled for maternal pre-pregnancy overweight (body mass index ≥ 27 kg/m^2) and extremes of offspring birth weight (≤ 2500 and ≥ 4000 grams). In JPS-1, peripheral blood samples were collected from offspring (N = 1,250) at an average age of 32 years, at which time various anthropometric measures were also taken. For specific aim 3, all JPS-1 offspring who were female (N = 613) women were included.

Summary and Potential Impact

This study seeks to use contemporary causal modeling method to investigate whether early life SES has a direct effect on adult outcomes amongst women, including cardiometabolic phenotype and birth outcomes. Additionally, it seeks to assess whether DNA methylation may have a potential role in mediating these associations. This work may provide evidence for the importance of early life in determining adult health.

(*End Introduction)*

Chapter 1 – Direct effects of maternal education at daughter's birth on daughter's adult cardiometabolic risk.

Abstract

Background: Studies of associations between maternal SES indicators and offspring cardiometabolic risk have had inconsistent results. We examined the direct effect of maternal education on daughter's adult cardiometabolic risk using two modelling methods, adjusting for confounders and life course mediators.

Methods: Using data from the National Longitudinal Study of Adolescent Health (1995-2009), we identified female respondents whose biological mothers were interviewed (N = 4,026). We defined maternal education as less than high school (HS), HS diploma or equivalent, or college degree at respondent's birth. Using multivariate regression and marginal structural models (MSM), we estimated the controlled direct effect of maternal education on respondent's adult (mean age = 28 years) body size, blood pressure, pulse, plasma glucose, C-reactive Protein (CRP), and risk of metabolic syndrome independent of mediating risk factors: adolescent maltreatment and overweight status, low adult education and household income, smoking and low physical activity. We evaluated sensitivity of our findings to model assumptions and missing data.

Results: Eighteen percent (N = 720) of mothers had less than HS education at respondent's birth. Multivariate regression and MSM suggested a 10-14% reduced CRP (MSM β = -13.8% [95% CI: -23.5%, -2.8%]) and a 30-40% reduced risk of metabolic syndrome (MSM OR = 0.60 [95% CI: 0.45, 0.80]) associated with higher maternal education, independent of mediators. MSM analyses suggested additional associations with reduced waist circumference (3 cm), BMI (1 kg/m2), and glycated hemoglobin (1.3%). Findings were robust to our sensitivity analyses.

Conclusions: Our models suggest a direct effect of maternal education on daughter's cardiometabolic risk. Further research should consider additional mechanisms responsible for maternal early-life risk exposures to offspring cardiometabolic risk.

Background

Maternal external environment around the time of pregnancy influences maternal health and is important in the developmental origins of offspring adult health and disease, due to organ development and metabolic programming events that occur during the perinatal period.[1, 2] For instance, low maternal educational attainment is known to be a contributor to pregnancy stress, *e.g.* through resource availability and psychosocial preparedness for childbearing, that may contribute directly to excess fetal glucocorticoid exposure[3-6] and subsequent adult cardiometabolic risk.[7] Moreover, among female offspring, this mechanism may result in intergenerational transmission of disease risk through poor pregnancy outcomes.[3-7] However, studies associating maternal education with offspring cardiometabolic outcomes in adulthood have shown mixed results. While Bouhanick, *et al*. found maternal completion of compulsory education (16 years) to be independently associated with reduced risk of offspring metabolic syndrome at age 45,[8] Kvaavik, *et al.* found no association between maternal education and offspring body mass index, lipids, or blood pressure at 15, 25, or 40 years.[9]

These inconsistent findings may be due to varying covariate adjustment while estimating direct effects of maternal education independent of known mediators and confounders. The relationship between early life and adult cardiometabolic outcomes is likely complex and mediators such as child-rearing environment[10, 11] and adult socioeconomic stressors[12, 13] may themselves affect others mediators such as adolescent obesity or risky health behaviors.[8-14] In such a setting, the likelihood of unmeasured mediator-outcome confounding is high and the estimation of direct effects through conventional covariate adjustment and mediation analysis can be biased.[15-17] Other methods such as marginal structural models (MSMs) may better account for known psychosocial and biological mediators between maternal education and adult cardiometabolic risk.[15-20] Nonetheless,

MSMs are still susceptible to unmeasured confounding and assumptions about causal structure, warranting additional sensitivity analyses.[21,22]

The objective of the current study is to estimate whether maternal education at the time of her daughter's birth, has a direct effect on the daughter's body size, blood pressure, pulse, plasma glucose, inflammatory markers, and overall risk of metabolic syndrome in young adulthood, independent of known psychosocial and biological mediators: childhood maltreatment, adolescent overweight, adult socioeconomic status (SES), and adult smoking and physical activity. We improve on past work by incorporating life-course social and biological determinants of adulthood cardiometabolic risk in a causal mediation framework and conducting multiple sensitivity analyses to test robustness of findings to weight and model misspecification.

Methods

Study Setting

The current study was conducted in the setting of the National Longitudinal Study of Adolescent Health (Add Health), a nationally-representative, longitudinal study of 7-12 grade United States adolescents that begun in 1994 (N = 90,118). In the Add Health study, in-home interviews were conducted with a core subset of respondents (randomly selected within school and sex strata) along with a parent, during which extensive information was collected on demographics, health, attitudes, behaviors, and environment. Additional respondents (and parents) were interviewed based on ethnic minority status, having siblings in the study, and/or being black with at least one college-educated parent (N = 20,745 individuals). Respondents first interviewed in Wave I were followed for up to three additional in-home interviews in 1996 (Wave II), 2001-2002 (Wave III), and 2007-2008 (Wave IV). Anthropometric measurements (Wave III) and capillary whole blood (Wave IV) were also collected. During Wave IV, 15,701 respondents (80.3% of eligible Wave I) were interviewed. Investigators calculated a grand sampling weight for respondents interviewed in all four waves to approximate the target population of U.S. adolescents in grades 7-11 in 1994-5.[23] This weight accounts for clustered sampling, attrition, and oversampling in longitudinal analyses.[23]

Additional information on respondent characteristics, sampling frame, and protocols can be found on the Add Health site: www.cpc.unc.edu/projects/addhealth/. All data used for the current study were obtained through a restricted data access agreement between the University of Washington Center for Studies in Demography and Ecology (CSDE) and the Inter-university Consortium for Political and Social Research (ICPSR) at the University of Michigan. Research on these data has been approved by the Institutional Review Board of the University of Washington.

Study Population

Since we are interested in the potential intergenerational transmission of disease risk, respondents were included in this current study if they were women, had a biological mother interviewed during Wave I, and participated in Wave IV interview. There were 5,375 respondents available based on these criteria. We excluded 129 (2.4%) respondents without a reported maternal education and 1,256 (23.4%) missing a Wave IV longitudinal grand sampling weight due to non-response in previous waves. Individuals missing this weight cannot be used for analysis, since their contribution to the target population cannot be estimated. Since non-response patterns are accounted for in the weights of the remaining individuals, the bias due to these exclusions should be minimal.[23] Our final analytic data set included 4,026 respondents.

Data Collection

Primary Exposure – During Wave I (1994-5), a respondent's mother was asked "How far did you go in school?" Responses were coded into three categories: less than high school diploma; high school diploma or GED certification, and completed college degree. Because we were interested in maternal education at or prior to respondent's birth, we capped completed education to less than high school if she gave birth at or prior to 16 years (n = 29) and high school diploma if she gave birth at or prior to 21 years (n = 93).

Primary Outcomes – Respondent's height, weight, waist circumference, blood pressure, and pulse rate were measured by study staff at Wave IV home visit. Mean arterial pressure (MAP = (systolic + 2 x diastolic) / 3), pulse pressure (PP = systolic – diastolic), and body mass index (BMI = weight in kilograms / height in meters squared) were calculated by study staff. Capillary whole blood was collected by finger stick, from which high-sensitivity C - reactive protein (hsCRP), glycated hemoglobin (HbA1c), and plasma glucose were assayed. Additional details for sample collection and quantification protocols can be found

under "Biological Data" at: http://www.cpc.unc.edu/projects/addhealth/design/wave4. We log-transformed hsCRP, HbA1c, and plasma glucose because their distributions were skewed.

Metabolic Syndrome - Metabolic syndrome was defined using the American Heart Association / National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines for women.[24] A respondent was defined to have evidence of metabolic syndrome by ATP III if she had three or more of the following: Waist circumference ≥ 88 cm, elevated triglycerides ≥ 150 mg/dL or on drug treatment, reduced HDL < 50 mg/dL, blood pressure greater than 130/85 mmHg, and/or fasting glucose ≥ 100 mg/dL or on drug treatment. To adapt these criteria to available data, we substituted self-reported high cholesterol for the lipid measures and HbA1c ≥ 5.7% for the glucose measure (since we did not have a fasting glucose measure). This cutoff for HbA1c is a potential marker for pre-diabetes.[25]

Psychosocial mediators – Childhood maltreatment[10,11] and adult socioeconomic status[12,13] are important psychosocial stressors in the respondent's life course that may lie in the causal pathway between maternal education and cardiometabolic risk. In line with previous investigations in Add Health,[26] we used self-reported incidents of neglect, physical abuse, and sexual abuse prior to 18 years of age to predict a maltreatment factor score. Factors scores were generated using principal component factor (PCF) analysis and quartimin rotations.[27-29] Adult socioeconomic status (SES) was measured prior to our outcomes using two variables from Wave III: Self-reported total household income in dollars or, if the woman could not provide an exact amount, one the following approximates: $5,000; $12,500; $17,500; $25,000; $35,000; $45,000; $62,500; or $100,000, based on the mid-point of categorical choices (capped at $100,000); and last completed year of education. A factor score for adult SES was predicted from these two indicated by PCF analysis and

quartimin rotations. Maltreatment and adult SES factor scores were also dichotomized at their medians to high/low categories.

Biological mediators – Higher adolescent BMI and adult behavioral risk factors such as smoking and lack of physical activity increase adult cardiometabolic risk[11, 12] and may be a consequence of lower maternal education and/or psychosocial stressors.[11] Adolescent BMI was calculated from Wave I (mean age = 15) self-reported height and weight. Based on American Academy of Pediatrics guidelines, respondents up to 17 years old at Wave I were classified as having high childhood BMI if their BMI was greater than 85 percent of other respondents of the same age (Cutoff range: 25.3 – 27.3 kg/m^2). Respondents 18 years or older were classified as high BMI if their BMI met or exceeded 25 kg / m^2, according to adult standards. A behavioral risk factor score was calculated from Wave IV self-report of number of cigarettes smoked per month and number of various recreational physical activity events in the past week, using PCF analyses and quartimin rotations. High behavioral risk was defined as having a score higher than the median factor score.

Confounders – Mother's age at respondent's birth is related to educational attainment and may be related to child-rearing practices and therefore psychosocial and biological stressors experienced by her daughter[30,31] Similarly, maternal self-reported race is related to educational opportunity and attainment, as well as social and biological stressors experienced by the daughter throughout her life course.[32,33] Maternal race was coded as non-Hispanic white, Hispanic white, black any ethnicity, or other non-white, with those reporting any mixed race not including black being accorded the last category. Both mother's age at respondent's birth and mother's self-reported race were included as confounders in all analyses.

Statistical Analysis

We first examined univariate demographic characteristics of the study population accounting for survey design. Next, we estimated the direct effect of maternal education (X_i) on the daughter's anthropometrics and cardiometabolic risk markers (Y_i) in early adulthood, independent of the daughter's own life experiences, using two modelling approaches: Multivariate-adjusted linear (for continuous outcomes) or logistic (for dichotomous outcomes) regression models including all measured predictors of outcomes (Traditional Approach) and linear or logistic marginal structural models (MSM) estimated by inverse probability weights using dichotomous mediators (Causal Approach).

Hypothesized causal structure - In each model we attempted to estimate the controlled direct effect of X_i on Y_i after accounting for confounding by mother's age at daughter's birth (G_i) and mother's self-reported race (R_i), as well as mediation / endogenous confounding by daughter's life-course psychosocial and biological stressors: high childhood maltreatment prior to 18 years (M_i), adolescent overweight (O_i), low adult SES (A_i), and high adult behavioral risk (B_i). The relationships between exposure, mediators, confounders, and outcome are constrained only by temporality (*Figure 1*): Mother's age and self-reported race precede all other factors, including mother's education at daughter's birth. Each of the mediators in the daughter's life course is hypothesized to also affect all subsequent mediators (*e.g.* child maltreatment affects adolescent overweight, adult SES, and adult behavioral risk).

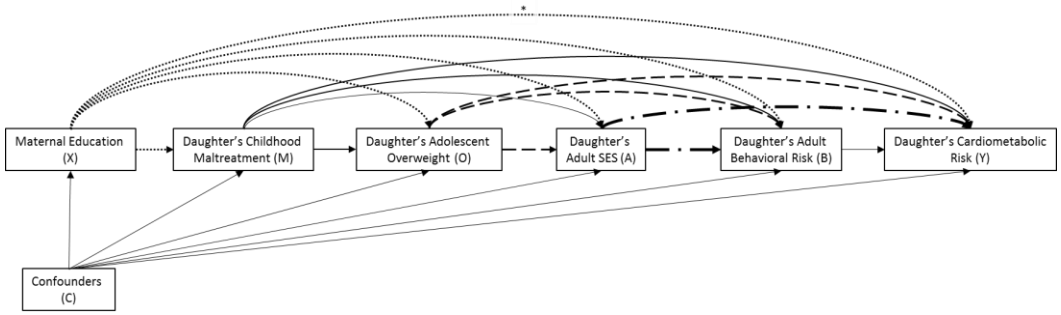

Figure 1. Causal diagram of hypothesized life course determinants of cardiometabolic risk. Each exposure and mediator is assumed to have an effect on all other mediators that occur subsequent to it. Directed edges are drawn with different patterns based on their source for visual effect only; they do not reflect any addition knowledge or specification about relationships. The asterisk (*) indicates the effect of interest: the direct effect of maternal education on daughter's cardiometabolic risk measures. Confounders (C) represent a vector including maternal age at daughter's birth (G) and maternal self-reported race (R).

Traditional Approach: Multivariate regression – We first estimated direct effects conventionally by fitting the following multivariate-adjusted linear regression model in which the primary exposure, confounders, and mediators are included as predictors of continuous outcomes (a corresponding logit model was fit for binary outcomes):

$$\mathrm{E}[Y_i \mid X_i = x, R_i = r, G_i = g, M_i = m, O_i = o, A_i = \mathrm{a}, B_i = \mathrm{b}] = \quad (1)$$

$$\beta_0 + \beta_1 X + \beta_2 R + \beta_3 G + \beta_4 M + \beta_5 O + \beta_6 A + \beta_7 B$$

Under strong assumptions of no model misspecification, no interaction, and no unmeasured confounding,[16] it is possible for equation (1) to give an unbiased estimate of the causal direct effect of maternal education on daughter's anthropometrics and cardiometabolic risk makers as well as the causal direct effects of mediators. However, our model (*Figure 1*) implies mediator adjustment induces confounding through the mediator's parents (*i.e.* collider stratification bias). We use this as a baseline to explore effect estimates from different covariates functional forms.

Causal Approach: MSM estimated by inverse probability weighting – Next, we tested the controlled direct effect of maternal education (X_i) on daughter's cardiometabolic risk markers (Y_i) by fitting the following regression model:

$$\mathrm{E}[Y_i \mid X_i = x, M_i = m, O_i = o, A_i = \mathrm{a}, S_i = s] = \quad (2)$$

$$\beta_0 + \beta_1 X + \beta_2 M + \beta_3 O + \beta_4 A + \beta_5 B$$

weighting individual subjects by the inverse probability of their exposure to their given strata of G0 education, the baseline confounders, maternal age and race, and probability of exposure to the four dichotomous mediators. No interaction terms were included, as there

was not consistent evidence of additive interaction between the exposure and each of the mediators. Stabilized weights[34] for G0 education (w_i^X) were estimated by multinomial logistic regression to predict the probability for a given strata of G0 education given *C* confounders. Stabilized inverse probability weights (IPW) for mediators were calculated using logistic regression, giving regard to temporality (*Figure 1*):

G0 education: $$w_i^X = \frac{P(X = x_i)}{P(X = x_i \mid C = c_i)} \quad (3)$$

Childhood maltreatment: $$w_i^M = \frac{P(M = m_i \mid C = c_i)}{P(M = m_i \mid X = x_i,\ C = c_i)} \quad (4)$$

Adolescent overweight: $$w_i^O = \frac{P(O = o_i \mid C = c_i)}{P(O = o_i \mid X = x_i,\ M = m_i,\ C = c_i)} \quad (5)$$

Adult SES: $$w_i^A = \frac{P(A = a_i \mid C = c_i)}{P(A = a_i \mid X = x_i,\ M = m_i,\ O = o_i,\ C = c_i)} \quad (6)$$

Adult behavioral risk: $$w_i^B = \frac{P(B = b_i \mid C = c_i)}{P(B = b_i \mid X = x_i,\ M = m_i,\ O = o_i,\ A = a_i,\ C = c_i)} \quad (7)$$

A correctly specified MSM gives the controlled direct effect of *X* on *Y* in a marginal population in which, potentially counter to the fact, all individuals are "unexposed" to the mediators, in our model, a marginal G1 population at lower risk of childhood maltreatment, adolescent overweight, low adult SES, and adult behavioral risk.

Incorporating survey design – To account for sampling dependency introduced by the survey design and produce correct standard errors,[23] each weight was estimated with the *svy* option after using the Wave IV grand sampling weights, clustering, and stratification variables for the *svyset* command. An overall weight ($W_{overall}$) was then generated for each subject using the product of the stabilized weights and the Wave IV grand sampling weight (w_i^{gsw}):

Overall Weight: $$W_{overall} = w_i^X * w_i^M * w_i^O * w_i^A * w_i^B * w_i^{gsw} \quad (8)$$

The *svy* option was subsequently used to fit model (2) using $W_{overall}$ for the *svyset* command. An analogous method was described by Brumback, *et al*. employing *PROC SURVEYREG* in SAS.[35]

Sensitivity Analyses

Like other causal modeling methods, MSM effect estimations are subject to biases from misspecified causal models, mismeasurement, and unmeasured confounding.[20,21] We examined qualitative robustness of our original MSM estimates to IPW modifications, alternate mediation models, and data replacement.

IPW Modification - We re-fit model (2) after truncating inverse probability weights at the 1st / 99th, 5th / 95th, and 10th / 90th percentiles. We also re-fit model (2) after changing the dependencies in the IPW equations (4-7) to reverse the presumed temporal relationship between childhood maltreatment and adolescent overweight and between adult SES and adult behavioral risk.

Alternate Mediation – We re-fit model (2) using all discretely binary mediators, rather than dichotomized factor scores. Namely, childhood maltreatment was recoded as any or no episodes of neglect, physical abuse, or sexual abuse, adult SES was recoded as high school graduate at Wave III or not, and adult behavioral risk was recoded as any smoking and no physical activity. Adolescent BMI remained dichotomized at 25 kg/m^2. Since approximately 20% of our respondents still lived with parents at Wave III, we also re-fit model (2) adding respondent's Wave VI (mean age = 28) SES as an additional mediator. Wave IV SES was defined by a factor score predicted from total household income, attained education, and an indicator if respondent would "still be in debt," "break even," or "have some left over" if she sold all of her assets to pay off her debts. This mediator was presumed to occur after Wave III SES, but prior to adult behavioral risk.

Data replacement – Finally, we tested sensitivity to missing values by alternately replacing all missing values for binary mediators to be alternately, an indicator of cardiometabolic risk (*e.g.* missing value for adult SES replaced with indicator of low adult SES) or an indicator of protection (*e.g.* missing value for adult SES replace with indicator of high adult SES).

All data processing and analysis were conducted in STATA 12.1 MP (College Station, TX).

Results

Twenty-three percent (N = 919), 59% (N = 2,387), and 18% (N = 720) of respondents had mothers who reported at least a college degree, at least a high school diploma or GED, or less than a high school education, respectively (*Table 1*). The corresponding proportions accounting for survey design are 21%, 62%, and 17%, respectively. Accounting for survey design, 72% percent and 15% of mothers fell into study-defined categories of non-Hispanic white and black, respectively. Daughters of mothers with less than a high school education had a 14% greater prevalence of metabolic syndrome than daughters of college-educated mothers (21% versus 7%, respectively). Daughters of mother's with less than a high school education were also more likely to experience psychosocial stressors (*e.g.* 13% greater prevalence of any childhood physical abuse) and behavioral risk factors (*e.g.* 9% greater prevalence of adult smoking) compared to daughters of college-educated mother*s*.

Using covariate adjustment for confounders and mediators, we estimated that each higher level of maternal education is independently associated with a 1 beat-per-minute (bpm) slower (-2.2, -0.2) resting pulse rate, 10% lower (-19%, -0.5%) hsCRP, and 28% lower risk (OR = 0.72, [95 CI: 0.55, 0.94]) of metabolic syndrome in the daughter (*Table 2*).

In MSM analyses, we found each higher level of maternal education had a direct effect of 3 cm decreased (-4.8, -1.6) waist circumference, 1 kg/m^2 decreased (-1.7, -0.2) BMI, 2 bpm slower (-2.7, -0.8) resting pulse rate, 14% lower (-23.55, -2.7%) hsCRP, and 1.3% lower (-1.9%, -0.7%) HbA1c, as well as 28% decreased risk (OR = 0.72, [95% CI: 0.57, 0.90])

of overweight and 40% decreased risk (0.45, 0.80) of metabolic syndrome in the daughter (*Table 3*).

Table 1. Study population characteristics, by maternal educational attainment.

% (N) / mean (SD)	Overall (N = 4,026)	*Maternal educational attainment* < High School Diploma (n = 720)	High School Diploma or GED (n = 2,387)	College Diploma or Higher (n = 919)
Mother				
% White	60.9% (2,434)	38.6% (276)	65.3% (1,550)	66.9% (608)
Age at daughter's birth (years)	25.9 (5.3)	24.5 (6.3)	25.3 (5.1)	28.6 (4.0)
Daughter – Childhood / Adolescent				
Ever neglected before age 18	49.9% (1,991)	50.9% (364)	50.8% (1,202)	46.8% (425)
Ever physically abused before age 18	15.2% (607)	19.7% (140)	15.5% (368)	10.9% (99)
Ever sexually abused before age 18	6.5% (261)	7.5% (53)	7.2% (170)	4.2% (38)
BMI at Wave I (kg/m^2)	22.2 (4.5)	23.2 (4.5)	22.3 (4.6)	21.3 (4.0)
Daughter - Adulthood				
Years of formal education by Wave III	13.4 (1.9)	12.3 (1.7)	13.3 (1.8)	14.4 (1.7)
% High school graduate by Wave III	90.1% (3,625)	78.2% (563)	90.7% (2,163)	97.9% (899)
Average annual household income in Wave III (dollars)*	31,510 (38,220)	28,533 (28,382)	31,418 (38,251)	34,079 (44,237)
% Daily smoker at Wave IV	17.8% (712)	18.9% (135)	20.1% (477)	10.9% (100)
Physical activity events in past week at Wave IV	5.5 (5.3)	5.1 (5.2)	5.4 (5.2)	6.3 (5.4)
Daughter - Adult Cardiometabolic Measures				
Height (cm)	163.7 (7.2)	161.9 (6.9)	163.8 (7.1)	164.9 (7.3)
Weight (kg)	77.9 (22.3)	81.4 (23.9)	78.5 (22.1)	73.5 (20.7)
Waist circumference (cm)	96.6 (18.2)	100.9 (18.7)	97.3 (18.2)	91.6 (16.5)
BMI (kg/m^2)	29.1 (8.1)	31.0 (8.9)	29.2 (7.9)	27.0 (7.4)
Overweight (BMI > 25 kg/m^2)	37.1% (1,475)	47.5% (335)	38.1% (902)	26.3% (238)
Systolic blood pressure (mmHg)	119.8 (12.5)	120.3 (12.8)	120.1 (12.5)	118.6 (12.3)
Diastolic blood pressure (mmHg)	76.8 (9.7)	77.3 (9.8)	76.9 (9.6)	75.9 (9.8)
Mean arterial pressure (mmHg)	91.1 (10.0)	91.6 (10.3)	91.3 (10.0)	90.1 (10.0)
Pulse rate (bpm)	75.9 (11.5)	76.7 (11.4)	76.4 (11.5)	74.0 (11.5)
Pulse pressure (mmHg)	43.0 (7.9)	43.0 (7.7)	43.2 (8.0)	42.6 (7.7)
Median random glucose (mg/dL)	101 (33.2)	100 (33.3)	101 (36.0)	100 (24.1)
Median HbA1c (%)	5.5 (0.76)	5.5 (0.77)	5.5 (0.78)	5.4 (0.56)
Median plasma hsCRP	2.9 (9.4)	4.1 (9.2)	3.0 (9.9)	2.3 (8.0)
Diagnosed high cholesterol / lipids	7.3% (292)	7.9% (57)	7.0% (167)	7.4% (68)
Evidence of metabolic syndrome**	12.3% (497)	17.9% (129)	12.4% (296)	7.8% (72)

*** Average household income is either the combined income of the daughter and her spouse / partner or the daughter and her family, if she lives with parents.**

**** Based on AHA/NCEP guidelines, we classified a woman as having evidence of metabolic syndrome if she has at least three of the following: waist circumference ≥ 88 cm, HbA1c ≥ 5.7%, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, and/or physician diagnosis of high cholesterol.**

Our sensitivity analyses using truncated inverse probability weights (*Table 4*), altered causal ordering of mediators (*Table 5*), discrete categorization of mediators (*Table 6*), an additional indicator of respondent's Wave IV SES (*Table 7*), and missing mediator value replacement (*Table 8*), did not substantially alter the MSM estimates of direct effects.

Table 2. Multivariate regression estimates of maternal education effect on daughter's cardiometabolic risk.

Linear Regression Estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Measures	*β, [95 % Confidence Interval]*	*p-value*
Height (cm)	0.91 [0.42, 1.40]	0.0003***
Weight (kg)	0.83 [-0.64, 2.30]	0.267
Waist circumference (cm)	-1.14 [-2.43, 0.15]	0.083
BMI (kg/m^2)	0.006 [-0.48, 0.49]	0.981
Systolic blood pressure (mmHg)	0.02 [-0.97, 1.01]	0.970
Diastolic blood pressure (mmHg)	-0.06 [-0.82, 0.71]	0.885
Mean arterial pressure (mmHg)	-0.03 [-0.83, 0.77]	0.939
Pulse rate (beats per minute)	-1.19 [-2.17, -0.21]	0.018*
Pulse pressure (mmHg)	0.08 [-0.51, 0.66]	0.801
Log hsCRP *(untransformed hsCRP)*	-0.11 [-0.21, -0.005] (-10.3% [-19.1%, -0.5%])	0.039*
Log HbA1c *(untransformed HbA1c)*	-0.006 [-0.013, 0.001] (-0.6% [-1.3%, 0.1%])	0.100
Log glucose *(untransformed random plasma glucose)*	-0.002 [-0.019, 0.015] (-0.2% [-1.9%, 1.5%])	0.788
Logistic regression estimates ($^{*}p < 0.05$; $^{**}p < 0.005$)		
Measures	*OR, [95 % Confidence Interval]*	*p-value*
Overweight (BMI > 25 kg/m^2) at Wave IV (mean age = 28)	1.06 [0.88, 1.27]	0.532
Evidence of metabolic syndrome†	0.72 [0.55, 0.94]	0.016*

Note: Models are the linear association between increasing maternal education from < high school to high school graduate (or, high school graduate to college graduate) and either continuous outcome (linear regression), or log odds of binary outcome (logistic regression).

Models are adjusted for: Maternal race and age at birth; Daughter's BMI at Wave I (mean age = 15); frequency of childhood neglect, physical or sexual abuse; years of education and household income at Wave III (mean age = 22); number of cigarettes smoked in the past month; and number of physical activity events in the last week.

† Based on AHA/NCEP guidelines, we classified women has having evidence of metabolic syndrome if she has at least three of the following: waist circumference ≥ 88 cm, HbA1c ≥ 5.7%, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, and/or physician diagnosis of high cholesterol.

Table 3. Marginal structural model estimates of maternal education direct effect on various cardiometabolic measures estimated by inverse probability weighting.

Linear Regression Estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*β, [95 % Confidence Interval]*	*p-value*
Height (cm)	1.06 [0.56, 1.55]	4.2×10^{-5}***
Weight (kg)	-1.60 [-3.60, 0.39]	0.115
Waist circumference (cm)	-3.18 [-4.78, -1.57]	0.0001***
BMI (kg/m^2)	-0.95 [-1.69, -0.21]	0.012*
Systolic blood pressure (mmHg)	-0.50 [-1.53, 0.54]	0.344
Diastolic blood pressure (mmHg)	-0.55 [-1.35, 0.24]	0.169
Mean arterial pressure (mmHg)	-0.53 [-1.36, 0.29]	0.204
Pulse rate (beats per minute)	-1.78 [-2.74, -0.81]	0.0004***
Pulse pressure (mmHg)	0.06 [-0.58, 0.70]	0.862
Log hsCRP *(untransformed hsCRP)*	-0.15 [-0.27, -0.028] (-13.8% [-23.5%, -2.8%])	0.016*
Log HbA1c *(untransformed HbA1c)*	-0.013 [-0.019, -0.007] (-1.3% [-1.9%, -0.7%])	0.0001***
Log glucose *(untransformed random plasma glucose)*	-0.006 [-0.021, 0.010] (-0.6% [-2.1%, 1.0%])	0.464
Logistic regression estimates ($^{*}p < 0.05$; $^{**}p < 0.005$)		
Outcome	*OR, [95 % Confidence Interval]*	*p-value*
Overweight (BMI > 25 kg/m^2) at Wave IV (mean age = 28)	0.72 [0.57, 0.90]	0.005**
Evidence of metabolic syndrome†	0.60 [0.45, 0.80]	0.001**

Note: Models are the linear association between increasing maternal education from < high school to high school graduate (or, high school graduate to college graduate) and either continuous outcome (linear regression) or log odds of binary outcome (logistic regression).

The controlled direct effect of maternal education on cardiometabolic outcomes are estimated by adjusting for the following mediators: high childhood maltreatment; adolescent overweight; low adult SES (education and income); and high behavioral risk (high smoking and no physical activity), while weighting the sample using the inverse probability of, jointly: each of these mediators, the primary exposure, and survey sampling.

The mediators are assumed to occur in the order listed above, meaning each mediator only has effects on the mediators subsequent to them. The effect is estimated for a marginal population where each individual has equal probability of exposure and mediators.

† Based on AHA/NCEP guidelines, we classified women has having evidence of metabolic syndrome if she has at least three of the following: waist circumference ≥ 88 cm, HbA1c ≥ 5.7%, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, and/or physician diagnosis of high cholesterol.

Discussion

We found robust evidence that attained maternal education at the time of a daughter's birth has a direct effect on the daughter's adult body size, pulse rate, inflammatory state, glucose control, and risk of metabolic syndrome. While some of these associations were observed

when using conventional covariate adjustment models, evidence for a direct effect of maternal education on daughter's adult cardiometabolic measures was uniformly stronger when estimated by MSM, which is more robust to unmeasured mediator-outcome confounding.[16] Moreover, the qualitative interpretation of estimates from the MSM approach did not change under any of our sensitivity analyses for modeling assumptions and missing data.

A number of studies have investigated associations between childhood socioeconomic status (SES) and adult cardiometabolic outcomes.[11, 18, 38] A systematic review by Tamayo, *et al.* found some evidence for an independent association between parental SES, including parental education, and type 2 diabetes and obesity among adult offspring.[11] The authors also identified childhood neglect and obesity and adult SES and health behaviors as important mediators.[11] However, various investigators have highlighted significant weaknesses in the overall body of evidence due to the substantial heterogeneity in choice of exposure measures,[11, 18] inconsistencies in accounting for life course psychosocial and adult SES measures,[11, 18] and reliance on conventional regression methods[18, 38] which are particularly vulnerable to unmeasured and endogenous confounding. For example, Bouhanick, *et al*. found offspring from the British National Child Development Study whose mothers had not completed compulsory education (16 years) by the time of the child's birth had a 47% (OR = 1.47 [95% CI: 1.30 to 1.67]) higher risk of metabolic syndrome at age 45 after adjusting for maternal antenatal characteristics.[8] However, Kvaavik, *et al*. found no association between maternal education (categories: elementary, high school, high/comprehensive school, some college/university, completed college/university), ascertained approximately 13 years postpartum (*i.e.* and not at the time of birth), and offspring body mass index, lipids, or blood pressure at 15, 25, or 40 years in a Norwegian cohort, with or without adjustment for offspring characteristics.[9] Our study improves on past work by focusing on maternal education at daughter's birth as the exposure of interest,

explicitly incorporating salient mediators identified in past literature, and implementing MSM and associated sensitivity analyses.

Maternal education at offspring birth may be a particularly important determinant of offspring cardiometabolic risk because of the association between lower maternal education attainment and exposure to greater stress during pregnancy.[7, 30] Lower maternal educational attainment may increase fetal exposure to cortisol[3, 41] and, in turn, this exposure to excess glucocorticoids may program poor life-time metabolic control and stress response in the offspring.[3, 4, 6] One recent study found lower maternal education to be associated with increased placental expression of glucocorticoid-related genes.[41] In related work, we found maternal education at offspring's birth to be related to glucocorticoid-related gene methylation in young adult women (Huang, *et al.*; *manuscript in progress*). Therefore, maternal educational attainment prior to offspring birth may a better indicator that paternal occupation or educational attainment. We utilized a measure of maternal education that was truncated based on her age at daughter's birth. In our analytic population, this affected 3% of mothers, limiting their educational attainment to either high school graduate or less than high school. In fact, when we did not perform this truncation, therefore utilizing maternal education as a measure of childhood (rather than perinatal) SES, our estimates of effect were slightly reduced (*results not shown*).

Another strength of our study was the inclusion of childhood maltreatment, prospectively collected adolescent BMI, adult SES, and adult smoking and physical activity, in our models. These are identified mediators of early life SES-adult cardiometabolic risk associations.[11] Moreover, we account for these mediators by employing a causal framework and MSM estimation by IPW. Prior studies employing traditional covariate adjustment for mediators may have produced biased estimates due to collider stratification: Using our proposed causal diagram as an example (*Figure 1*), the common practice of adjusting for adolescent overweight[11] would bias the association between maternal education and child

maltreatment, and consequently bias estimates of the direct effect of maternal education, even if child maltreatment is additionally adjusted for.[16, 39] Indeed, we found effect estimates to be smaller, and possibly negligible for waist circumference and HbA1c, when using the "traditional approach" of simple covariate-adjustment of our mediators (*Table 2*). Similarly, Nandi, *et al.* found a direct effect of childhood SES on risk of cardiovascular disease and diabetes was only observed using MSM but not when using covariate adjustment.[18] Our study extends this work to other identified mediators, suggesting that their observed direct effects on cardiovascular disease may be explain in part by risk factor development in early adulthood.

Two other strengths are worth noting: First, despite our study population exclusions, mothers in our study were similar to the distribution of race and educational attainment of all U.S. adult women in 1994-5 as estimated by the U.S. Census Bureau.[36, 37] This suggests that effects estimated may also be generalizable to the cohort of U.S. female offspring aging into young adulthood in 2008-9. Additionally, our work corroborates past work in the Add Health population which found an association between higher parental education (highest degree or certificate between mother and father) and a 5% lower C-reactive protein concentration among white women.[40] We extend this previous study by investigating maternal education at the time of daughter's birth, better characterizing exposure at a critical period of development, and including salient mediators within a causal framework.

Potential limitations to our study deserve mention. First, our current study is based on complete case analyses. While there was relatively complete data on covariates, we were missing survey weights on a substantial proportion (23.4%) of our potential study population. To the extent that individuals who were lost to follow-up by Wave IV may differ from our available population, our estimates may be biased. However, individuals missing weights did not differ substantially from our analytic population with regards to maternal education, daughter's mediators, or outcome measures (*data not shown*). Additionally, 187

(4.6%) subjects were missing Wave III adult SES measures. However, our data replacement techniques (*Table 8*) showed replacing all mediator values with either extreme (*i.e.* indicator of risk or protection) did not substantially alter results. However, we cannot exclude the possibility that some other pattern of missing-ness may alter our interpretations. Additionally, it is possible a combination of one or more unmeasured confounders may fully explain our findings. Initially we had intended to perform a quantitative bias analysis for this possibility, in line with related work regarding pregnancy outcomes (Huang, *et al.*; manuscript in progress). However, due to the strengths of associations found, the bias analysis was judged to be unhelpful: For example, to fully explain the observed reduction in waist circumference, a confounder(s) would have to be unbalanced by more than 30% across educational levels and cause a 5 centimeter change. Nonetheless, we explored the possibility that several other potential confounders available from the data, including maternal report of breastfeeding duration and respondent's age, might explain our findings. However, neither variable changed estimates for any of our models.

Finally, we may have introduced residual confounding in our MSM estimates due to use of factor scores and dichotomized mediators. To verify that our results were not simply due to the parameterization of our mediators, we ran unweighted regression models adjusted for confounders and dichotomized mediators and found results similar to our conventionally adjusted models. Also, similar findings from MSM using discrete mediators (*Table 6*) and adding a Wave IV adult SES mediator (*Table 7*) reduces the likelihood that residual confounding may explain our findings.

In summary, we incorporated existing knowledge about child- and adulthood socioeconomic and biological determinants of cardiometabolic risk to conduct a causal framework-based investigation of maternal education at offspring birth, an indicator of perinatal exposure to SES-related stressors, and cardiometabolic risk in young adult women. We provide evidence

for the direct effect of higher maternal education on thinness, reduced inflammatory markers, better glucose control, and lower overall risk of metabolic syndrome, independent of important mediating factors: childhood maltreatment, adolescent overweight, adult SES, and smoking and physical activity. Future empirical work should also consider the relevant causal components of education, improve measurement of other life course variables, and consider additional causal estimation methods that can relax more modeling assumptions.

More broadly, this study contributes to the empirical evidence that interventions to prevent socioeconomic disparities must consider the early life and correspondingly, ascertaining the effect of such interventions may require measuring outcomes across multiple generations. Our findings suggest traditional covariate adjustment may mask early life SES-adult disease associations due to causal relationships between risk factors across the life course. The findings of this study and others employing MSM to estimate effects of early life SES on adult health suggest causal frameworks should be incorporated explicitly in future similar investigations.

Additional Tables for Sensitivity Analyses

Table 4. Marginal structural model estimates of maternal education direct effect on various cardiometabolic measures estimated by inverse probability weighting – Weights truncated at the 5th and 95th percentiles.

Linear Regression Estimates (*p < 0.05; **p < 0.005; ***p < 0.0005)		
Outcome	*β, [95 % Confidence Interval]*	*p-value*
Height (cm)	1.14 [0.69, 1.59]	$1.6 \times 10^{-6***}$
Weight (kg)	-1.84 [-3.47, -0.20]	0.028^{*}
Waist circumference (cm)	-3.16 [-4.49, -1.84]	$6.1 \times 10^{-6***}$
BMI (kg/m^2)	-1.06 [-1.67, -0.45]	0.001^{**}
Systolic blood pressure (mmHg)	-0.54 [-1.48, 0.40]	0.260
Diastolic blood pressure (mmHg)	-0.58 [-1.34, 0.18]	0.135
Mean arterial pressure (mmHg)	-0.57 [-1.34, 0.21]	0.153
Pulse rate (beats per minute)	-1.77 [-2.72, -0.82]	0.0003^{***}
Pulse pressure (mmHg)	-0.04 [-0.54, 0.63]	0.887
Log hsCRP	-0.16 [-0.27, -0.05]	0.004^{**}
(untransformed hsCRP)	(-14.9% [-23.6%, -5.2%])	
Log HbA1c	-0.013 [-0.019, -0.007]	$3.3 \times 10^{-5***}$
(untransformed HbA1c)	(-1.3% [-1.9%, -0.7%])	
Log glucose	-0.004 [-0.019, 0.010]	0.538
(untransformed random plasma glucose)	(-0.4% [-1.9%, 1.0%])	
Logistic regression estimates (*p < 0.05; **p < 0.005; ***p < 0.0005)		
Outcome	*OR, [95 % Confidence Interval]*	*p-value*
Overweight (BMI > 25 kg/m^2) at Wave IV (mean age = 28)	0.72 [0.59, 0.88]	0.001^{**}
*Evidence of metabolic syndrome†† *	0.64 [0.48, 0.85]	0.002^{**}

Note: Models are the linear association between increasing maternal education from < high school to high school graduate (or, high school graduate to college graduate) and either continuous outcome (linear regression) or log odds of binary outcome (logistic regression).

The controlled direct effect of maternal education on cardiometabolic outcomes are estimated by adjusting for the following mediators: high childhood maltreatment; adolescent overweight; low adult SES (education and income); and high behavioral risk (high smoking and no physical activity), while weighting the sample using the inverse probability of, jointly: each of these mediators, the primary exposure, and survey sampling.

The mediators are assumed to occur in the order listed above, meaning each mediator only has effects on the mediators subsequent to them. The effect is estimated for a marginal population where each individual has equal probability of exposure and mediators. This model was estimated by truncating the estimated weights at the 5th and 95th percentile to limit influential individuals at the extremes.

† Based on AHA/NCEP guidelines, we classified women has having evidence of metabolic syndrome if she has at least three of the following: waist circumference ≥ 88 cm, HbA1c ≥ 5.7%, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, and/or physician diagnosis of high cholesterol.

Table 5. Marginal structural model estimates of maternal education direct effect on various cardiometabolic measures estimated by inverse probability weighting – Presumed causal ordering changed.

Linear Regression Estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*β, [95 % Confidence Interval]*	*p-value*
Height (cm)	1.06 [0.57, 1.55]	$3.9 \times 10^{-5***}$
Weight (kg)	-1.62 [-3.61, -0.36]	0.107
Waist circumference (cm)	-3.20 [-4.79, -1.61]	0.0001^{***}
BMI (kg/m^2)	-0.96 [-1.69, -0.23]	0.011^{*}
Systolic blood pressure (mmHg)	-0.50 [-1.53, 0.53]	0.337
Diastolic blood pressure (mmHg)	-0.55 [-1.34, 0.24]	0.168
Mean arterial pressure (mmHg)	-0.54 [-1.36, 0.29]	0.201
Pulse rate (beats per minute)	-1.78 [-2.74, -0.81]	0.0004^{***}
Pulse pressure (mmHg)	0.05 [-0.58, 0.69]	0.872
Log hsCRP *(untransformed hsCRP)*	-0.15 [-0.27, -0.03] (-14.0% [-23.7%, -3.0%])	0.014^{*}
Log HbA1c *(untransformed HbA1c)*	-0.012 [-0.019, -0.007] (-1.3% [-1.9%, -0.7%])	0.0001^{***}
Log glucose *(untransformed random plasma glucose)*	-0.006 [-0.021, 0.010] (-0.6% [-2.1%, 1.0%])	0.464
Logistic regression estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*OR, [95 % Confidence Interval]*	*p-value*
Overweight (BMI > 25 kg/m^2) at Wave IV (mean age = 28)	0.72 [0.57, 0.90]	0.004^{**}
Evidence of metabolic syndrome†	0.60 [0.45, 0.91]	0.001^{**}

Note: Models are the linear association between increasing maternal education from < high school to high school graduate (or, high school graduate to college graduate) and either continuous outcome (linear regression) or log odds of binary outcome (logistic regression).

The controlled direct effect of maternal education on cardiometabolic outcomes are estimated by adjusting for the following mediators: adolescent overweight; high childhood maltreatment; high behavioral risk (high smoking and no physical activity); and low adult SES (education and income), while weighting the sample using the inverse probability of, jointly: each of these mediators, the primary exposure, and survey sampling.

The mediators are assumed to occur in the order listed above, meaning each mediator only has effects on the mediators subsequent to them. As shown by the list, this model differs from Tables 3 and 4 in that the presumed order of the two childhood mediators (maltreatment and overweight) and the two adulthood mediators (SES and risk factors) are both swapped.

† Based on AHA/NCEP guidelines, we classified women has having evidence of metabolic syndrome if she has at least three of the following: waist circumference ≥ 88 cm, HbA1c ≥ 5.7%, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, and/or physician diagnosis of high cholesterol.

Table 6. Marginal structural model estimates of maternal education direct effect on various cardiometabolic measures estimated by inverse probability weighting – Discrete mediators.

Linear Regression Estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*β, [95 % Confidence Interval]*	*p-value*
Height (cm)	0.99 [0.51, 1.48]	0.0001***
Weight (kg)	-1.25 [-3.21, 0.70]	0.206
Waist circumference (cm)	-2.48 [-3.94, -1.02]	0.001**
BMI (kg/m^2)	-0.81 [-1.52, -0.11]	0.023*
Systolic blood pressure (mmHg)	-0.30 [-1.28, 0.67]	0.538
Diastolic blood pressure (mmHg)	-0.36 [-1.10, 0.38]	0.338
Mean arterial pressure (mmHg)	-0.34 [-1.11, 0.42]	0.379
Pulse rate (beats per minute)	-1.84 [-2.93, -0.75]	0.001**
Pulse pressure (mmHg)	0.06 [-0.60, 0.71]	0.864
Log hsCRP *(untransformed hsCRP)*	-0.16 [-0.29, -0.024] (-14.5% [-25.0%, -2.4%])	0.021*
Log HbA1c *(untransformed HbA1c)*	-0.013 [-0.021, -0.005] (-1.3% [-2.1%, -0.5%])	0.002*
Log glucose *(untransformed random plasma glucose)*	-0.004 [-0.022, 0.014] (-0.4% [-2.2%, 1.4%])	0.659
Logistic regression estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*OR, [95 % Confidence Interval]*	*p-value*
Overweight (BMI > 25 kg/m^2) at Wave IV	0.72 [0.59, 0.88]	0.002**
Evidence of metabolic syndrome	0.62 [0.48, 0.80]	0.0003***

Note: This model is similar to that from Table 3 with the exception that values for binary mediators are redefined as follows: High childhood maltreatment = any episodes of neglect, physical, or sexual abuse; High adolescent BMI = ≥ 25 kg/m^2; low early adult SES = high school education or less; and high adult risk factors = any self-reported cigarette smoking and no physical activity. Thus, no factor scores were used.

Table 7. Marginal structural model estimates of maternal education direct effect on various cardiometabolic measures estimated by inverse probability weighting – Add Low Wave IV SES as a mediator.

Linear Regression Estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*β, [95 % Confidence Interval]*	*p-value*
Height (cm)	0.88 [0.35, 1.41]	0.001**
Weight (kg)	-1.27 [-3.55, 1.01]	0.274
Waist circumference (cm)	-2.80 [-4.72, -0.89]	0.004**
BMI (kg/m^2)	-0.76 [-1.61, -0.08]	0.076
Systolic blood pressure (mmHg)	-0.19 [-1.30, 0.91]	0.732
Diastolic blood pressure (mmHg)	-0.35 [-1.21, 0.51]	0.417
Mean arterial pressure (mmHg)	-0.30 [-1.19, 0.59]	0.508
Pulse rate (beats per minute)	-1.76 [-2.77, -0.76]	0.001**
Pulse pressure (mmHg)	0.16 [-0.52, 0.84]	0.640
Log hsCRP *(untransformed hsCRP)*	-0.13 [-0.27, 0.01] (-12.1% [-23.6%, 1.0%])	0.069
Log HbA1c *(untransformed HbA1c)*	-0.012 [-0.021, -0.004] (-1.2% [-2.1%, -0.4%])	0.005*
Log glucose *(untransformed random plasma glucose)*	-0.014 [-0.031, 0.004] (-1.4% [-3.1%, 0.4%])	0.131
Logistic regression estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*OR, [95 % Confidence Interval]*	*p-value*
Overweight (BMI > 25 kg/m^2) at Wave IV	0.78 [0.58, 1.05]	0.104
Evidence of metabolic syndrome	0.72 [0.52, 1.01]	0.057

Note: This model is similar to that from Table 3 with the exception low SES at Wave IV (mean age = 28) was included as an additional mediator, causally situated subsequent to low SES at Wave III but prior to Wave IV risk. SES at Wave IV was estimated by principle components factor analysis of last school completed, total household income, and a self-report of whether an individual would "still be in debt," "break even," or "have some left over" if they sold all their assets and used all savings to pay off debts, all at time of Wave IV interview. The predicted factor score was then dichotomized at the median with lower than median scores considered low SES.

Table 8. Marginal structural model estimates of maternal education direct effect on various cardiometabolic measures estimated by inverse probability weighting – Missing mediator values replaced.

Linear Regression Estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*β, [95 % Confidence Interval]*	*p-value*
Height (cm)	1.02 [0.53, 1.50]	0.0001^{***}
Weight (kg)	-1.84 [-3.79, 0.11]	0.064
Waist circumference (cm)	-3.23 [-4.79, -1.67]	0.0001^{***}
BMI (kg/m^2)	-1.02 [-1.73, -0.32]	0.005^{**}
Systolic blood pressure (mmHg)	-0.58 [-1.54, 0.37]	0.230
Diastolic blood pressure (mmHg)	-0.60 [-1.37, 0.17]	0.123
Mean arterial pressure (mmHg)	-0.60 [-1.38, 0.19]	0.136
Pulse rate (beats per minute)	-1.57 [-2.49, -0.66]	0.001^{**}
Pulse pressure (mmHg)	0.02 [-0.58, 0.62]	0.955
Log hsCRP *(untransformed hsCRP)*	-0.11 [-0.23, 0.01] (-10.1% [-20.2%, 1.2%])	0.076
Log HbA1c *(untransformed HbA1c)*	-0.013 [-0.019, -0.007] (-1.3% [-1.9%, -0.7%])	$3.4 \times 10^{-5***}$
Log glucose *(untransformed random plasma glucose)*	-0.002 [-0.017, 0.012] (-0.2% [-1.7%, 1.2%])	0.737
Logistic regression estimates ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)		
Outcome	*OR, [95 % Confidence Interval]*	*p-value*
Overweight (BMI > 25 kg/m^2) at Wave IV (mean age = 28)	0.73 [0.60, 0.88]	0.001^{**}
Evidence of metabolic syndrome††	0.61 [0.47, 0.80]	0.0003^{***}

Note: This model is identical to that from Table 3 with the except that missing values for the mediators high childhood maltreatment, high childhood BMI, low adult SES, and high adult risk factors were assumed to an indicator of risk. For example, if a subject was missing a value for high childhood BMI, this model assumes the individual did indeed have a BMI ≥ 25 kg/m^2.

(*End Chapter 1*)

Chapter 2 – Direct effect of grandmaternal educational attainment on birth weight and associated bias analyses

Abstract

Background: Distinguishing early life determinants of offspring low birth weight from more proximal determinants (*i.e.* life course characteristics) has been challenging. We examined the direct effect of grandmother's (G0) education at the time of mother's (G1) birth on offspring (G2) birth weight (BW).

Methods: Using the National Longitudinal Study of Adolescent Health (1995-2009), we identified G1 and their first, live singleton (G2) (N = 1,681 pairs). G0 education was defined as less than high school (HS), HS diploma or equivalent, or college degree at G1 birth. We fit a structural equation model (SEM) incorporating G1 mediators: childhood maltreatment, pre-pregnancy overweight, low adult education and income, and prenatal smoking. Using marginal structural models (MSM), we estimated the controlled direct effect of G0 education on G2 BW independent of these mediators. We evaluated sensitivity to alternative models incorporating pre-term birth, modified weights, and data replacement. We used quantitative bias analysis to examine unmeasured confounding of the direct effect.

Results: Mean BW (G2) was 3,259 grams and increased across G0 education (3,171; 3,267; and 3,339 grams, respectively). SEM estimated 54-grams increased G2 BW per higher level of G0 education (95% Confidence Interval: -14.0, 122.1), while MSM estimated 89-grams increased G2 BW (95% CI: 20.8, 156.5) in a marginal population. This estimate did not change substantively under sensitivity scenarios. Quantitative bias analysis suggested a single, unmeasured confounder would have to differ by more than 15% between exposure groups and affect BW by more than 150 grams to completely explain findings.

Conclusions: Our models suggest a direct effect of grandmaternal education on grandchild birth weight. This effect is robust to some model misspecification and unmeasured confounding.

Background

Early life socioeconomic differences may contribute to a woman's risk of low birth weight delivery, despite adult health[1] and socioeconomic attainment.[2] According to Developmental Origins of Health and Disease (DOHaD) theory, exposures occurring *in utero*, during which organogenesis and epigenetic programming occur, are potential determinants of adult metabolic capacity.[3, 4] Lower maternal education during the perinatal period, an important indicator of low socioeconomic status and related stressors, may adversely affect intrauterine fetal development and programming of daughters[5, 6] who may, in turn be at risk of adverse reproductive outcomes, including low offspring birth weight.[6-10] However, the effects of grandmaternal education on the health and social trajectory of the mother may also explain birth outcome differences[11, 12] and evidence of direct effects are inconsistent.[10, 13] One possible explanation is the lack of appropriate causal methods in past investigations of grandmaternal education and birth weight.[14]

Incorporating perinatal exposures in life course investigations of adult health and disease outcomes is now well recognized.[14] However, accounting for the complex causal structure between exposures with numerous mediators to outcomes remains challenging[14, 15]: Standard covariate-adjusted regression models introduce collider stratification bias in the presence of endogenous confounding and unmeasured confounders.[15, 16] Moreover, developmental programming effect may produce heterogeneity in the response to mediators,[17] suggesting violations of the consistency assumption when conditioning on them. Structural equation models (SEMs) have been favored to explicitly model complex, socio-biological causal structures,[18] however they are limited by strong assumptions regarding both modeled and un-modeled relationships between covariates.[19] Alternatively, marginal structural models (MSMs) estimated by inverse probability weighting may be used to obtain controlled direct effects of early life exposures.[15,19,20] MSMs are superior to standard regression in addressing mediators and endogenous confounding[21] and superior to

SEMs in requiring fewer assumptions about relationships amongst covariates.[15] Nonetheless, MSM are still susceptible to unmeasured confounding and assumptions about causal structure.[22-24] Consequently, sensitivity and quantitative bias analyses are still indicated to strengthen inference.[22, 24]

This study aims to examine whether grandmother's (G0) education at the time of the mother's (G1) birth, has a direct effect on offspring birth weight (G2), using data from the National Longitudinal Study of Adolescent Health (Add Health). This study improves on past work in four ways: (1) prior life-course, social and biological determinants of birth weight are incorporated explicitly in a causal mediation framework, (2) effect estimates are compared to standard multivariate regression and SEM estimates, (3) sensitivity analyses are performed to test robustness to weight and model misspecification, and (4) quantitative bias analysis is implemented to estimate robustness to unmeasured confounding under simplifying assumptions.

Methods

Study Design

The National Longitudinal Study of Adolescent Health (Add Health) is a nationally-representative, longitudinal study of 7-12 grade United States adolescents begun in 1994 (N = 90,118). In-home interviews were conducted with a core subset of respondents (randomly selected within school and sex strata) along with a parent, during which extensive information were collected on demographics, health, attitudes, behaviors, and environment. Additional respondents (and parents) were interviewed based on ethnic minority status, having siblings in the study, and/or being black with at least one college-educated parent (N = 20,745 individuals). Respondents first interviewed in Wave I were followed for up to three additional in-home interviews in 1996 (Wave II), 2001-2002 (Wave III), and 2007-2008 (Wave IV). Anthropometric measurements (Wave III) and capillary whole blood (Wave IV) were also collected. During Wave IV, 15,701 respondents (80.3% of eligible Wave I) were interviewed.

Investigators calculated a grand sampling weight for respondents interviewed in all four waves to approximate the target population of U.S. adolescents in grades 7-11 in 1994-5.[25] This weight accounts for clustered sampling, attrition, and oversampling in longitudinal analyses.[25] Additional information on respondent characteristics, sampling frame, and protocols can be found on the Add Health site: www.cpc.unc.edu/projects/addhealth/. All data used for the current study were obtained through a restricted data access agreement between the University of Washington Center for Studies in Demography and Ecology (CSDE) and the Inter-university Consortium for Political and Social Research (ICPSR) at the University of Michigan. Research on these data has been approved by the Institutional Review Board of the University of Washington.

Study Population

Individuals (G1) were eligible for this current study if they were women, had a biological mother (G0) interviewed during Wave I, participated in both Wave I and IV interviews, and had at least one live birth during the course of the study. Since first born offspring are hypothesized to be more susceptible to adverse *in utero* programming from maternal constraint,[26] we matched respondents to their first reported singleton birth (G2). There were 2,352 women who met eligibility criteria. We excluded 60 (2.6%) respondents without a recorded exposure status and 428 (18%) individuals missing a Wave IV grand sampling weight. Consequently, we included 1,876 respondents (G1) and their matched births (G2) for the current study.

Measures

Primary Exposure and Outcome – During Wave I (1994-5), a respondent's mother (G0) was asked "How far did you go in school?" with responses we coded into three categories: less than high school diploma; high school diploma or equivalency (*i.e.* GED) certificate, and completed college degree. Because we were interested in G0 education at or prior to G1 birth, we capped completed education to high school diploma if she gave birth at or prior to 21 years (n = 86). G2 birth weight (grams) was reported by G1 during Wave IV interview. When compared to birth records, maternal recall of child birth weight has previously been found to be fairly accurate.[10]

Psychosocial stress mediators – Childhood maltreatment[27, 28] and adult socioeconomic status[29] are important psychosocial stressors in the respondent's (G1) life course that may be in the causal pathway between G0 education and G2 birth weight. In line with previous investigations in Add Health,[28] we use self-reported incidents of neglect, physical abuse, and sexual abuse prior to 18 years of age to predict a maltreatment factor score. Factors scores were generated using principle component factor analysis and quartimin rotations.[30, 31] Adult socioeconomic status (SES) closest to the time of G2 birth was measured using three variables: (1) self-reported total household income during Wave IV reported in dollars or, if

the woman could not provide an exact amount, one the following approximates: $5,000; $12,500; $17,500; $25,000; $35,000; $45,000; $62,500; or $100,000, based on the mid-point of categorical choices (capped at $100,000); (2) attained education categorized by last school type completed; and (3) an indicator of whether G1 reported she would be in debt in response to the question "Suppose you and others in your household were to sell all of your major possessions (including your home), turn all of your investments and other assets into cash, and pay off all of your debts. Would you have something left over, break even, or be in debt?" Using these three measures, a factor score for adult SES was predicted through principle component factor analysis and quartimin rotations. If the respondent gave birth to G2 prior to 2001, a Wave III factor score was calculated instead excluding the 3rd (debt) measure, which was not asked in Wave III. Correlations between the two adult SES factor scores was moderate ($r = 0.578$; $n = 1,665$). For the purposes of weighting, maltreatment and adult SES factor scores were dichotomized at their median to high/low categories.

Biological mediators – Higher pre-pregnancy BMI is consistently associated with maternal education and higher birth weights.[32, 33] G1 pre-pregnancy BMI was calculated from either: Wave II self-reported height and weight if she gave birth at or prior to 2001, or study staff-measured height and weight from Wave III, otherwise. Correlation between the two BMI measures was high (unadjusted Pearson's $r = 0.76$; $p < 0.0001$). Pre-pregnancy overweight was categorized as a BMI ≥ 25 kg/m^2. Prenatal smoking is associated with maternal education and lower birth weight.[32-34] We coded it as any or none based on self-reported smoking during the index pregnancy.

Confounders – Grandmother's (G0) age at mother's (G1) birth is related to grandmother's educational attainment and may influence childrearing practices and therefore life-course psychosocial and biological stressors experienced by the mother (G1).[35, 36] Similarly, G0 self-reported race is related to G0 educational opportunity and attainment, as well as social

and biological stressors experienced by G1 throughout her life course.[37,38] G0 race was coded as non-Hispanic white, Hispanic white, black any ethnicity, or other non-white, with those reporting any mixed race not including black being accorded the last category. Both G0 age at G1 birth and G0 self-reported race were included as confounders in all analyses.

Statistical Analysis

We first examined univariate demographic characteristics of the study population accounting for survey design. Next, we examined the possibility that *in utero* SES may affect future pregnancy outcome by estimating the direct effect of grandmaternal (G0) education (X_i) on the birth weight (Y_i) of her daughter's (G1) child (G2) that is not mediated by the daughter's (G1) life experiences using three modeling approaches: A multivariate-adjusted linear regression model including measured predictors of G2 birth weight (Approach 1), a linear structural equation model (SEM) using dichotomous mediators (Approach 2), and a linear marginal structural model (MSM) estimated by inverse probability weights again using dichotomous mediators (Approach 3). All data processing and analysis were conducted in STATA 12.1 MP (College Station, TX).

Hypothesized causal structure - In each model we attempted to estimate the controlled direct effect of X_i on Y_i after accounting for confounding by G0 age at G1 birth (G_i) and G0 self-reported race (R_i), as well as mediation / endogenous confounding by G1 life-course psychosocial and biological stressors: high childhood maltreatment prior to 18 years (M_i), pre-pregnancy overweight (O_i), low adult SES (A_i), and any prenatal smoking (S_i). The relationships between exposure, mediators, confounders, and outcome are constrained only by temporality (*Figure 1*): G0 age at G1 birth and self-reported race precede all other factors, including G0 education at G1 birth. Each G1 mediator is hypothesized to also affect all subsequent mediators (*e.g.* child maltreatment affects pre-pregnancy overweight, adult SES, and prenatal smoking).

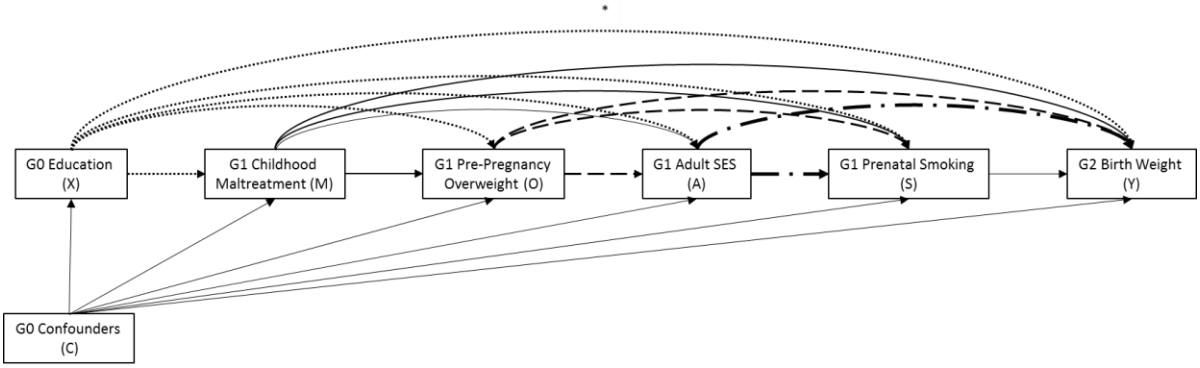

Figure 1. Causal diagram of hypothesized life course determinants of birth weight. Each exposure and mediator is assumed to have an effect on all other mediators that occur subsequent to it. Directed edges are drawn with different patterns based on their source for visual effect only; they do not reflect any addition knowledge or specification about relationships. The asterisk (*) indicates the effect of interest: the direct effect of G0 education on G2 birth weight. Confounders (C) represent a vector including G0 age at G1 birth (G) and G0 race (R).

Approach 1: Multivariate linear regression – We first estimated direct effects conventionally by fitting the following multivariate-adjusted linear regression model in which the primary exposure, confounders, and mediators are included as predictors of G2 birth weight:

$$E[Y_i \mid X_i = x, R_i = r, G_i = g, M_i = m, O_i = o, A_i = a, S_i = s] = \quad (1)$$

$$\beta_0 + \beta_1 X + \beta_2 R + \beta_3 G + \beta_4 M + \beta_5 O + \beta_6 A + \beta_7 S$$

Under strong assumptions of no model misspecification, no interaction, and no unmeasured confounding,[15] it is possible for (1) to give an unbiased estimate of the causal direct effect of G0 education on G2 birth weight as well as the causal direct effects of mediators. However, our model (*Figure 1*) implies mediator adjustment induces confounding through the mediator's parents (*i.e.* collider stratification bias). We use Approach 1 as a baseline to explore effect estimates using different covariate functional forms and incorporate multiple imputation for missing data.

Approach 2: SEM – We next estimated direct and indirect effects of G0 education by fitting an SEM of our hypothesized causal structure using dichotomous forms of our mediators (*Figure 2*). Like Approach 1, SEM makes strong assumptions of no model misspecification

and no unmeasured confounding with additional assumptions about covariate relationships.[19] We use Approach 2 to explore qualitative verification of our hypothesized causal structure and serve as a reference for sensitivity analyses.

Approach 3: MSM estimated by inverse probability weighting – Finally, we tested the controlled direct effect of G0 education (X_i) on G2 birth weight (Y_i) by fitting the following regression model:

$$E[Y_i \mid X_i = x, M_i = m, O_i = o, A_i = a, S_i = s] = \beta_0 + \beta_1 X + \beta_2 M + \beta_3 O + \beta_4 A + \beta_5 S \quad (2)$$

weighting individual subjects by the inverse probability of their exposure to their given strata of G0 education and probability of exposure to the four dichotomous mediators. No interaction terms were included, as there was not consistent evidence of additive interaction between each of the mediators and the exposure (*Supplemental Table*). Stabilized weights[39] for G0 education (w_i^X) were estimated by multinomial logistic regression to predict the probability for a given strata of G0 education given a vector of *C* confounders. Stabilized weights for mediators were calculated using logistic regression, giving regard to temporality (*Figure 1*):

G0 education: $$w_i^X = \frac{P(X = x_i)}{P(X = x_i \mid C = c_i)} \quad (3)$$

Childhood maltreatment: $$w_i^M = \frac{P(M = m_i \mid C = c_i)}{P(M = m_i \mid X = x_i,\ C = c_i)} \quad (4)$$

Pre-pregnancy overweight: $$w_i^O = \frac{P(O = o_i \mid C = c_i)}{P(O = o_i \mid X = x_i,\ M = m_i,\ C = c_i)} \quad (5)$$

Adult SES: $$w_i^A = \frac{P(A = a_i \mid C = c_i)}{P(A = a_i \mid X = x_i,\ M = m_i,\ O = o_i,\ C = c_i)} \quad (6)$$

Prenatal smoking: $$w_i^S = \frac{P(S = s_i \mid C = c_i)}{P(S = s_i \mid X = x_i,\ M = m_i,\ O = o_i,\ A = a_i,\ C = c_i)} \quad (7)$$

A correctly specified MSM gives the controlled direct effect of *X* on *Y* in a marginal population in which, potentially counter to the fact, all individuals are "unexposed" to the

mediators. In our model, this is a marginal G1 population at lower risk of childhood maltreatment, pre-pregnancy overweight, low adult SES, and any prenatal smoking.

Incorporating survey design – To account for sampling dependency introduced by the survey design and produce correct standard errors,[25] each weight was estimated with the *svy* option after using the Wave IV grand sampling weights, clustering, and stratification variables for the *svyset* command. An overall weight ($W_{overall}$) was then generated for each subject using the product of the stabilized weights and the Wave IV grand sampling weight (w_i^{gsw}):

Overall Weight: $$W_{overall} = w_i^X * w_i^M * w_i^O * w_i^A * w_i^S * w_i^{gsw} \quad (8)$$

The *svy* option was subsequently used to fit model (2) using $W_{overall}$ for the *svyset* command. An analogous method was described by Brumback, *et al*. employing *PROC SURVEYREG* in SAS.[40]

Sensitivity Analyses

Like other causal modeling methods, MSM effect estimations are subject to biases from misspecifed causal models, mismeasurement, and unmeasured confounding.[11, 41] We addressed this through several sensitivity analyses: Specifically, we examine qualitative robustness of estimates to altered model specification including mediation by pre-term birth, data replacement techniques, and quantitative bias analysis informed by SEM.

Model specification – Pre-term birth is an important mediator of low birth weight and may be of great causal interest.[42, 43] Consequently, we re-fit our model adding an indicator for pre-term birth (< 37 weeks) and a corresponding probability weight to model (2). Additionally, we examined sensitivity of model (2) to: truncating weights at the 1st / 99th, 5th / 95th, and 10th / 90th percentiles; including interaction terms in estimating weights (3) - (7); and reversing the presumed temporal relationship of adult SES and pre-pregnancy

overweight by adding adult SES to the denominator of (5) and removing pre-pregnancy overweight from the denominator of (6).

Data replacement – Out of our total sample of 1,876, we had missing observations of pre-pregnancy overweight and low adult SES for 78 (4.2%), maltreatment scores for 28 (1.5%), prenatal smoking for 3 (0.16%) women. Subjects missing mediator values did not differ systematically from those with complete data. To analyze the sensitivity of our analysis to covariate values missing at random conditional on available data, we re-fit model (1) using multiple imputation by chained equations to generate ten sets of possible values for missing binary mediators. For model (2), sensitivity to missingness was assessed by replacing missing values for G1 mediators and reweighting. Data replacement was conducted in three ways: first, all missing values were assumed to be indicative of risk (*i.e.* missing value for adult SES replaced with indicator of low adult SES); second, missing values of Wave III pre-pregnancy overweight and Wave IV adult SES were replaced with their values from Waves II and III, respectively; and third, all values for these two mediators were replaced with their values from Waves II and III, respectively.

Quantitative Bias Analyses – The no unmeasured mediator-outcome confounding assumption is critical to an unbiased estimate of controlled direct effect.[12] To assess the robustness of model (2) to unmeasured confounding, we simulated the effects of a hypothetical, binary, mediator-outcome confounder.[44-46] Assuming an unmeasured binary confounder *U* with no effect modification by G0 education, we specified ranges for two sensitivity parameters *g* and *d* with parameter *g* corresponding to the effect of *U* on G2 birth weight (*i.e.* change in birth weight grams) and *d* to the prevalence difference of confounder *U* between levels of exposure. By subtracting each combination of *g x d* from the β_1 coefficient and its 95% confidence limits estimated by model (2), we defined bounds within which our findings remain valid under the specified scenario. We set the ranges *g* and *d* to be feasible values drawn from our SEM modelling and consistent with the literature. For

example, since prenatal smoking is a strong determinant of birth weight observed in the literature,[33,34] the upper limits for *g* were set to +/- 150 grams, close to the effect of prenatal smoking observed in our SEM (113 grams). Similarly, we set the upper limit of *d*, to be +/- 20%, close to the difference in prevalence of pre-natal smoking between high and low G1 adult SES estimated in our SEM (15%).

Results

Overall, 20.5%, 63.3%, and 16.2% of grandmothers (G0) in the analytic population reported at least < HS, HS or GED, or College diploma, respectively (*Table 1*). This represented 80.9% and 14.3% of the target population, respectively. Taking survey design into account, 71.1% and 16.2% of grandmothers fell into study-defined categories of non-Hispanic white and non-Hispanic black. Mean birth weight for G2 was 3,265 grams (*Table 1*).

Using covariate-adjusted linear regression, we estimated a 54-gram higher G2 birth weight for each higher level of G0 education (β = 54.1, [95% CI: -14.0, 122.1]; *Table 2*), independent of mediators. After multiple imputation (analytic population N = 1,856; 99% of full sample), we estimated a 48-gram (95% CI: -16.5, 111.9) direct effect by covariate-adjusted linear regression, which was not substantially different than complete case analysis (*Table 2*).

Table 1. Study population characteristics, by grand-maternal (G0) education. [a]				
		Grand-maternal (G0) educational status		
% (N) / mean (SD)	Overall (N = 1,681)	< High School Diploma (n = 345)	High School Diploma or GED (n = 1,064)	College Diploma or Higher (n = 272)
Grandmother (G0)				
G0 % white	61.5 % (1,033)	46.4 % (160)	66.4 % (706)	61.4 % (167)
G0 age at G1 birth (years)	24.8 (5.2)	24.2 (5.9)	24.7 (5.1)	26.1 (4.8)
Mother (G1)				
G1 ever neglected	50.0 % (840)	49.0 % (169)	50.5 % (537)	49.3 % (134)
G1 ever physically abused	18.1 % (304)	20.3 % (70)	17.7 % (188)	16.9 % (46)
G1 ever sexually abused	8.1 % (136)	8.1 % (28)	8.0 % (85)	8.5 % (23)
G1 BMI at Wave II (kg/m^2)	22.7 (4.5)	23.3 (4.5)	22.6 (4.5)	22.0 (4.2)
G1 BMI at Wave III (kg/m^2)	26.7 (6.7)	27.4 (6.8)	26.6 (6.6)	26.0 (7.0)
G1 % high school graduate	88.4 % (1,486)	79.4 % (274)	89.4 % (951)	96.0 % (261)
G1 % in debt	22.6 % (375)	23.9 % (81)	22.0 % (231)	23.2 % (63)
G1 average annual household income (in 2008 dollars)[b]	58,842 (41,878)	47,681 (31,895)	58,761 (42,601)	72,880 (45,670)
G1 % any prenatal smoking	19.3 % (325)	19.1 % (66)	20.9 % (222)	13.6 % (37)
Child (G2)				
Gestational age (weeks)[c]	39.1 (2.4)	39.2 (2.1)	39.1 (2.5)	38.9 (2.2)
% preterm birth[d]	9.2 % (155)	9.0 % (31)	9.4 % (100)	8.8 % (24)
G2 birth weight (grams)	3,265 (611.1)	3,220 (579.0)	3,268 (622.9)	3,309 (602.4)
% low birth weight	9.3 % (156)	9.0 % (31)	9.4 % (100)	9.2 % (25)

[a] *Means, standard deviations, and percentages presented here correspond to those of the sample, i.e. survey weighting was not taken into account.*

[b] *Average household income is either the combined income of the individual (G1) and her spouse / partner or the individual (G1) and her family, if she lives at home. If an individual responded by selecting an income category, she was assigned the mean of the category; i.e. a person reporting "5,000 to 9,999" would be assigned 7,500 dollars.*

[c] *Estimated from the question "How many weeks early or late was your baby born?" with a response "on time" interpreted as 40 weeks.*

[d] *Estimated as a gestational age < 37 weeks based on the question above.*

Table 2. Multivariate linear regression estimate of effect on G2 birth weight. (N = 1,681)		
Model	*β, [95 % Confidence Interval]*	*p-value*
No Factor Scores[1]	45.4, [-28.0, 118.8]	0.223
Childhood Maltreatment Factor Score Only[2]	47.2, [-25.9, 120.2]	0.204
Adult SES Factor Score Only[3]	45.6, [-26.5, 117.6]	0.213
Both Factor Scores[4]	47.5, [-24.0, 119.1]	0.191
Dichotomized Mediators[5]	54.1, [-14.0, 122.1]	0.118

Dichotomized Mediators After Multiple Imputation (N = 1,856)	47.7, [-16.5, 111.9]	0.144

Note: All models are adjusted for G0 age at G1 birth and G0 self-reported race.
[1] Adjusted for number of events of neglect, physical abuse, and sexual abuse before age 18 (childhood maltreatment); G1 pre-pregnancy BMI (closest measurement before delivery); G1 education, household income, and 'break even' indicator at Wave IV (adult SES); and any G1 pre-natal smoking.
[2] Adjusted for a childhood maltreatment factor score estimated from number of events of neglect, physical abuse, and sexual abuse before age 18 and other covariates from [1].
[3] Adjusted for a factor score estimated from G1 education, household income, and 'break even' indicator at Wave IV and other covariates from [1].
[4] Adjusted for maltreatment and adult factor scores, G1 pre-pregnancy BMI, and any G1 prenatal smoking.
5 Adjusted for high childhood maltreatment (factor score higher than the median), G1 pre-pregnancy overweight (BMI > 25 kg/m^2), low adult SES (factor score lower than the median), and any G1 prenatal smoking.

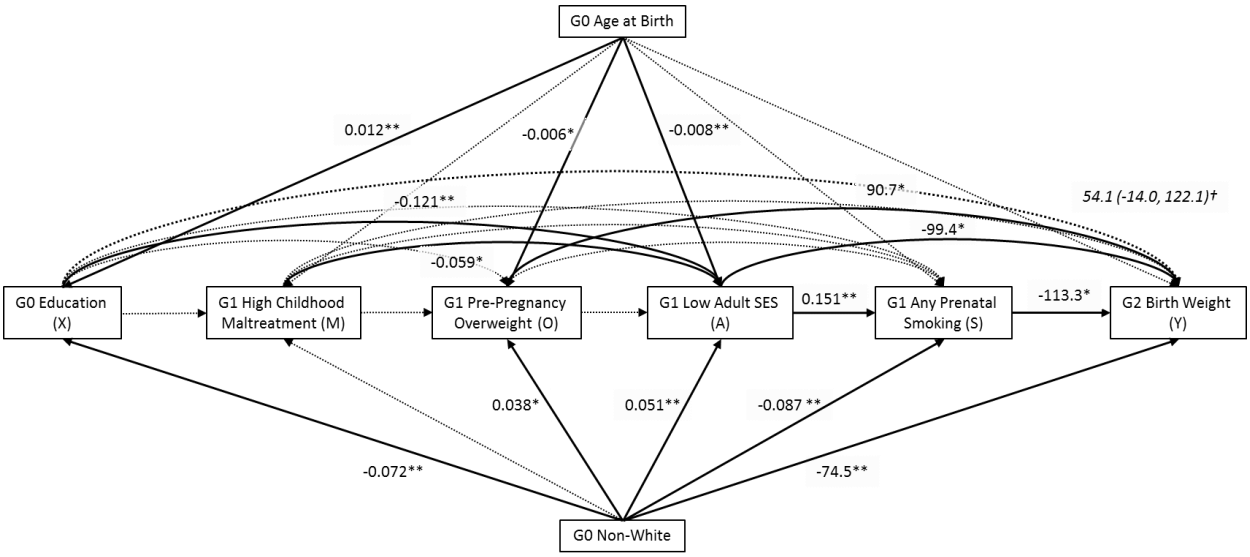

Figure 2. Structural equation model with unstandardized coefficients. For better visual clarity, unstandardized point estimates with $p < 0.05$ are shown with their edges highlighted. The point estimate and 95% confidence interval (†) for the main effect are also shown. *$p < 0.05$; **$p < 0.005$.

Using MSM estimated by inverse probability of treatment and mediator weights, we estimated a controlled direct effect of 89 grams increased (95% CI: 20.8, 156.5) G2 birth weight for each level higher of G0 education (*Table 3*) in a marginal population at lower risk of high childhood maltreatment, pre-pregnancy overweight, low adult SES, and any prenatal smoking. Even after incorporating pre-term birth as a mediator between G0 education and birth weight, we found evidence consistent with a direct effect of G0 education on G2 birth weight (*Table 4*). Using MSM, we estimated a controlled direct effect of 67-grams (95% CI: 3.8, 130.5) increased G2 birth weight per level higher G0 education. Additionally, weight

truncation at the 1st/99th, 5th/95th, and 10th/90th percentiles, introducing interaction terms between G0 education and each mediator, and refitting the model with adult SES preceding pre-pregnancy overweight, each did not change estimates greatly. (*Table 4*) Replacing all missing mediators values as either "high" (*i.e.* high maltreatment, pre-pregnancy overweight, low adult SES, and any prenatal smoking) or "low" (the opposite) risk had minimal impact on the MSM-estimated direct effect (*Table 4*), This was also true of the two other replacement scenarios (*Table 4*).

Table 3. Marginal structural model estimates of effect on G2 birth weight. (N = 1,681)		
	β, [95 % Confidence Interval]	*p-value*
G0 Education	88.7, [20.8, 156.5]	0.010
High G1 Childhood Maltreatment	-41.4, [-120.7, 37.9]	0.306
G1 Pre-pregnancy Overweight (> 25 kg/m^2)	73.7, [-10.7, 158.1]	0.087
Low G1 Adult SES	-118.6, [-198.2, -38.9]	0.004
Any G1 Prenatal Smoking	-118.0 [-208.6, -27.4]	0.011

Table 4. Marginal structural model estimates of G0 education effect on G2 birth weight, under various scenarios.

Scenario	*N*	*β, [95 % Confidence Interval]*	*p-value*
Altered causal structure			
Added Pre-term delivery (< 37 weeks)	1,680	67.2, [3.8, 130.5]	0.038
Order of G1 pre-pregnancy BMI and adult SES reversed	1,681	89.1, [21.3, 157.0]	0.010
Weight manipulation			
Weight truncation at 1st / 99th percentile	1,681	85.7, [19.5, 152.0]	0.011
Weight truncation at 5th / 95th percentile	1,681	88.5, [23.9, 153.1]	0.007
Weight truncation at 10th / 90th percentile	1,681	86.9, [23.1, 150.7]	0.008
Weights estimated with interactions	1,681	71.3, [0.583, 142.0]	0.048
Data replacement			
Replace missing measures as "high risk"	1,856	84.9, [22.3, 147.4]	0.008
Replace missing measures as "low risk"	1,856	83.3, [20.6, 145.9]	0.009
Replace only missing measures of G1 pre-pregnancy BMI and adult SES with older measures	1,795	81.7, [16.7, 146.7]	0.014
Use older G1 pre-pregnancy BMI (Wave II) and adult SES (Wave III) measures	1,685	93.5, [25.8, 161.2]	0.007

Under the hypothetical bias scenario, we found that a single binary confounder would have differ to in prevalence between exposure groups by at least 15% and have an effect on G2

birth weight of 150 grams to fully explain our findings (*Table 5*). As previously noted,[44-46] this does not preclude the possibility that a non-binary confounder or set of confounders may also fully explain our findings.

Table 5. The effect of an unmeasured binary confounder on the estimated controlled direct effect of G0 education on G2 birth weight, a quantitative bias analysis. (Adjusted β, [95% CI])

	g = -150 g	***g = -100 g***	***g = -50 g***	***g = 50 g***	***g = 100 g***	***g = 150 g***
d = -20%	58.7 [-9.2, 126.5]	68.7 [0.8, 136.5]	78.7 [10.8, 146.5]	98.7 [30.8, 166.5]	108.7 [40.8, 176.5]	118.7 [50.8, 186.5]
d = -15%	66.2 [-1.7, 134]	73.7 [5.8, 141.5]	81.2 [13.3, 149]	96.2 [28.3, 164]	103.7 [35.8, 171.5]	111.2 [43.3, 179]
d = -10%	73.7 [5.8, 141.5]	78.7 [10.8, 146.5]	83.7 [15.8, 151.5]	93.7 [25.8, 161.5]	98.7 [30.8, 166.5]	103.7 [35.8, 171.5]
d = -5%	81.2 [13.3, 149]	83.7 [15.8, 151.5]	86.2 [18.3, 154]	91.2 [23.3, 159]	93.7 [25.8, 161.5]	96.2 [28.3, 164]
d = 5%	96.2 [28.3, 164]	93.7 [25.8, 161.5]	91.2 [23.3, 159]	86.2 [18.3, 154]	83.7 [15.8, 151.5]	81.2 [13.3, 149]
d = 10%	103.7 [35.8, 171.5]	98.7 [30.8, 166.5]	93.7 [25.8, 161.5]	83.7 [15.8, 151.5]	78.7 [10.8, 146.5]	73.7 [5.8, 141.5]
d = 15%	111.2 [43.3, 179]	103.7 [35.8, 171.5]	96.2 [28.3, 164]	81.2 [13.3, 149]	73.7 [5.8, 141.5]	66.2 [-1.7, 134]
d = 20%	118.7 [50.8, 186.5]	108.7 [40.8, 176.5]	98.7 [30.8, 166.5]	78.7 [10.8, 146.5]	68.7 [0.8, 136.5]	58.7 [-9.2, 126.5]

Shading indicates the 95% Confidence Interval encompasses zero: To explain the observed direct effect of G0 education on G2 birth weight, a hypothetical unmeasured/mis-measured binary confounder would have to be lower amongst more-highly-educated grandmothers by 15% and decrease G2 birth weight by 150 grams (or vice versa). As a comparison, any G1 pre-natal smoking was 6.4% lower amongst college-educated grandmothers as compared to less than high-school education grandmothers and produced a 118 gram reduction in G2 birth weight.

Discussion

Overall, we found fairly robust evidence for a small controlled direct effect of grand-maternal (G0) education at the time of a mother's (G1) birth on the birth weight of the child (G2) independent of the mother's mediating life course circumstances: We found evidence that an improvement of grandmother's education from less than high school to college graduate increases child's birth weight by approximately 180 grams (2 * 89 grams), in a marginal population where all mothers were at equal risk of childhood maltreatment, high pre-pregnancy BMI, low adult SES, and any prenatal smoking. Our findings are strengthened by our analytic methods; the robustness of estimates to model alternation, data replacement, and quantitative bias analyses; as well as the consistency of secondary findings (*e.g.* association of prenatal smoking and lower birth weight) with past literature.

Lower grandmaternal educational status may be related to an adverse fetal programming environment for the mother[5-10]: Lower maternal education may cause the fetus to experience excess glucocorticoids (*e.g.* cortisol)[6] which may program offspring HPA axis to chronically higher cortisol secretion in response to stressors.[7] In women, higher chronic levels of cortisol can in turn lead to low birth weight offspring.[7] However, prior empirical studies on the effect of grandparental education on birth weight have not provided consistent evidence[10, 13]: In a study of predominantly African American and low-income Whites, Astone, *et al.* found high grandmaternal education to be associated with a 181 gram increased birth weight among high school or less mothers.[10] However, Kwok, *et al*. found no association between grandparental education and birth weight after adjusting for parental education.[13] One potential limitation to past studies is use of simple covariate-adjustment models in the presence of endogenous confounders.[21] While the use of MSM to estimating direct effect of SES on subsequent health outcomes in the presence of such confounding is increasingly common,[20, 47] we believe the current study is the first to use MSM to provide

evidence for intergenerational effects of grandmaternal education on birth weight while applying multiple sensitivity and quantitative bias analysis to address known limitations.

Several strengths are worth mentioning: First, prominent determinants of birth weight including pre-pregnancy BMI, prenatal smoking, and self-reported race[33] and mediation by childhood maltreatment,[27, 28] were explicitly included in our model. Second, we addressed the possibility of incorrect model specification and mismeasurement in our model by adding pre-term delivery as a mediator, altering the causal order of intermediates, and data replacement. We found these processes to have little influence on effect estimates. For example, it is plausible that adult SES actually influences pre-pregnancy BMI. Neither swapping the causal ordering of these mediators (through re-weighting) nor using prior measures of these mediators appeared to greatly affect our estimates. Our quantitative bias analysis suggests that a single binary confounder would have been fairly strong and imbalanced across exposure groups to fully explain our estimated direct effect. Fourth, confidence in our findings is also improved by the concurrence of secondary model findings with past literature: Notably, we observed greater effect sizes and strengths of association between G2 birth weight and the proximate mediators, prenatal smoking and adult SES, than the earlier mediators, pre-pregnancy BMI and high childhood maltreatment in our MSM. Also, the magnitude of birth weight reduction due to prenatal smoking was in line with findings from past meta-analyses.[33, 48] Furthermore, our study agrees with past studies in the Add Health population which found G1 adult SES,[28] prenatal smoking,[28] and G1 obesity[49] to be directly associated with G2 birth weight.

Some limitations to our study are worth highlighting: In spite of several sensitivity and biases analyses, model misspecification and unmeasured cofounding are still possible. Recent publications have questioned the ability of conventional observation methods to completely account for these issues.[50, 51] Overall, we have attempted to address these limitations through sensitivity analysis in order to estimate an average causal effect for a

marginal population in the presence of model misspecification and the presence of unmeasured confounding. However, our study of relevant mediators and covariates was not exhaustive and our ability to incorporate several covariates potentially important to birth weight was limited. For example, we did not include information regarding respondent's (G1) infection history or exposure to air pollution. Notably, the Add Health study was not designed to investigate characteristics surrounding the respondent's (G1) birth, consequently we have few variables related to grandmaternal (G0) pregnancy and perinatal health. For example, we did not have information on grandmaternal nutrition or prenatal smoking. When breastfeeding duration was incorporated into our MSM analyses, point estimates and confidence intervals were virtually unchanged. Since our study relies almost solely on self-report (with the exception of Wave III BMI), bias due to measurement error is also a concern. Specifically, recall of childhood maltreatment and prenatal smoking may be differentially misclassified by grandmaternal and maternal SES. Notably, this may explain the unexpected finding of association between high childhood maltreatment and greater proportion of high SES in our SEM. However, women have been shown to be reliable reporters of infant birth weight.[52] Additionally, while dichotomizing mediators was necessary in order to generate weights, this may have resulted in residual confounding and non-exchangeability in our target marginal population. Nonetheless, when we dichotomized at other cut points, such as defining everyone below the 80th percentile of adult SES factor score as low SES and any maltreatment as high maltreatment, estimates did not substantially change. In fact, defining low adult SES as the bottom 20th percent of adult SES factor scores only strengthened the estimated association. Finally, it is possible G0 education captures early post-natal experiences beyond *in utero* experience. We attempted to address this by including childhood maltreatment and pre-pregnancy BMI, which in many cases were measured in adolescence, as mediators in our model. To the extent that early childhood rearing practices have an impact on G2 birth weight, we are likely to see them mediated through these pathways.

Developmental Origins researchers have been cautioned to not ignore social, life-course context in their investigations[11] and our study attempts to address this concern by explicitly examining prominent social and biological determinants of birth weight. We addressed assumptions of no-residual confounding and model specification assumptions through sensitivity analysis and still found evidence for the direct effect of grandmaternal education on offspring birth weight. Consequently, we believe we have provided evidence that supports early life social conditions should be a target for intervention and measurement in order to address disparities in pregnancy outcomes. Future empirical work should consider the relevant causal components of education, possible biological mechanisms (such as epigenetics), improved measurement of life course variables, and consider additional causal estimation methods, including those that relax more modeling assumptions.

(*End Chapter 2*)

Chapter 3 - Early life socioeconomic status, candidate gene DNA methylation, and adult cardiometabolic phenotype in young adult women: the Jerusalem Perinatal Family Follow-Up Study.

Abstract

Background: Early life socioeconomic status (SES) has been related to adult cardiometabolic health. However, potential mechanisms such as epigenetics have not been well described.

Methods: Among 613 adult women (mean age = 32 years) participants of the Jerusalem Perinatal Study Family Follow-Up we investigated associations between early life SES and DNA methylation at five cardiometabolic and stress response genes: *ABCA1*, *INSIGF*, *LEP*, *HSD11B2*, and *NR3C1* (two regions). Early life SES was characterized by father's occupational class (6 being 'lowest' to 1 being 'highest') and years of maternal and paternal education. Sequenom MassARRAY was used to profile methylation. We used multivariate linear regression to test associations between measures of early life SES and average, region-specific methylation, adjusted for maternal age at birth, prenatal smoking, country of origin, and parity. We examined whether methylation mediates the SES-adult phenotype relationship using exploratory product-of-coefficients mediation analyses under two presumed causal structures: with and without adjustment for a woman's life course characteristics (adolescent overweight status, years of education, religiosity, marital status, number of children, and frequency of alcohol and cigarette use). All models were adjusted for age at blood draw and original stratified sampling criteria, maternal pre-pregnancy BMI and participant birth weight.

Results: Average methylation ranged from 5.7% for *HSD11B2* to 77.3% for *INS-IGF*. Each higher paternal occupational class was associated with a 0.5 %-point (95% CI: 0.004, 0.9; p = 0.048) and 0.4 %-point (95% CI: 0.1, 0.8; p = 0.022) higher *ABCA1* and *NR3C1* Exon 1F region 2 methylation, respectively. Each additional year of maternal education was associated with a 0.08 %-point (95% CI: 0.02, 0.1; p =0.012) higher *HSD11B2* methylation. Only *HSD11B2* or *NR3C1* Exon 1F region 2 appeared to be related to adult

phenotype after adjustment for life course characteristics. However, there was not consistent evidence that methylation at these regions mediated early life SES-adult phenotype relationships (for all tests of indirect effect, $p > 0.05$).

Discussion: We found evidence for associations between early life SES and adult methylation at regions in *ABCA1*, *HSD11B2*, and *NR3C1*. However, we did not find evidence for DNA methylation to mediate the relationships between early life SES and adult cardiometabolic phenotype. Future larger, replication studies in this population should consider addition SES measures including income, immigration status, and military service, sampling DNA at different time points and tissues, and study sampling structures that better allow for causal mediation analyses.

Background

Early life exposure to adverse environments such as poverty, famine, and war may increase adult susceptibility to early mortality,[1] cardiovascular disease,[2,3] and, among women, poor pregnancy outcomes.[4,5 6] Experiences of psychosocial stress *in utero* may have an important role in such relationships[7] due to the putative programming of the fetal epigenome through DNA methylation, the process by which methyl groups are added to cysteine residues of cytosine-guanine (CpG) dinucleotides in the DNA sequence.[8,9] Methylation of gene promoter regions is a common gene expression regulatory mechanism with profound implications in physiological processes such as X chromosome silencing as well as pathologic processes such as Silver-Russell syndrome, where intra-uterine growth restriction is caused by abnormal imprinting of the insulin-like growth factor (*IGF2*).[10] In the context of early life exposures and development origins of adult disease, DNA methylation can play a key mediating role because (a) it is influenced by the intrauterine environment[11-13]; (b) it is a key component of fetal epigenetic programming[8-14]; and (c) it is mitotically stable and can persist throughout the adult life,[6-10] and (d) it can be transmitted across generations.[6-14] Importantly, DNA methylation of growth, metabolism, and stress response genes[8,11-14] may be programmed by excess exposure to the glucocorticoid cortisol resulting from prenatal stressors including low socioeconomic status (SES).[7,15]

Several studies have found associations between maternal experiences of war[16] and intimate partner violence[17] and higher glucocorticoid receptor (*NR3C1*) methylation in neonates and adolescents, respectively. *In utero* exposure to famine has been associated with lower methylation at *INSIGF*, a shared promoter for *IGF2*, among adults.[12,18] Moreover, less acute sources of adversity[19] including low childhood socioeconomic position[20-22] may also be associated with differential adult methylation: In a U.S. birth cohort, Tehranifar, *et al.* found lower family income at birth to be associated with a 19.7% lower methylation of the *Sat2* repetitive DNA element in peripheral blood of adult women.[21] However, there is a

lack of studies on associations between early life socioeconomic status (SES) and adult methylation status of specific cardiometabolic and stress response genes, such as *NR3C1* and *INSIGF*,[23,24] and whether such associations might mediate early life SES-adult health relationships. Investigating such associations may provide mechanistic evidence for fetal or intergenerational effects of adverse socioeconomic conditions.[25-28] Moreover, investigating these associations amongst women is important as intergenerational effects might be transmitted through a woman's own pregnancy outcomes.[6,26,27]

To this end, we investigated associations between measures of early life SES (measured by father's occupational class and parental education) and variations of DNA methylation in specific cardiometabolic (*ABCA1*, *INSIGF*, *LEP*) and stress-related genes (*HSD11B2*, *NR3C1*) among young adult women (mean age = 32 years) participants of a birth cohort study. In line with previous studies, we hypothesized that lower early life SES would be associated with reduced *HSD11B2* and increased *NR3C1* methylation in young adulthood, as evidence of an adaptive response to excess perinatal glucocorticoids.[7,30,31] Further, we hypothesized that lower early life SES would be associated with lower *INSIGF* and higher *ABCA1* and *LEP* methylation.[29] In secondary analyses, we investigated associations between DNA methylation and adult cardiometabolic profiles and whether methylation mediated early life SES and adult cardiometabolic phenotype relationships, using two models for mediation.

Methods

Study setting and population - This study was conducted in setting of the Jerusalem Perinatal Study (JPS) which included all 17,003 births to residents of Jerusalem between 1974 and 1976.[32] Around the time of subjects' birth in 1974-76, maternal demographic, medical history, and pregnancy course information, as well as subject's birth weight were abstracted from birth certificates or maternity ward logs. Additional information on maternal immigration history, years of education, pre-pregnancy weight, height, smoking status, paternal occupation and years of education were collected by interview of the mother one or two days postpartum. In the JPS Family Follow-Up Study (JPS-1), a well characterized cohort of 1,400 mother-offspring dyads, oversampled on the basis of maternal pregnancy body mass index (ppBMI ≥ 27 kg/m^2) and offspring birth weight (≤ 2500 grams or ≥ 4000 grams), were identified and recruited from JPS participants. Offspring were only selected to participate in this follow-up if records showed they had been born as singletons, at ≥ 36 weeks of gestation, and without any congenital malformations. Between 2007 and 2009, JPS-1 offspring were interviewed and examined as young adults (mean age = 32 years old). All JPS-1 female offspring with available blood samples (N = 613) were included in the current study. JPS and JPS-1 study protocols were approved by the University of Washington (Seattle, WA, USA) and Hadassah-Hebrew University Medical Center (Jerusalem, Israel) Institutional Review Boards and participants provided informed consent.

Data Collection

JPS-1 subjects were asked via telephone interview about years of education, marital status, religiosity (secular, traditionalist, religious, and ultra-orthodox), medical history and medications, and frequency of alcohol or cigarette use. They were also asked whether they had any children, and if so, whether any of them were born at term, but weighing less than 2.5 kilograms. In a subsequent physical exam, anthropometrics and blood pressure as well as a peripheral blood sample were collected by standardized procedures (see below).[32,33]

Early life socioeconomic status (SES) exposures – Prenatal socioeconomic stress may be a complex construct with multiple dimensions.[34] In line with previous studies, we chose four measures for early life SES: We defined our primary exposure as paternal occupational type, as reported by the mother during post-partum interview (Savitsky, *et al*., *In Review*). These occupational types were categorized into 6 classes, with 6 being the lowest class comprising manual occupations and 1 being the highest class comprising professional occupations. Paternal occupational class was dichotomized to 'low' (4-6) and 'high' (1-3) to match previous work. Since we did not hypothesize a specific threshold for the effect of socioeconomic related stress based on occupational class, we also evaluated paternal occupational class ordinally (*i.e.* 6 to 1). Additionally, we were interested in parental education as measures of early life SES. We defined maternal or paternal education as total number of years of education completed by mother or father, respectively. Past studies have found education to be highly correlated to other measures of prenatal socioeconomic status[34] and is one of the strongest predictions of offspring methylation.[24]

Candidate gene region selection and methylation profiling – Promoter regions from five genes of interest were selected based on prior literature on maternal perinatal adversity and offspring methylation as well as their putative role in cardiometabolic function and stress response: *ABCA1* (cholesterol transport protein),[23,29] *HSD11B2* (glucocorticoid-inactivating enzyme),[40-42] *INS-IGF2* (insulin and insulin-like growth factor),[23,24,29] *LEP* (leptin, an energy balance hormone),[29] and *NR3C1* (glucocorticoid receptor).[16,17,43] Peripheral blood samples were used for DNA methylation profiling conducted at the Roswell Park Cancer Institute's Genomics Shared Resource (Buffalo, NY, USA). Quantitative methylation analysis was performed with the Sequenom MassARRAY Compact System (San Diego, CA, USA) using standard, previously described methods.[44-46] Briefly: 1 µg of genome DNA for each individual and CpG region of interest was bisulfite converted using the EZ DNA Methylation Kit (Zymo Research, Orange, CA). Converted DNA was then amplified by PCR using primers flanking

the regions of interest designed in MethPrimer.[47] PCR products were then cleaved using Transcleave (Sequenom), purified with Clean Resin (Sequenom), and spotted onto a 384-well SpectroCHIP (Sequenom) plate. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) was conducted using a MassARRAY Analyzer Compact (Sequenom) to analyze cleavage products, with resultant methylation calls performed by the EpiTyper software v1.0 (Sequenom) and written to an Oracle 8i database. Each cleavage product corresponds to one CpG unit consisting of several proximal CpG sites with the output value being the proportion of sites in the unit that were methylated. Average methylation for each region of interest was then calculated by taking the arithmetic mean of all analyzed CpG units from the region to form one summary measure expressed as a percentage methylated (*i.e.* out of the entire region). Control runs of known methylation of 0%, 50%, and 100% were run for each CpG gene to assess for profiling bias. CpG units failing > 25% of methylation calls were excluded (N = 3 for ABCA1, 2 for LEP, 4 for NR3C1 Region 1, and 2 for Region 2). No subjects were excluded on the basis of failed methylation calls.

Adult Cardiometabolic Risk / Phenotype Outcomes – At the woman's in-person physical exam around age 32, measurements were collected including height, weight, waist and pelvic circumference, systolic and diastolic blood pressures by study staff according to previously described protocols.[32] We calculated body mass index (BMI) by the formula: (measured weight in kilograms) / (measured height in meters)^2. We calculated waist to hip ratio (WHR) by the formula: (measured waist circumference in centimeters) / (measured pelvic circumference in centimeters) x 100%. Additionally, a fasting (≥ 8 hours since the last meal) peripheral blood sample was collected, immediately spun, and assayed for biomarkers in plasma including total cholesterol, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol assayed on the VITROS 5,1 FS Chemistry System (Ortho Clinical Diagnostics).[32]

We constructed three binary young adult health outcomes: obesity, metabolic syndrome, and any, term low birth weight offspring. Obesity was defined as a calculated BMI ≥ 30 kg/m^2. Metabolic syndrome defined based on International Diabetes Federation (IDF) criteria[48]: central obesity defined by waist circumference ≥ 90 cm or BMI ≥ 30 kg/m^2 and at least two of the following: triglycerides > 150 mg/dL; HDL < 50 mg/dL; SBP > 130 mmHg; DBP > 85 mmHg; and/or fasting glucose > 100 mg/dL. During the young adult phone interview, a woman was asked whether she had any children and, if so, whether any were born at or near term, but weighing less than 2.5 kilograms (coded "yes" or "no").

Early life confounders – Data on maternal pre-pregnancy overweight (ppBMI ≥ 27 kg/m^2), age at birth, immigrant from West, any prenatal smoking, and parity collected by post-partum interview as well as a woman's birth weight abstracted from medical records[32] were included as potential confounders. Previous studies have shown maternal ppBMI, age, and race[34] as well as maternal prenatal smoking and offspring birth weight[24] to be relevant confounder of early life SES-methylation associations. Because of the relative homogeneity of the study population[35] and similar to prior work (Savitzky, *In Review*), we used maternal immigration status *in lieu* of a racial classification. In past studies of this population, country of origin has been associated with SES and health outcomes.[35] Finally, due to the potential relationship between birth order and putative fetal programing,[36] we also adjusted for maternal parity. Since methylation is dependent on age,[9] we adjusted for a woman's age at blood draw in all analyses.

Life course factors – While it is hypothesized that methylation status is established early in life,[8,9] it is possible life course factors may mediate early life SES and adult methylation associations or predict adult methylation status independently. Such factors include: childhood overweight in grades 4-6 (approximately ages 10 to 12 years), years of education, religiosity, marital status, childbearing and parity, and any alcohol and tobacco use as self-reported by the woman during telephone interview (~age 32). These life course

factors may lie on the causal pathway between early life SES and adult methylation (and between early life SES and adult outcomes[33]) and yet they may also serve as confounders in the relationship between adult methylation and phenotype. Contemporary thought suggests including endogenous confounders in regression models may result in over-adjustment by removing some of the mediated effect.[37] Since we are primarily interested in the total effect of early life SES, we exclude these life course factors in our primary analysis of early life SES-methylation associations. In assessing associations between adult methylation and phenotype, however, we adjust for these factors as potential confounders.

Use of life course factors in mediation analyses - To explore the potential role of DNA methylation in mediating early life SES-adult phenotype relationships, we must make assumptions regarding the causal relationship of life course factors to adult methylation status. Since the precise role of DNA methylation as cause, mediator, or consequence of life course health processes is still in question[38,39] and we only have measurement of adult methylation, we propose two models in our study: For an "early programming" model, we assume that methylation marks are set in early life, even though they are observed in adulthood (*Figure 1*). In assessing mediation through this models, we again do not adjust for life course factors as they can only be a consequence of DNA methylation status. For a "late effect" model, we assume that methylation marks are a consequence of life course factors (*Figure 2*). To test this model using comparable mediation methods, we adjust for life course factors despite prior reservations. To assess whether the early life SES-methylation association may be unduly biased by these adjustments, we compare the qualitative interpretation of SES-methylation associations with and without adjustment for life course factors.

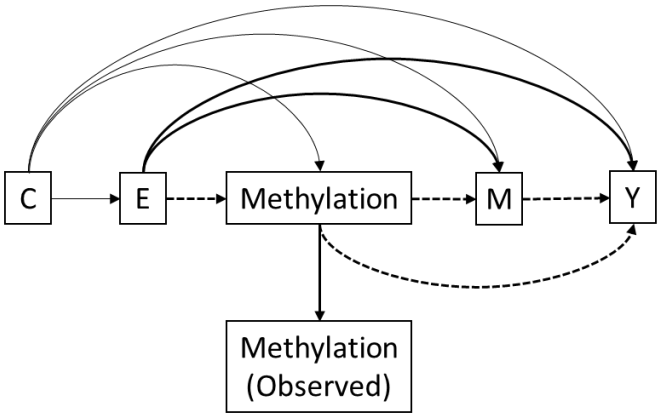

Figure 1. Methylation is established in early life. Under this causal diagram, a woman's methylation status is established early in life by birth SES (E) and perinatal characteristics (C) and independent of subsequent life course mediators (M) such as childhood overweight, attained education, marital status, religiosity, childbearing, or substance use. The mediated effect of birth SES (E) on adult phenotype (Y) through methylation status (i.e. the indirect effect) is given by the dashed lines – any effect through life course mediators is captured by the total effect of methylation.

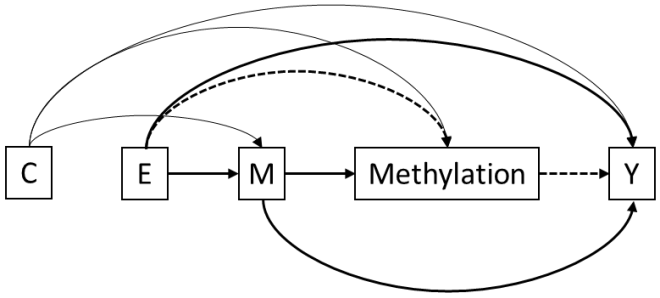

Figure 2. Methylation is affected by life course mediators. Under this causal diagram, a woman's methylation status is determined by birth SES (E), perinatal characteristics (C), and life course mediators (M). The mediated effect of birth SES (E) on adult phenotype (Y) is given by the dashed lines – only the effect through life methylation.

Statistical Analysis – To estimate associations between early life SES and young adult methylation, we fit three multivariable linear regression models for average methylation at each of the six candidate gene regions (*ABCA1*, *HSD11B2*, *INSIGF*, *LEP*, *NR3C1* Exon 1-F Region 1 and Region 2) predicted by each of the four exposures (low / high paternal occupational class; increasing paternal occupational class; increasing maternal years of education; increasing paternal years of education) and: (1) adjusting for woman's age at blood draw and the stratification variables maternal pre-pregnancy overweight (≥ 27 kg/m^2)

and woman's birth weight category (≤ 2500 grams, 2501 to 3999 grams, ≥ 4000 grams); (2) also adjusting for early life confounders; and (3) also adjusting for life course factors. As mentioned above, adjustment for life course factors was primarily done to assess whether our "late effect" mediation model may be unduly biased. Up to 19 individuals (maternal education and woman's cigarette use) were missing information for each variable, so multiple imputation by chained equations was used to estimate the influence of values missing at random (MAR) conditional on observed covariates on our estimates of early life SES-adult methylation associations.

To estimate associations between adult methylation and phenotype, we fit two multivariable linear regression models for each of our continuous adult outcomes predicted by percent methylation at each of the six promoter regions: (1) Adjusted for woman's age at blood draw and stratification variables; and (2) also adjusted for both confounders and mediators, as they serve as potential confounders of the methylation-phenotype relationship. Similarly we fit two multivariable logistic regression models for each of the binary adult outcomes: risk of obesity (BMI ≥ 30 kg/m^2), risk of metabolic syndrome, and risk of reporting any term, offspring weighing less than 2.5 kilograms, predicted by percent methylation at each of the six promoter regions. Risk of low birth weight offspring was estimated only amongst women who had reported any births. Continuous biomarkers (*e.g.* cholesterol, HDL, LDL) were not log transformed in accordance with past studies[32] as they did not deviate substantially for normality.

Finally, for promoter regions that showed significant associations with a measure of early life SES and one or more adult outcomes, we explored potential mediation of that specific early life SES-adult phenotype relationship. We did this by estimating the indirect effects of the given early life SES measure on our set of adult outcomes, as mediated through percent methylation, using multivariate linear regressions and the standard product of coefficients approach,[49] which has been formalized in a causal framework under assumptions of no

residual confounding (sequential randomization) and no interaction.[49,50] As discussed in the section on life course factors, we operationalized two alternative causal structures for the developmental origins hypothesis: In the "early programming" model, we assumed that adult methylation status is a measure of methylation status established early in life (*Figure 1*) and therefore independent of life course factors (childhood overweight, educational attainment, religiosity, adult marital status, and any alcohol or cigarette use). In this model, we estimated the direct effect of early life SES on adult phenotype conditional only on adult methylation. Implicitly, the estimates of indirect effect through methylation capture the effects of methylation on "subsequent" life course measures. In the "late effects" model, we allow that adult methylation may be affected by life course mediators (*Figure 2*) and adjust for them in models for both direct and indirect effects. In this way, we focus on the components of the effect of early life SES and methylation that do not involve measured life course events. Confidence intervals for calculated indirect effects were obtained by bootstrapping standard errors. We conducted all data analyses using Stata MP 13.1 (StataCorp, College Station, TX, USA) and $p < 0.05$ was chosen as our indicator of model-dependent statistical significance. Because we chose a small set of specific exposures and candidate genes *a priori* based on previous work, and in the interest of not increasing type II error, we elected not to perform any multiple testing corrections. Subsequently, we describe implications for this in the Discussion.

Results

Overall, women with available blood samples included in our study did not differ substantially from the total female population (N = 715) of the JPS Family Follow-Up with regard to maternal characteristics and adult cardiometabolic measures, though women in our study sample were slightly less likely to have been low birth weight (13.1% vs. 14.5%), married (78.7% vs. 80.2%), or ultra-Orthodox (18.7% vs. 20.4%). Moreover, our study population was similar in characteristics to women from a previous study[32] in this population (*Table 1a*). In our study, high paternal occupational class was associated with an average of 3 more years of parental education each compared to low occupational class. Wives of high occupational class fathers were much more likely to have been immigrants from the West (22.7% versus 8.6%) and less likely to report any prenatal smoking (8.4% vs. 18.4%). Women with high occupational class fathers were more likely to report being overweight in the 4^{th} to 6^{th} grades (22.4% vs. 18.8%), had more years of education, were more likely to be married and identify as ultra-Orthodox, and have had more children, compared to low early life SEP women. Overall, adult methylation was tightly controlled: with the exception of the *LEP* promoter region, interquartile ranges were less than 10% methylation (*Table 1b*). Compared to women with low early life SEP, high early life SEP women had higher mean methylation at *ABCA1* (20.2% vs. 19.6%), *INSIGF* (77.4% vs. 77.2%), *LEP* (22.7% vs. 21.5%), and *NR3C1* Region 2 (10.7% vs. 9.1%) and lower methylation at *NR3C1* Region 1 (6.4% vs. 6.8%), though differences appeared slight (*Table 1b*).

Table 1a. Study population characteristics, by father's occupational class.			
		Father's Occupational Class	
	Overall (N = 613)	Low (Class 4-6) (n = 256)	High (Class 1-3) (n = 357)
Socioeconomic Status (SES) at Birth			
% Low paternal occupational class[1]	41.8% (256)	--	--
Maternal education (years)	11.8 (3.3)	10.0 (3.0)	13.0 (3.0)
Paternal education (years)	12.2 (4.0)	9.6 (2.9)	14.0 (3.6)
Maternal Perinatal Characteristics			
Age at delivery (years)	28.3 (5.8)	27.8 (6.0)	28.7 (5.6)
% Immigrant from the West	16.8% (103)	8.6% (22)	22.7% (81)
Pre-pregnancy BMI (kg/m^2)	24.3 (3.9)	24.5 (4.0)	24.2 (3.8)
% Any smoking during pregnancy	12.6% (77)	18.4% (47)	8.4% (30)
Parity[2]	2.0 (2.0)	2.0 (1.8)	2.0 (2.2)
Daughter's Perinatal Characteristics			
Birth weight (grams)	3,298 (599)	3,302 (597)	3,296 (602)
% Low birth weight (< 2500 grams)	13.1% (80)	11.3% (29)	14.3% (51)
Daughter's Life Course Mediators			
% Childhood overweight[3]	20.9% (128)	18.8% (48)	22.4% (80)
Years of education	14.9 (2.6)	14.4 (2.5)	15.3 (2.6)
% Married	78.7% (474)	76.8% (192)	80.1% (282)
% Ultra-Orthodox	18.7% (112)	6.5% (16)	27.4% (96)
Number of children	2.4 (2.1)	1.9 (1.6)	2.7 (2.4)
Daughter's Adult Phenotype			
Height (cm)	162.0 (6.1)	161.7 (6.1)	162.2 (6.2)
Weight (kg)	68.0 (14.7)	68.0 (15.1)	68.0 (14.5)
BMI (kg/m^2)	25.9 (5.4)	26.0 (5.4)	25.9 (5.5)
Waist to Hip Ratio (%)	78.7 (6.2)	78.6 (5.8)	78.7 (6.5)
Systolic blood pressure (mmHg)	99.6 (10.5)	99.3 (11.4)	99.9 (9.8)
Diastolic blood pressure (mmHg)	69.6 (8.7)	69.5 (9.8)	69.6 (7.9)
Serum total cholesterol (mg/dL)	183.7 (33.8)	186.0 (35.7)	182.1 (32.3)
Serum HDL (mg/dL)	57.0 (15.1)	56.4 (14.7)	57.5 (15.3)
Serum LDL (mg/dL)	108.1 (28.5)	110.2 (29.8)	106.6 (27.4)
% Obese (≥ 30 kg/m^2)	20.0% (122)	20.4% (52)	19.6% (70)
Metabolic syndrome[4]	5.1% (31)	6.3% (16)	4.2% (15)
% Any term, low birth weight children	13.5% (61)	13.5% (25)	13.4% (36)

[1] Defined by father's occupational class being in the bottom half (i.e. 4-6).
[2] Number of self-reported previous live births, including those who have since died.
[3] Self-reported as "slightly overweight" or "significantly overweight" in grades 4 to 6 (around age 10 to 12 years).
[4] Based on International Diabetes Federation (2006) criteria, a subject has metabolic syndrome if she: Has central obesity defined as waist circumference ≥ 90 cm (~80th percentile) or BMI ≥ 30 kg/m^2 AND at least two of the following: triglycerides > 150 mg/dL, HDL < 50 mg/dL, systolic blood pressure > 130 mmHg, diastolic blood pressure > 85 mmHg, and/or fasting glucose > 100 mg/dL.
Note: All measures reported as mean (standard deviation) other than proportions, which are given as % (N).

Table 1b. Detailed Methylation (%) Distribution of Candidate Gene Promoter Regions.										
Gene (N)	Location (GRCh37 / human genome build 19)	# CpG sites	Overall (N = 613)						Low Class (n = 256)	High Class (n = 357)
			Mean % (SD)	Min	25%ile	Median	75%ile	Max	Mean % (SD)	Mean % (SD)
ABCA1 (N = 436)	Chromosome 9: 107,690,502-107,690,821	27	20.0% (7.3)	6.6	14.5	18.6	24	58.5	19.6% (7.2)	20.2% (7.4)
HSD11B2 (N = 573)	Chromosome 16: 67,464,230-67,464,442	6	5.7% (2.1)	0.5	4.5	5.3	6.3	22.8	5.7% (2.2)	5.7% (2.1)
INS-IGF (N = 563)	Chromosome 11: 2,182,336-2,182,640	4	77.3% (5.4)	44.5	75	77.8	80.5	97.5	77.2% (5.4)	77.4% (5.3)
LEP (N = 470)	Chromosome 7: 127,881,051-127,881,408	32	22.2% (11.7)	2.5	13.4	20.3	29.7	62.6	21.5% (11.2)	22.7% (11.9)
NR3C1 Exon 1-F.1 (N = 508)	Chromosome 5: 142,783,506-142,783,905	47	6.5% (2.7)	2.8	5.4	6.2	7	48.8	6.8% (3.7)	6.4% (1.7)
NR3C1 Exon 1-F.2 (N = 317)	Chromosome 5: 142,783,885-142,784,247	33	10.1% (6.3)	1.2	6.1	8.7	12.3	62.4	9.1% (5.1)	10.7% (7.0)

Paternal class-adult methylation associations - Adjusting for woman's age at blood draw and stratification variables (maternal pre-pregnancy overweight and woman's birth weight category), high paternal occupational class (1-3) was associated with higher *NR3C1* Region 2 methylation (β = 1.5; p = 0.027), relative to low class (*Table 2a*). However, after adjusting for additional early life confounders (maternal age at birth, any prenatal smoking, country of origin, and parity), this association was no longer significant (*Table 2b*). Each higher category of paternal occupational class was associated with a 0.5 percentage-point higher *ABCA1* (p = 0.033) and *NR3C1* Region 2 (p = 0.008) promoter methylation, respectively (*Table 2a*). These associations remained statistically significant after adjusting for woman's age, stratification variables, and other early life confounders (*Table 2b*).

Education-adult methylation associations – Each greater year of maternal education was associated with a 0.7 percentage-point higher *HSD11B2* promoter methylation (β = 0.07; p = 0.013; *Table 2a*). This persisted after additional adjustment for early life confounders (*Table 2b*). Each greater year of paternal education was associated with higher *NR3C1* Region 2 methylation (β = 0.02; p = 0.026; *Table 2a*). However, after adjusting for early life confounders, this relationship was no longer statistically significant (*Table 2b*).

Table 2a. Associations Between Early Life SES and Percent Candidate Gene Methylation.[1]						
Exposure	*ABCA1*	*HSD11B2*	*INS-IGF*	*LEP*	*NR3C1-1F.1*	*NR3C1-1F.2*
	β (95% CI), p-value (p < 0.05 shaded)					
High Paternal Occupational Class[2]	0.5 (-0.9, 1.9) p = 0.450	-0.04 (-0.4, 0.3) p = 0.808	0.2 (-0.7, 1.1) p = 0.681	1.2 (-1.0, 3.4) p = 0.275	-0.4 (-0.9, 0.2) p = 0.172	1.5 (0.2, 2.9) p = 0.027
Increasing Paternal Occupational Class[3]	0.5 (-0.04, 0.9) p = 0.033	-0.02 (-0.1, 0.1) p = 0.778	0.1 (-0.2, 0.4) p = 0.517	0.4 (-0.4, 1.1) p = 0.326	-0.1 (-0.3, 0.1) p = 0.291	0.5 (0.1, 0.9) p = 0.008
Mother's Years of Education	0.2 (-0.02, 0.4) p = 0.085	0.07 (0.01, 0.1) p = 0.013	-0.04 (-0.2, 0.1) p = 0.595	0.3 (-0.04, 0.6) p = 0.089	0.02 (-0.07, 0.1) p = 0.683	0.1 (-0.04, 0.3) p = 0.125
Father's Years of Education	0.1 (-0.05, 0.3) p = 0.154	0.03 (-0.01, 0.08) p = 0.159	-0.09 (-0.2, 0.03) p = 0.139	0.2 (-0.1, 0.4) p = 0.254	-0.02 (-0.08, 0.04) p = 0.581	0.02 (0.02, 0.3) p = 0.026

[1] *Adjusted for woman's age at blood draw, maternal pre-pregnancy overweight (≥ 27 kg/m^2), and woman's birth weight category (≤ 2500 grams, 2501 to 3999 grams, ≥ 4000 grams).*
[2] *High early life SEP (Father's Occupational Class 1-3) versus low early life SEP (Class 4-6).*
[3] *Per unit father's occupational class increase,* i.e. *From class 6 (Low) to class 1 (High).*
NOTE: Coefficients represent percentage-point change per unit exposure.

Table 2b. Associations Between Early Life SES and Percent Candidate Gene Methylation, Adjusted for Maternal Characteristics.[1]						
Exposure	*ABCA1*	*HSD11B2*	*INS-IGF*	*LEP*	*NR3C1-1F.1*	*NR3C1-1F.2*
	β (95% CI), p-value (p < 0.05 shaded)					
High Paternal Occupational Class	0.4 (-1.1, 1.8) p = 0.600	0.0002 (-0.4, 0.4) p = 0.999	0.3 (-0.7, 1.2) p = 0.589	0.8 (-1.4, 3.0) p = 0.490	-0.4 (-1.1, 0.2) p = 0.156	1.3 (-0.02, 2.6) p = 0.053
Increasing Paternal Occupational Class	0.5 (0.004, 0.9) p = 0.048	-0.008 (-0.1, 0.1) p = 0.902	0.1 (-0.2, 0.4) p = 0.449	0.2 (-0.5, 1.0) p = 0.520	-0.1 (-0.3, 0.1) p = 0.272	0.4 (0.1, 0.8) p = 0.022
Mother's Years of Education	0.2 (-0.003, 0.5) p = 0.053	0.08 (0.02, 0.1) p = 0.012	-0.1 (-0.2, 0.1) p = 0.454	0.2 (-0.1, 0.6) p = 0.177	0.02 (-0.1, 0.1) p = 0.691	0.08 (-0.1, 0.3) p = 0.429
Father's Years of Education	0.1 (-0.1, 0.3) p = 0.219	0.04 (-0.003, 0.1) p = 0.068	-0.1 (-0.2, 0.04) p = 0.159	0.1 (-0.2, 0.4) p = 0.505	-0.02 (-0.1, 0.04) p = 0.473	0.1 (-0.02, 0.3) p = 0.081

[1] *In addition to 2a, also adjusted for: maternal age at birth, any maternal smoking, maternal country of origin, mother's parity.*

Sensitivity of models to life course factor adjustment – After further adjusting for potential life course mediators: childhood overweight, years of education, marital status, religiosity, number of children, alcohol and cigarette use and imputing missing values, qualitative associations between early life SES and adult methylation remained virtually identical: Increasing paternal occupational class was associated with a 0.6 percentage-point higher (p = 0.018) *ABCA1* and 0.5 percentage-point higher (p = 0.039) *NR3C1* Region 2 methylation (*Table 2c*). Each higher year of maternal education was associated with a 0.08 percentage-point higher (p = 0.008) *HSD11B2* methylation (*Table 2c*).

Table 2c. Sensitivity Analysis - Associations Between Early Life SES and Candidate Gene Methylation, Adjusted for Life Course Factors, Missing Data Imputed.[1]

Exposure	*ABCA1*	*HSD11B2*	*INS-IGF*	*LEP*	*NR3C1-1F.1*	*NR3C1-1F.2*
	β (95% CI), p-value (p < 0.05 shaded)					
High Paternal Occupational Class	0.7 (-0.8, 2.3) p = 0.338	0.002 (-0.4, 0.4) p = 0.994	0.5 (-0.5, 1.4) p = 0.330	0.8 (-1.5, 3.0) p = 0.503	-0.6 (-1.5, 0.3) p = 0.169	1.3 (-0.2, 2.8) p = 0.100
Increasing Paternal Occupational Class	0.6 (0.1, 1.1) p = 0.018	-0.01 (-0.1, 0.1) p = 0.888	0.2 (-0.1, 0.5) p = 0.250	0.2 (-0.6, 1.0) p = 0.601	-0.2 (-0.4, 0.1) p = 0.264	0.5 (0.03, 0.9) p = 0.036
Mother's Years of Education	0.2 (-0.02, 0.5) p = 0.066	0.08 (0.02, 0.1) p = 0.008	-0.04 (-0.2, 0.1) p = 0.622	0.2 (-0.2, 0.6) p = 0.345	0.03 (-0.1, 0.1) p = 0.478	0.09 (-0.1, 0.3) p = 0.436
Father's Years of Education	0.2 (-0.04, 0.4) p = 0.112	0.05 (-0.01, 0.1) p = 0.081	-0.1 (-0.2, 0.1) p = 0.270	p = 0.1 (-0.2, 0.4) p = 0.456	-0.04 (-0.1, 0.06) p = 0.425	0.1 (-0.1, 0.3) p = 0.164

[1] *In addition to 2b, also adjusted for: woman's childhood overweight (10 – 12 years), years of education, marital status, religiosity, number of children, alcohol and cigarette use. Missing values for maternal education, pre-pregnancy overweight, smoking, and parity; father's education; and woman's marital status, years of education, religiosity, number of children, alcohol use, and cigarette use were imputed ten times using multiple imputation by chained equations. As a result, each regression only was missing a maximum of three subjects.*

Adult methylation-phenotype associations

HSD11B2 – Adjusted for maternal and life course confounders, each 1%-point higher *HSD11B2* promoter methylation was associated with 0.5 kg (95% CI: -1.0, -0.05) lower weight, 1.4 mg/dL (95% CI: -2.4, -0.3 lower cholesterol, 1.3 mg/dL (95%CI: -2.2, -0.4) lower LDL, and 12% higher risk (OR = 1.12, [95% CI: 1.00, 1.26]) of having any low birth weight offspring (*Table 3*).

NR3C1 Exon 1F Region 1 - Adjusted for maternal and life course confounders, each 1%-point higher *NR3C1* Exon 1F Region 1 methylation was associated with 0.3 mmHg higher (95% CI: 0.08, 0.5) diastolic blood pressure in the fully adjusted model (p = 0.010; *Table 3b*).

NR3C1 Exon 1F Region 2 - Adjusted for maternal and life course confounders, each 1%-point higher *NR3C1 Exon 1F* Region 2 methylation was associated with 0.1 cm (95%CI: 0.02, 0.2) greater height (p = 0.019; *Table 3*).

Additional associations and analyses - No significant associations were found for *LEP* or *INS-IGF2*. Multiple imputation for missing values did not change estimates of association with adult phenotype greatly (*not shown*) and were substantively similar to Table 3.

Table 3. Associations Between Candidate Gene Methylation and Adult Phenotype, Adjusted for Confounders. [1]

Phenotype Measure	*ABCA1*	*HSD11B2*	*INS-IGF*	*LEP*	*NR3C1-1F1*	*NR3C1-1F2*
			β (95% CI), p-value (p < 0.05 shaded)			
Height (cm)	-0.05 (-0.1, 0.03) p = 0.261	-0.1 (-0.4, 0.1) p = 0.246	0.001 (-0.1, 0.1) p = 0.976	0.03 (-0.02, 0.07) p = 0.297	0.01 (-0.2, 0.2) p = 0.919	0.1 (0.02, 0.2) p = 0.019
Weight (kg)	-0.04 (-0.2, 0.1) p = 0.642	-0.5 (-1.0, -0.05) p = 0.030	-0.2 (-0.4, 0.04) p = 0.115	0.05 (-0.06, 0.2) p = 0.349	0.2 (-0.2, 0.6) p = 0.276	0.05 (-0.2, 0.3) p = 0.659
BMI (kg/m^2)	-0.0002 (-0.1, 0.1) p = 0.994	-0.1 (-0.3, 0.02) p = 0.086	-0.06 (-0.1, 0.01) p = 0.091	0.01 (-0.03, 0.05) p = 0.637	0.07 (-0.06, 0.2) p = 0.294	-0.01 (-0.1, 0.08) p = 0.833
Waist-to-hip ratio (%)	0.04 (-0.04, 0.1) p = 0.339	0.01 (-0.2, 0.2) p = 0.930	-0.01 (-0.1, 0.08) p = 0.793	-0.01 (-0.06, 0.04) p = 0.609	-0.05 (-0.2, 0.1) p = 0.530	-0.004 (-0.1, 0.1) p = 0.949
Systolic blood pressure (mmHg)	0.01 (-0.1, 0.1) p = 0.877	0.02 (-0.4, 0.4) p = 0.933	-0.07 (-0.2, 0.1) p = 0.405	-0.07 (-0.2, 0.006) p = 0.070	0.2 (-0.1, 0.4) p = 0.268	0.04 (-0.2, 0.2) p = 0.688
Diastolic blood pressure (mmHg)	-0.01 (-0.1, 0.1) p = 0.863	-0.07 (-0.4, 0.3) p = 0.664	-0.08 (-0.2, 0.06) p = 0.280	-0.04 (-0.1, 0.02) p = 0.168	0.3 (0.08, 0.5) p = 0.010	-0.03 (-0.2, 0.1) p = 0.741
Cholesterol (mg/dL)	-0.1 (-0.6, 0.4) p = 0.649	-1.4 (-2.4, -0.3) p = 0.013	-0.01 (-0.5, 0.5) p = 0.960	0.07 (-0.2, 0.4) p = 0.621	0.5 (-0.3, 1.4) p = 0.234	0.5 (-0.2, 1.1) p = 0.189
HDL (mg/dL)	0.01 (-0.2, 0.2) p = 0.931	0.1 (-0.4, 0.7) p = 0.596	0.002 (-0.3, 0.3) p = 0.987	-0.02 (-0.2, 0.1) p = 0.728	-0.08 (-0.4, 0.3) p = 0.657	-0.04 (-0.2, 0.2) p = 0.675
LDL (mg/dL)	-0.1 (-0.5, 0.3) p = 0.615	-1.3 (-2.2, -0.4) p = 0.005	-0.03 (-0.4, 0.4) p = 0.904	0.1 (-0.1, 0.3) p = 0.365	0.5 (-0.1, 1.1) p = 0.111	0.5 (-0.1, 1.1) p = 0.120
Risk Measure			*OR (95% CI), p-value (p < 0.05 shaded)*			
Risk of obesity (BMI > 30 kg/m^2)	1.02 (0.98, 1.06) p = 0.290	0.91 (0.81, 1.02) p = 0.113	0.96 (0.91, 1.00) p = 0.054	1.00 (0.98, 1.02) p = 0.785	1.03 (0.96, 1.11) p = 0.401	1.00 (0.96, 1.04) p = 0.972
Risk of Metabolic Syndrome	0.95 (0.89, 1.02) p = 0.132	0.79 (0.58, 1.08) p = 0.137	1.00 (0.92, 1.08) p = 0.951	0.99 (0.95, 1.04) p = 0.773	0.92 (0.58, 1.48) p = 0.743	1.02 (0.92, 1.13) p = 0.691
Risk of Any Low Birth Weight Offspring	0.96 (0.92, 1.00) p = 0.061	1.12 (1.00, 1.26) p = 0.043	1.04 (0.98, 1.11) p = 0.179	0.98 (0.95, 1.01) p = 0.192	0.84 (0.65, 1.08) p = 0.171	0.95 (0.87, 1.03) p = 0.213

[1] *In addition to covariates in 4a, also adjusted for: maternal age at birth, parity, prenatal smoking, and immigrant from West; and woman's self-reported overweight at age 10-12, years of education, religiosity, marital status, number of children, frequency of alcohol consumption, and number of cigarettes smoked per day.*

Exploratory mediation analyses - Since *HSD11B2* and *NR3C1* Exon 1F Region 2 methylation were related to both exposure and outcome in our models, we explored their potential to mediate any relationships between early life SES and adult phenotype in our population. We did not find strong evidence that an effect of maternal education on adult phenotype was mediated by *HSD11B2* through either the "early programming" (*e.g.* Direct effect of maternal education on metabolic syndrome risk p = 0.042, mediated risk through *HSD11B2* p = 0.211; *Table 4a*) or "late effects" (*Table 4b*) models. When assuming methylation status is set in early life by the "early programming" model, there was a suggestion of an indirect effect of increasing paternal class on 0.5 cm (95% CI: 0.0002, 0.1) increased height mediated through higher *NR3C1* Exon 1F Region 2 methylation (p = 0.049; *Table 5a*). This effect was not statistically significant when assuming the "late effects" model (*Table 5b*).

Table 4a. Effects of increasing maternal education on daughter's adult phenotype as mediated by HSD11B2, "early programming" model.[1]

Cardiometabolic Measure	*Total Effect of Increasing Years of Education[2]*	*Effect of Increasing Education Not Mediated by HSD11B2 Methylation[3]*	*Indirect Effect Mediated Through HSD11B2[4]*	*Percent Change Relative to Direct Effect[5]*
	β (95% Confidence Interval), p-value (p < 0.05 shaded)			
Height (cm)	0.1 (-0.07, 0.3) p = 0.231	0.1 (-0.05, 0.3) p = 0.180	-0.01 (-0.03, 0.004) p = 0.158	-10%
Weight (kg)	-0.2 (-0.6, 0.2) p = 0.361	-0.2 (-0.5, 0.2) p = 0.453	-0.03 (-0.07, 0.001) p = 0.055	+15%
BMI (kg/m^2)	-0.1 (-0.3, 0.05) p = 0.172	-0.1 (-0.3, 0.06) p = 0.211	-0.01 (-0.02, 0.004) p = 0.169	+10%
Waist to Hip Ratio (%)	-0.01 (-0.2, 0.2) p = 0.896	-0.01 (-0.2, 0.2) p = 0.889	0.001 (-0.02, 0.02) p = 0.934	-10%
Systolic Blood Pressure (mmHg)	0.02 (-0.3, 0.3) p = 0.909	0.01 (-0.3, 0.3) p = 0.934	0.005 (-0.03, 0.04) p = 0.772	+50%
Diastolic Blood Pressure (mmHg)	0.07 (-0.2, 0.4) p = 0.617	0.08 (-0.2, 0.4) p = 0.592	-0.005 (-0.04, 0.02) p = 0.725	-6.3%
Cholesterol (mg/dL)	0.1 (-0.8, 1.0) p = 0.808	0.2 (-0.7, 1.1) p = 0.637	-0.1 (-0.2, 0.02) p = 0.113	-50%
HDL (mg/dL)	0.4 (-0.07, 0.8) p = 0.101	0.4 (-0.08, 0.8) p = 0.106	0.005 (-0.04, 0.05) p = 0.845	+1.3%
LDL (mg/dL)	0.04 (-0.7, 0.8) p = 0.918	0.1 (-0.6, 0.9) p = 0.719	-0.1, (-0.2, 0.01) p = 0.078	-100%
Cardiometabolic Risk	*OR (95 % Confidence Interval), p-value (p < 0.05 shaded)*			
Obese (BMI ≥ 30 kg/m^2)	0.97 (0.90, 1.05) p = 0.437	0.97 (0.90, 1.05) p = 0.504	0.99 (0.99, 1.00) p = 0.195	-1%
Metabolic syndrome	0.85 (0.73, 0.99) p = 0.035	0.86 (0.74, 0.99) p = 0.042	0.98 (0.96, 1.01) p = 0.211	-2%
Any low birth weight offspring	0.87 (0.77, 0.99) p = 0.041	0.86 (0.75, 0.98) p = 0.023	1.02 (1.00, 1.04) p = 0.108	+2%

[1] All models adjusted for maternal factors: age at daughter's birth; whether she emigrated from the West, any prenatal smoking, parity, and pre-pregnancy BMI, as well as daughter's age and birth weight. Models are estimated based on individuals with full data on maternal education, methylation, and outcome.

[2] Total effect of years of maternal education on adult cardiometabolic measures.

[3] Direct effect of years of maternal education on adult cardiometabolic measures, adjusted for percent HSD11B2 methylation. This assumes methylation is stable and not affected by woman's life course mediators (i.e. childhood overweight and adult marital status, educational attainment, number of children, religiosity, and any smoking or drinking).

[4] Standard errors bootstrapped over 50 replications.

[5] Represents the proportion by which the mediated pathway through HSD11B2 methylation reduces or increases the observed direct effect. (e.g. If the direct effect of education is negative and the indirect effect is also negative, the percent change is positive.)

[6] Percent change in odds of the outcome (calculated by OR – 1).

Table 4b. Effects of increasing maternal education on daughter's phenotype as mediated by HSD11B2, "late effect" model.[1]

Cardiometabolic Measure	*Total Effect of Increasing Education[2]*	*Effect of Increasing Education Not Mediated by HSD11B2 Methylation[3]*	*Indirect Effect Mediated Through HSD11B2[4]*	*Percent Change Relative to Direct Effect[5]*
	β (95% Confidence Interval), p-value (p < 0.05 shaded)			
Height (cm)	0.1 (-0.08, 0.3) p = 0.267	0.1 (-0.07, 0.3) p = 0.214	-0.01 (-0.03, 0.01) p = 0.310	-10%
Weight (kg)	0.05 (-0.3, 0.4) p = 0.806	0.09 (-0.3, 0.5) p = 0.642	-0.04 (-0.1, 0.01) p = 0.140	-44%
BMI (kg/m^2)	-0.02 (-0.2, 0.1) p = 0.808	-0.01 (-0.1, 0.1) p = 0.942	-0.01 (-0.03, 0.007) p = 0.214	+100%
Waist to Hip Ratio (%)	0.03 (-0.2, 0.2) p = 0.721	0.04 (-0.2, 0.2) p = 0.721	-0.0003 (-0.02, 0.02) p = 0.978	-0.8%
Systolic Blood Pressure (mmHg)	0.2 (-0.2, 0.5) p = 0.298	0.2 (-0.2, 0.5) p = 0.304	0.001 (-0.03, 0.03) p = 0.949	+0.5%
Diastolic Blood Pressure (mmHg)	0.2 (-0.1, 0.4) p = 0.293	0.2 (-0.1, 0.5) p = 0.269	-0.01 (-0.04, 0.02) p = 0.581	-5%
Cholesterol (mg/dL)	0.2 (-0.7, 1.1) p = 0.678	0.3 (-0.6, 1.2) p = 0.471	-0.1 (-0.3, 0.03) p = 0.110	-33%
HDL (mg/dL)	0.04 (-0.4, 0.5) p = 0.871	0.02 (-0.4, 0.5) p = 0.918	0.01 (-0.05, 0.08) p = 0.685	+50%
LDL (mg/dL)	0.3 (-0.5, 1.1) p = 0.486	0.4 (-0.4, 1.3) p = 0.302	-0.1 (-0.3, 0.03) p = 0.109	-25%
Cardiometabolic Risk				
Obese (BMI ≥ 30 kg/m^2)	1.01 (0.93, 1.10) p = 0.819	1.02 (0.94, 1.10) p = 0.705	0.99 (0.98, 1.01) p = 0.370	-1%[6]
Metabolic syndrome	0.91 (0.79, 1.05) p = 0.189	0.92 (0.80, 1.06) p = 0.260	0.98 (0.94, 1.02) p = 0.314	-2%[6]
Any low birth weight offspring	0.87 (0.75, 1.00) p = 0.048	0.85 (0.73, 0.98) p = 0.026	1.02 (0.99, 1.04) p = 0.127	+2%[6]

[1] All models adjusted for maternal factors: age at daughter's birth; whether she emigrated from the West, any prenatal smoking, parity, and pre-pregnancy BMI, as well as daughter's age and birth weight. Also adjusted for daughter's life course mediators: childhood overweight, years of education, marital status, religiosity, number of children, and frequency of alcohol and cigarette use. Models are estimated based on individuals with full data on maternal education, methylation, and outcome.

[2] Total effect of years of maternal education on daughter's cardiometabolic measures, adjusted for daughter's life course mediators.

[3] Direct effect of years of maternal education on daughter's cardiometabolic measures, adjusted for mediators and percent HSD11B2 methylation.

[4] Standard errors bootstrapped over 50 replications.

[5] Represents the proportion by which the mediated pathway through HSD11B2 methylation reduces or increases the observed direct effect.

[6] Percent change in odds of the outcome (calculated by OR – 1).

Table 5a. Effects of increasing paternal occupational class on daughter's phenotype as mediated by NR3C1 Exon 1F.2, "early programming" model.[1]

Cardiometabolic Measure	*Total Effect of Increasing Class[2]*	*Effect of Increasing Class Not Mediated by NR3C1 Exon 1F-2 Methylation[3]*	*Indirect Effect Mediated Through NR3C1 Exon 1F-2[4]*	*Percent Change Relative to Direct Effect[5]*
	β (95% Confidence Interval), p-value (p < 0.05 shaded)			
Height (cm)	-0.04 (-0.5, 0.4) p = 0.854	-0.1 (-0.5, 0.4) p = -0.675	0.05 (0.0002, 0.1) p = 0.049	-50%
Weight (kg)	-0.9 (-2.0, 0.2) p = 0.096	-1.0 (-2.1, 0.1) p = 0.077	0.06 (-0.09, 0.2) p = 0.406	-6%
BMI (kg/m^2)	-0.3 (-0.7, 0.08) p = 0.120	-0.3 (-0.7, 0.08) p = 0.110	0.01 (-0.05, 0.07) p = 0.737	-3%
Waist to Hip Ratio (%)	0.2 (-0.3, 0.7) p = 0.363	0.2 (-0.3, 0.7) p = 0.357	-0.01 (-0.08, 0.07) p = 0.878	-5%
Systolic Blood Pressure (mmHg)	-0.4 (-1.2, 0.3) p = 0.265	-0.5 (-1.2, 0.3) p = 0.253	0.02 (-0.09, 0.1) p = 0.720	-4%
Diastolic Blood Pressure (mmHg)	-0.4 (-1.1, 0.3) p = 0.291	-0.4 (-1.1, 0.3) p = 0.290	-0.0003(-0.08, 0.08) p = 0.994	+0.1%
Cholesterol (mg/dL)	-2.3 (-5.4, 0.8) 0.147	-2.4 (-5.6, 0.7) p = 0.127	0.1 (-0.3, 0.5) p = 0.501	-4%
HDL (mg/dL)	0.06 (-1.1, 1.3) p = 0.923	0.09 (-1.1, 1.3) p = 0.885	-0.03 (-0.1, 0.07) p = 0.551	-33%
LDL (mg/dL)	-1.8 (-4.4, 0.8) p = 0.183	-1.9 (-4.5, 0.7) p = 0.146	0.2 (-0.09, 0.4) p = 0.196	-7%
Cardiometabolic Risk	*OR (95 % Confidence Interval), p-value (p < 0.05 shaded)*			
Obese (BMI ≥ 30 kg/m^2)	0.85 (0.69, 1.04) p = 0.120	0.85 (0.69, 1.04) p = 0.112	1.00 (0.97, 1.03) p = 0.807	--
Metabolic syndrome	0.85 (0.60, 1.20) p = 0.361	0.85 (0.60, 1.20) p = 0.346	1.01 (0.92, 1.12) p = 0.787	-1%[6]
Any low birth weight offspring	0.88 (0.64, 1.20) p = 0.412	0.89 (0.65, 1.21) p = 0.453	0.99 (0.96, 1.02) p = 0.570	+1%[6]

[1] All models adjusted for maternal factors: age at daughter's birth; whether she emigrated from the West, any prenatal smoking, parity, and pre-pregnancy BMI, as well as daughter's age and birth weight. Models are estimated based on individuals with full data on maternal education, methylation, and outcome.

[2] Total effect of increasing early life SEP as defined by father's occupational class at birth on adult cardiometabolic measures.

[3] Direct effect of increasing early life SEP on adult cardiometabolic measures, adjusted for percent NR3C1 Exon 1F-2 methylation. This assumes methylation is stable and not affected by woman's life course mediators.

[4] Standard errors bootstrapped over 50 replications.

[5] Represents the proportion by which the mediated pathway through NR3C1 Exon 1F-2 methylation reduces or increases the observed direct effect.

[6] Percent change in odds of the outcome (calculated by OR – 1).

Table 5b. Effects of increasing paternal occupational class on daughter's phenotype as mediated by NR3C1 Exon 1F.2, "late effects" model.[1]

Cardiometabolic Measure	*Total Effect of Increasing Class[2]*	*Effect of Increasing Class Not Mediated by NR3C1 Exon 1F-2 Methylation[3]*	*Indirect Effect Mediated Through NR3C1 Exon 1F-2[4]*	*Percent Change Relative to Direct Effect[5]*
	β (95% Confidence Interval), p-value (p < 0.05 shaded)			
Height (cm)	-0.01 (-0.5, 0.5) p = 0.955	-0.07 (-0.5, 0.4) p = 0.770	0.06 (-0.02, 0.1) p = 0.125	-86%
Weight (kg)	-1.1 (-2.2, 0.01) p = 0.053	-1.1 (-2.2, 0.02) p = 0.045	0.04 (-0.09, 0.2) p = 0.547	-4%
BMI (kg/m^2)	-0.4 (-0.01, 0.8) p = 0.056	-0.4 (-0.8, 0.01) p = 0.055	-0.0002 (-0.05, 0.05) p = 0.993	+0.1%
Waist to Hip Ratio (%)	0.1 (-0.3, 0.6) p = 0.587	0.1 (-0.3, 0.6) p = 0.577	-0.004 (-0.05, 0.04) p = 0.882	-4%
Systolic Blood Pressure (mmHg)	-0.3 (-1.0, 0.4) p = 0.405	-0.3 (-1.1, 0.4) p = 0.378	0.02 (-0.09, 0.1) p = 0.684	-7%
Diastolic Blood Pressure (mmHg)	-0.3 (-1.0, 0.4) p = 0.441	-0.3 (-1.0, 0.4) p = 0.454	-0.01 (-0.1, 0.1) p = 0.851	+3%
Cholesterol (mg/dL)	-2.4 (-5.7, 1.0) p = 0.165	-2.6 (-6.0, 0.7) p = 0.125	0.3 (-0.2, 0.7) p = 0.273	-12%
HDL (mg/dL)	-0.2 (-1.4, 1.0) p = 0.765	-0.2 (-1.4, 1.0) p = 0.791	-0.02 (-0.1, 0.09) p = 0.713	+10%
LDL (mg/dL)	-1.7 (-4.4, 1.1) p = 0.233	-1.9 (-4.7, 0.8) p = 0.170	0.3 (-0.06, 0.6) p = 0.115	-16%
Cardiometabolic Risk	*OR (95 % Confidence Interval), p-value (p < 0.05 shaded)*			
Obese (BMI ≥ 30 kg/m^2)	0.82 (0.64, 1.04) p = 0.103	0.81 (0.64, 1.04) p = 0.100	1.00 (0.98, 1.03) p = 0.890	--
Metabolic syndrome	1.27 (0.85, 1.89) p = 0.246	1.27 (0.85, 1.85) p = 0.245	1.01 (0.61, 1.67)[4] p = 0.976	-1%
Any low birth weight offspring	0.88 (0.64, 1.22) p = 0.460	0.90 (0.65, 1.25) p = 0.527	0.99 (0.94, 1.03) p = 0.572	+1%

[1] All models adjusted for maternal factors: age at daughter's birth; whether she emigrated from the West, any prenatal smoking, parity, and pre-pregnancy BMI, as well as daughter's age and birth weight. Models are estimated based on individuals with full data on maternal education, methylation, and outcome.

[2] Total effect of increasing early life SEP (6 through 1) as defined by father's occupational class at birth on adult cardiometabolic measures.

[3] Direct effect of increasing early life SEP on daughter's cardiometabolic measures, adjusted for mediators and percent NR3C1 Exon 1F-2 methylation.

[4] Standard errors bootstrapped over 50 replications. One parameter's standard error did not bootstrap well and a different seed number was required.

[5] Represents the proportion by which the mediated pathway through NR3C1 Exon 1F-2 methylation reduces or increases the observed direct effect.

[6] Percent change in odds of the outcome (calculated by OR – 1).

Discussion

Overall, we found some evidence that lower early life SES, defined by lower paternal occupational class or fewer years of maternal education, were associated with reduced methylation at candidate genes *ABCA1*, *HSD11B2*, and *NR3C1* Exon 1F. To our knowledge, the current study is the first to report associations between early life SES and methylation at these genes amongst young adult women. Moreover, findings for both early life SES-adult methylation (*Tables 2a-d*) were fairly consistent across models, including after sensitivity measures such as adjustments for life course factors and multiple imputation. This suggests DNA methylation is influenced by early life environment, associated with adult disease risk, and potentially independent of life course events. This is consistent with a hypothesis of early life programming of cardiometabolic risk. However, in exploratory analysis, we did not find substantial evidence for average methylation in these regions mediating birth SES-adult phenotype relationships.

While most studies of early life adversity have assessed methylation more globally,[19-21,51] several studies have investigated associations with candidate genes. For example, Appleton, *et al*. found an association between mothers with high school or greater educational attainment and a 9 percentage-point higher maternal-side placental methylation of the *HSD11B2* glucocorticoid inactivating enzyme relative to mothers with less than a high school education ($\beta = 8.8$; $p < 0.05$; $N = 444$) after adjusting for maternal age, pre-pregnancy BMI, race, infant sex, and birth weight percentile.[34] We found a positive association between maternal educational attainment and adult female offspring *HSD11B2* leukocyte methylation adjusted for similar characteristics, albeit an order of magnitude lower (*e.g.* 12 years of education * 0.08 = ~1 percentage point higher for high school education). Despite a noted discordance between methylation of different offspring tissues,[41,52] our findings suggest plausibility of a systemic, compensatory mechanism in response to early life adversity that persists into young adulthood, complementing findings by Appleton, *et al*.

Several studies have reported associations between maternal perinatal stress with differential *NR3C1* methylation in newborn and adolescent offspring: Using principle components analysis, Mulligan, *et al*. found a positive association between maternal exposure to war stress and the first principle component of newborn *NR3C1* promoter methylation from umbilical cord blood (Pearson's correlation r = 0.57, p = 0.003).[16] Contrastingly, Radtke, *et al*. found intimate partner violence experienced by the mother during pregnancy was associated with a 2 percentage-point reduction in adolescent (mean age = 14.1 years) offspring *NR3C1* promoter methylation ($p < 0.05$).[17] We found evidence that lower paternal occupational class (early life SEP) was related to reduced young adult *NR3C1* Exon 1F Region 2 methylation (β = -0.5 [95% CI: -0.9, -0.030]; p = 0.036). This region corresponds to a 363 base pair sequence (Chromosome 5: 142,783,885-142,784,248; GRCh37 human genome build 19) 3' of the identified Exon 1F regulatory region for the NR3C1 glucocorticoid receptor.[52] Extending previous methylation profiling work in newborns[16] and adolescents,[17] our findings support the plausibility that early socioeconomic adversity may result in different *NR3C1* methylation profiles in adults. This is relevant since response to glucocorticoids could be an important pathway relating early life adversity to adult cardiometabolic risk.[7,31, 53]

We also found evidence for an association between higher early life SES and increased young adult *ABCA1* methylation (adjusted 0.5 %-points per higher paternal occupational class, p = 0.048). One previous study by Tobi, *et al*. found an associations between famine exposure in early gestation and higher ABCA1 methylation (0.7 %-points relative unexposed siblings, p = 0.017) amongst older adults (~58 years of age).[29] This study also found famine to be associated with increased *INSIGF* and decreased *LEP* methylation.[29] However, we did not find associations between birth SES and *LEP* or *INSIGF* promoter methylation. Famine exposure may influence DNA methylation through different pathways than social stressors, including through overt, maternal nutritional deprivation of methyl donors like

folate.[18,29,54] Therefore, famine exposure may have different associations to methylation profiles than early life socioeconomic status as we have measured it. Moreover, *ABCA1*, *LEP*, and *INSIGF* methylation been shown to be extra sensitive to external environment over time despite substantial concordance in studies of monozygotic twins.[55] Our findings that associations with these gene regions did not exist even after adjusting for potential life course mediators suggests life course, postnatal factors may be substantially important for adult DNA methylation in these genes relative to early life SES.

In this current study, we did not find strong evidence for candidate gene methylation playing a specific mediating role in early life SES-adult outcome relationships. We can identify several reasons for this: It is possible that our exploratory mediation model is incorrect due to improper model specifications or residual confounding. For example, while we adjusted for birth weight, for consistency with other studies[34] and to account for stratified sampling, birth weight is likely an important mediator which captures much of the variance relating birth SES to adult outcome.[14] While we did not find systematic evidence for simple additive interaction between exposure and mediators, there may be a more complex un-modelled relationship.[49] Finally, the effect of early life SES may be exerted by pathways not captured by average methylation in these candidate gene regions.[28]

There are several notable strengths to our study: First, we use state-of-the-art mass spectrometry methods to profile methylation at candidate genes previously found to be related to early life conditions and also pertinent to adult cardiometabolic function. Additionally, our study of 613 women is one of the largest attempting to relate *in utero* environment and candidate gene methylation in adult subjects. Consequently, we were able to detect effect sizes that were quite small. Moreover, we investigated potential associations amongst women given the potential importance of early life factors on reproductive outcomes and intergenerational transmission of health. Finally, our study is conducted amongst a well-characterized population with extensive information on maternal

demographics, pregnancy course, and offspring characteristics and biomarkers at an average age of 32 years.[32] This study of births from 1974-76 in Jerusalem consists of a unique population subject to many historical stressors: The growth of the state of Israel since its formation in 1948 has been marked by large influxes of refugees and immigrants driven by religious and political pressures, as well as social strife from multiple wars.[35] In our study population, 47% of mothers were born somewhere other than Israel and experienced several wars during their lifetimes.[35,56] On the other hand, Israel has developed very rapidly as a modern market economy with female education averaging 16 years and a GDP per capital exceeding $36,000 USD as of 2013, making it 37th in the world.[57] Persistent changes in DNA methylation subsequent to early life adversity may operate cyclically, over generations, to produce disproportionate morbidity and mortality.[21,26,27] This phenomenon has particular implications for nations undergoing rapid transitions to industrial/post-industrial economies. Recent improvements in later life conditions may mask increased susceptibility to morbidity due to early life and intergenerational adversity.[58] Consequently, this population is ideal to study the effects of adverse maternal experience in the context of general socioeconomic improvement for the offspring generation. This context is unique and indeed our results show that adjustment for offspring life course factors changes associations very little.

However, some limitations of the current study deserve mention. Notably, caution is needed in interpreting and generalizing our findings. For example, Israeli women are required to serve in the military following completion of the twelfth and final year of compulsory education. Women may defer such service if they are admitted to a University or other higher educational training opportunity, identify as ultra-Orthodox, or are pregnant. As a result, approximately half all women serve in the military[32] in a manner that is dependent on education, childbearing, and religiosity. While our adjustment models account for these factors, the dynamics of life course stressors experienced by women in our study may be

unique to Israeli citizens. For example, our paternal occupational class measure groups fathers whose occupations are Yeshiva students (therefore, religious or ultra-Orthodox) as class 2, or 'high' SES. Previous studies have found this to be problematic (Savitsky, *et al.*, In Review), as Yeshiva students and their families are generally poor and more likely to have poorer health. Despite this, we found that candidate gene methylation assocations generally occurred in the same direction based on different measures of SES, lending confidence to a more general association between stresses of early life SES and adult methylation status. Another limitation is our ability to only capture candidate gene DNA methylation in peripheral blood leukocytes at age 32. It is possible that developmental effects of early life SES may be exerted in different tissues, different genes, and at earlier ages.[28,38,52] While we did not see substantial changes of estimates of associations when adjusting for life course mediators, we also did not have measures of newborn or infant methylation to directly test hypotheses regarding stable, early life programming. Nonetheless, early life methylation changes have been shown to occur systematically, across multiple organs, and persist over the life course.[9,28,29] Finally, in an effort to limit Type II error at the cost of a higher potential for Type I error,[59] we opted not to implement any adjustments for multiple hypothesis tests. We felt this was justified since our exposures, outcomes, and covariates were specified *a priori* from past research, with a reasonable expectation to find significant results. One correction we might have considered performing was for testing four measures of early life SES against each gene. Had we applied a Benjamin-Hochberg correction[60] with a false discovery rate of 5%, each early life SES-adult methylation association would have to meet the threshold of $p < 0.0125$ (0.25 * 0.05). Under this criterion, the positive association between maternal years of education and *HSD11B2* methylation would still be statistical significant ($p = 0.012$), though associations with *ABCA1* and *NR3C1* would not be (*Table 2b*).

In summary, we found some evidence of association of early life SES with young adult methylation in cardiometabolic and stress response-related genes amongst young adult women. In this young adult population, we did not find significant evidence for mediation of the early life SES-adult phenotype relationship by average methylation at our candidate gene regions. Future studies should evaluate the potential causal mechanisms (if any) and consequences of these relationships. This can include different parameterizations of early life SES sensitive to the specific relevance of the measure to mothers, measures of peripheral blood methylation at different time points (including early gestation, birth, and later life), and attempts to implement causal modeling and sensitivity analyses more robust to interactions and unmeasured confounding. The potential for methylation to be used for the early identification of cardiometabolic risk in individuals highlights the significance of future research in this area.

(*End Chapter 3*)

Discussion

Executive Summary

Parental socioeconomic status (SES) experienced by a woman *in utero* may directly affect her adult health and reproductive outcomes through epigenetic mechanisms that are independent of her life course experiences. Using a U.S.-national, longitudinal cohort, Add Health, we investigated the effect of mother's education on cardiometabolic risk and pregnancy outcomes among women averaging 30 years of age. Using an Israeli birth cohort, JPS-1, we investigated associations between parental education and father's occupational class on DNA methylation at cardiometabolic genes in 32-year old women.

(1) Using marginal structural models estimated by inverse probability weighting, we found young adult women whose mothers had higher educational attainment (*e.g.* college versus high school) had 40% lower risk (Odds Ratio = 0.60, 95% Confidence Interval: 0.45, 0.80) of metabolic syndrome, independent of childhood maltreatment, adolescent overweight, adult SES, and behavioral risk. Additionally, there was evidence that women with mothers who have higher education attainment were taller, thinner, and had a smaller waist, lower resting pulse rate, lower levels of inflammatory markers, and better blood sugar control.

(2) Additionally, women born to mothers with higher educational attainment had children delivered who were 90 grams heavier (95% CI: 20.8, 156.5), independent of childhood maltreatment, pre-pregnancy overweight, adult SES, and prenatal smoking. Moreover, results from (1) and (2) were robust to several sensitivity analyses including model alteration, data replacement, and quantitative bias analyses.

(3) Finally, we found that lower paternal occupational class was associated with reduced methylation at regions of the *ABCA1* cholesterol transporter and the *NR3C1* glucocorticoid receptor genes, after adjusting for numerous parental and offspring characteristics. Similarly, fewer years of mother's education was associated with reduced *HSD11B2*

glucocorticoid-inactivating enzyme gene methylation. There was also evidence that reduced *HSD11B2* methylation was associated with increased cardiometabolic risk markers. However, such associations did not appear to mediate early life SES and adult phenotype relationships.

Overall, there appears to be substantial evidence that early life SES is independently related to women's adult health, reproductive outcomes, and DNA methylation, however the mechanisms relating them require further elucidation.

Strengths and Limitations

A major strength of this dissertation is the use of rich longitudinal data sets, containing many measures of women's health, socioeconomic status, and behaviors across her life course. Subsequently, marginal structural modeling (MSM) estimated by inverse probability weighting were used to estimate direct effects of early life SES while controlling for these inter-related mediators without introducing substantial bias. Moreover, several sensitivity analyses procedures were implemented specifically to address known limitations of the MSM method, namely assumptions about causal structure and unmeasured confounding. Finally, the likelihood of epigenetic mechanisms playing a role in this effect was tested directly by state-of-the-art quantification of candidate gene methylation through mass spectrometry.

Notable limitations include the inability to control for other potential predictors of adult cardiometabolic phenotype including maternal smoking (in Add Health), nutritional factors, or environmental exposures such as air pollution. Also, we were unable to measure DNA methylation in either different tissues or different time points. Such measures would lend themselves to analysis better suited to evaluate the potential mediating effect of DNA methylation. Moreover, the stratified sampling method of the JPS study made implementing MSM difficult, thus we used a product of coefficients approach to mediation analysis, despite its known limitations in the presence of possible interactions and endogenous confounding.

Finally, we lacked biomarkers and other measures of early life exposure, such as maternal or fetal levels of cortisol. Thus, we could not investigate ways in which measures of early life SES may be directly related to the intrauterine environment.

Public Health Significance

Overall, this study suggests the importance of maternal education attainment for the health of her offspring. This was observed as increased cardiometabolic risk and lower birth weight amongst women whose mothers had lower education at the time of their birth. This is coupled with evidence that the effect is independent of the woman's experiences through her own life and may be associated with epigenetic programming. This suggests any effects of early life socioeconomic differences on adult health cannot be corrected merely by improving the behaviors or socioeconomic conditions of adult women at risk of cardiometabolic disease or poor pregnancy outcome. Consequently, policies seeking to address women's health disparities in adulthood should consider population level interventions to improve women's SES while measuring outcomes in the next generation.

Next Steps

In our study, the use of MSM did not necessarily suggest where bias may have been introduced by traditional adjustment, perhaps we corrected endogenous confounding by racial perceptions when adjusting for race. More work should be done to describe factors that confound life course-outcome relationships that are also a consequence of the early life SES (*i.e.* endogenous confounders). Incorporating these factors will further strengthen evidence for causal effects of early life SES. Additionally, a natural extension of our current work is to implement MSM analyses with DNA methylation in a population where data collection, including methylation assays at different time points, may be more amenable. Samples should be drawn from multiple tissues, profiled across more gene regions, and assessed for differential expression, in order to strengthen mechanistic evidence.

Finally, our study suggests that public health intervention to address health disparities is incomplete without addressing early life or intergenerational conditions. Consequently, future efforts should be directed at identifying specific aspects of early life socioeconomic status, particularly maternal education, as targets for interventions. Furthermore, future work should consider how DNA methylation marks may be used as early indicators of adult health risk and how such biomarkers may also be used to inform interventions.

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Chapter 1 – Direct effects of maternal education at daughter's birth on daughter's adult cardiometabolic risk

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