Published in final edited form as:

Psychosom Med. 2016 ; 78(9): 1053–1065. doi:10.1097/PSY.0000000000000411.

Epigenetic Mediators Between Childhood Socioeconomic Disadvantage and Mid-Life Body Mass Index: The New England Family Study

Eric B. Loucks, PhD^{1,*}, Yen-Tsung Huang, MD, ScD^{1,*}, Golareh Agha, PhD², Su Chu, MSc¹, Charles B. Eaton, MD, MSc^{1,3}, Stephen E. Gilman, ScD^{4,5}, Stephen L. Buka, ScD¹, Karl T. Kelsey, MD, ScM¹

¹Brown University School of Public Health, Department of Epidemiology, Providence, RI, USA

²Harvard T.H. Chan School of Public Health, Department of Environmental Health, Boston, MA, USA

³Brown University Warren Alpert Medical School, Department of Family Medicine, Providence, RI, USA

⁴Health Behavior Branch, Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, MD, USA

⁵Department of Social & Behavioral Sciences and Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

Abstract

OBJECTIVE—Childhood socioeconomic disadvantage is associated with adulthood obesity risk, however epigenetic mechanisms are poorly understood. Objectives were to evaluate whether associations of childhood socioeconomic disadvantage with adulthood body mass index (BMI) are mediated by DNA methylation.

METHODS—Participants were 141 men and women from the New England Family Study, prospectively followed prenatally through mean age 47 years. Epigenome-wide DNA methylation was evaluated in peripheral blood and adipose tissue obtained at adulthood, using the Infinium HumanMethylation450K BeadChip. Childhood socioeconomic status (SES) at age 7 years was assessed directly from parent reports. Offspring adiposity was directly assessed using BMI at mean age 47 years. Associations of SES, DNA methylation, and BMI were estimated using least square estimators. Statistical mediation analyses were performed utilizing joint significance test and bootstrapping.

RESULTS—Of CpG sites significant at the 25% false discovery rate level in epigenome-wide methylation-BMI analyses, 91 sites in males and 71 sites in females were additionally significant for SES-methylation associations ($P < 0.001$) in adipose tissue. Many involved genes biologically relevant for development of obesity, including fatty acid synthase (*FASN*), transmembrane protein

Corresponding Author: Eric B. Loucks, Ph.D., Department of Epidemiology, Brown University School of Public Health, 121 South Main St., Providence, RI, USA 02912, Phone: 401-863-6283, Fax: 401-863-7713, eric.loucks@brown.edu.

*Shared first authorship

88 (*TMEM88*), signal transducer and activator of transcription 3 (*STAT3*), and neuritin 1 (*NRN1*). There was no evidence of epigenetic mediation in peripheral blood leukocytes.

CONCLUSIONS—DNA methylation at specific genes may be mediators of associations between childhood socioeconomic disadvantage and mid-life BMI in adipose tissue. Findings motivate continued efforts to study if and how childhood socioeconomic disadvantage is biologically embedded at the level of the epigenome in regions etiologically relevant for adiposity.

Keywords

DNA methylation; epigenetics; adiposity; body mass index; socioeconomic disadvantage; socioeconomic status

INTRODUCTION

Adiposity is an important risk factor for many aging-related conditions such as type 2 diabetes, cardiovascular disease, cancer, injuries, physical function disabilities, and premature mortality (1,2). With current trends, total healthcare costs attributable to obesity could reach \$957 billion by 2030, accounting for approximately 18% of US health expenditures (3). The etiology of adiposity is multifactorial, and known to have genetic (4,5) and environmental determinants (6,7). What is becoming increasingly understood is that the origins of obesity are detectable early in the life course (8–10). While obesity disproportionately affects lower socioeconomic groups in adulthood, evidence suggests socioeconomic inequalities in obesity may also originate early in the life course, particularly among women (11). For example, a systematic review found inverse associations between childhood SES and adulthood obesity in 70% (14 of 20) of studies in females and 27% (four of fifteen) in males, indicating possible sex differences in the relation between childhood SES and adulthood adiposity (11).

Focus has turned to understanding the biological mechanisms by which genetic and environmental factors operate. Epigenetic mechanisms have been demonstrated to link the internal genetic landscape and external environmental influences, and it is recognized that adiposity and related cardiometabolic risk may arise as results of dysregulated cellular and metabolic programming via epigenetic mechanisms (12,13). Better knowledge of the precise nature of the alterations that give rise to this phenotype will improve the etiologic understanding of adiposity, and could potentially provide treatment targets. Several prior studies investigated epigenetic underpinnings of adiposity, as assessed by DNA methylation (14–23), however almost all studies used only blood (14–21,23). Blood may not be the most relevant tissue for research on the epigenetics of adiposity. Investigating DNA methylation in more targeted tissues, such as adipose tissue, may better elucidate the epigenetic mechanisms involved (24). Using adipose tissue, we recently showed that adiposity phenotypes (e.g. body mass index, android fat mass) were associated with DNA methylation in several genes that are biologically relevant to the development of adiposity, such as lipase E (*LIPE*), amine oxidase copper containing 3 (*AOC3*), extracellular superoxide dismutase 3 (*SOD3*), aquaporin 7 (*AQP7*), hypoxia-inducible factor 3 alpha (*HIF3A*), and cholesteryl ester transfer protein (*CETP*) (25). In contrast, blood DNA methylation profiles were not associated with adiposity, either before or after adjustment for leukocyte cell mixture effects

(25). Research on epigenetic regulation of adiposity is still in the early phase, consequently many relevant genomic sites under epigenetic control likely remain to be discovered.

There is increasing interest in elucidating biological pathways by which adverse early life exposures affect adiposity, with strong plausibility for epigenetic mechanisms. Epigenetics play a central role during fetal development, where methylation is associated with somatic lineage differentiation. Early life is thus a sensitive period during which external environmental stimuli may have considerable influence on the establishment of epigenetic patterning. However, few studies have investigated whether socioeconomic status during childhood is related to DNA methylation profiles (26–31). Beyond this, none have had the opportunity to investigate DNA methylation profiles in adipose tissue (in contrast to blood), which may be the optimal strategy to investigate these hypotheses. Furthermore, to our knowledge, no studies to date have performed formal mediation analyses evaluating whether epigenetic methylation patterns may be mediators of observed associations between early life socioeconomic disadvantage and later life adiposity. Consequently, objectives of this study were to evaluate whether associations of childhood socioeconomic disadvantage with adulthood body mass index are mediated by alterations in DNA methylation in CpG (cytosine-phosphate-guanine) sites, assessed in peripheral blood leukocytes and subcutaneous adipose tissue samples obtained in adulthood. If DNA methylation explains part of the association between childhood disadvantage and adult adiposity, this should be detected by statistical mediation analyses. The mediation analyses themselves are not dispositive of a causal effect of disadvantage on methylation and a resulting causal effect of methylation on adiposity; such inferences require further assumptions regarding confounding, including no unmeasured SES-methylation confounders, and no unmeasured methylation-adiposity confounders.

METHODS

Sample

Study participants were from the New England Family Study (NEFS), which comprises a series of follow-up studies of subsamples of the 17,921 offspring of pregnant women enrolled in the Collaborative Perinatal Project (CPP) in the study's Providence, Rhode Island, and Boston, Massachusetts, sites (United States) between 1959 and 1966 (32). The current NEFS sub-study, named the Longitudinal Effects on Aging Perinatal (LEAP) Project, is comprised of Providence-born participants assessed between 2010–2011. Of 796 participants eligible for assessment (i.e. not deceased, not incarcerated, had assessments taken at age 7 years, were located, and lived within 100 miles of the clinical assessment site), we were able to establish contact with 522 (76%) of the participants within the relatively brief 13-month data collection period, and invited them to participate in the study. Of these 522 participants, 19% (n=95) refused to participate, and a further 5% (n=27) agreed to participate but were unable to schedule assessments within the data collection period. This left 400 participants on whom assessments were made. Of these, 316 had adequate adipose tissue biopsy performed, 68 refused, and 16 had inadequate biopsy specimens. Blood and adipose tissue DNA methylation analyses were performed on a representative sample of 144 of these 316 participants. With the exception of adipose tissue for one participant, samples

yielded adequate DNA methylation data for blood and adipose tissue analyses for all 144 participants. Of the 143 participants with both adipose and blood methylation data, 2 were missing age 7 SES. Consequently, analyses were performed on 141 participants for adipose tissue analyses, and 142 participants for blood analyses. Characteristics of participants with DNA methylation data did not differ significantly from LEAP participants without methylation data, except that mean BMI was greater in participants with vs. without DNA methylation data (31.5 vs. 29.7 kg/m²; $p=0.02$). This was likely because adipose tissue samples of adequate amount for methylation assays were more readily obtained when there were larger amounts of adipose tissue to harvest from. The study protocol was approved by the institutional review boards at Brown University and Memorial Hospital of Rhode Island. All participants provided informed consent.

Tissue Sample Collection, Methylation Profiling, and Data Processing

Subcutaneous adipose tissue samples were collected from the upper outer quadrant of the buttock using a 16-gauge needle and disposable syringe. DNA was extracted from adipose tissue samples using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) and the Zymo Genomic DNA Clean & Concentrator Kit, according to the manufacturers' protocol. Whole blood samples were centrifuged to obtain buffy coat, and peripheral blood leukocyte DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA), according to the manufacturers' protocol. DNA was sodium-bisulfite converted using the EZ-96 DNA Methylation-Direct and EZ DNA Methylation-Direct kits (Zymo Research, Orange, CA; as per manufacturer's instructions). Blood and adipose tissue samples were randomized across plates, and analyzed using the Infinium HumanMethylation450 BeadChip array (Illumina, San Diego, CA) at the Genomics Core Facility at the UCSF Institute for Human Genetics (San Francisco, CA), following standard Illumina protocols. Control probe data was used to assess the quality of the array data (total signal, distribution of detection p-values, fraction of missing values). One adipose tissue sample was excluded due to having a detection p-value >0.05 for $>1\%$ of probes.

The DNA methylation data were out-of-band background corrected, dye bias-corrected (33), and further normalized using the Beta-Mixture Quantile Dilation (BMIQ) approach (34) (in order to obtain similar ranges for type I vs. type II probes on the infinium array).

Methylation beta values for each CpG was calculated as $M/(M+U+\epsilon)$, where M and U refer to the signal from the probe corresponding to the methylated and unmethylated target CpG, respectively, and $\epsilon=100$ in order to protect against division-by-zero. We also removed non-CpG sites (rs and ch probes), CpG sites located on sex chromosomes, CpG sites with SNPs in probe (35) (at the flanking G, within 10 nt of the target CpG, and 11–50 nt away from the target CpG), CpG sites that are considered cross-reactive (36) and CpG sites that had a detection p-value >0.05 for more than 1% of samples. The 'rs' (representSNPassays) probes are normal SNP probes; they do not measure methylation. The 'ch' probes represent non-CpG methylation sites.

Independent Variable

Socioeconomic status (SES) was assessed prospectively at age 7 by averaging the percentile of both parents' educational attainment, occupation, and income relative to the US population (37).

Dependent Variables

Weight and height measures were obtained from participants wearing light clothing without shoes, using a calibrated stadiometer and weighing scale operated by trained nurse researchers. Heads were positioned in the Frankfurt plane. Body mass index (BMI) was calculated as weight per height squared (i.e. kg/m²).

Covariates

Age was directly assessed via date of birth (recorded directly in this prenatal cohort), subtracted from clinic visit date. Sex, race/ethnicity, and smoking status (current number of cigarettes smoked daily) were self-reported in adulthood. Childhood weight and height and resulting BMI (defined above) were directly assessed at age 7 years by medical staff according to a standard protocol (38).

Epigenome-wide associations between blood DNA methylation and adiposity could be confounded by differential prevalence of leukocytes from varying developmental lineages (24,39). Consequently, all epigenome-wide association studies (EWAS) analyses in blood were performed with adjustment for blood leukocyte distribution using the method developed by Houseman *et al.* (40). Estimates of cell type proportion for each of major 6 cell types (CD8T, CD4T, NK, B-cell, Monocytes, Granulocytes) were adjusted in the EWAS analyses as covariates.

Statistical Analyses

Variables including age, race, BMI at age 7 and cigarette smoking were compared between high and low SES groups in men and women, separately, using t-tests or chi-square tests.

We conducted the joint significance test (41) by two-stage EWAS analyses to identify CpG sites where methylation levels statistically mediate the association of SES with adult BMI. Specifically, we performed the first stage EWAS analyses to detect associations between CpG methylation and adult BMI. In stage 1 EWAS, with false discovery rate (FDR) (42) of 25%, we selected the candidate CpG sites with significant CpG-BMI associations. Among these we then conducted second stage EWAS analyses for the association of CpG methylation with SES. The candidate CpG sites with FDR<25% from the first stage EWAS, and p<0.001 in the second stage analyses, were reported as candidate mediating CpG sites. In the formal mediation analyses, suggestive evidence for mediation was inferred if the indirect effect p was <0.05. Note that in the light of the large number of tests performed in this study, these alpha thresholds are liberal. It should be noted that a more lenient FDR threshold of 25% was implemented due to our goal of performing mediation analyses, where data are subsetted twice; once for subsetting significant associations of BMI with methylation patterns (i.e. stage 1), and then from those significant associations, a second subset is identified that is also significantly associated with SES (i.e stage 2). To preserve the

most signals from the first stage, we allowed for a 25% false discovery that would be further decreased in the second stage analyses. The FDR cutoff of 0.25 is arbitrary but not uncommon in practice (43–46). Similarly, the second stage cutoff of $p < 0.001$ is arbitrary, but also within typical range of other studies that vary from 0.05 to 0.0005 (47–49). While we followed the convention of statistical significance cutoffs, we note that the optimal theoretical justification is still limited in literature. The candidate loci were further scrutinized with their biological plausibility to avoid false positive findings. Given the moderate sample size, these reasonably liberal cut-offs minimized the likelihood of false negative findings. In addition, among the CpG sites selected as candidate mediating sites, we emphasized particular attention on instances where several of the CpG sites were annotated to the same gene. These multiple hits occurring within the same gene could suggest that particular gene, if important for gene regulation and as a mediator between early life SES and later life body mass index, may have altered methylation patterns at multiple sites. As a technical note, mediation analyses make the genome-wide adjustment even more challenging since the second stage is a mixture of validation and additional investigation. The two-stage joint significance test was proposed in 2002 for low-dimensional analyses (41). To our knowledge, this study is the first to utilize the approach in a high-dimensional setting. To provide better statistical justification in the two-stage high-dimensional mediation analyses, we have another ongoing theoretical study, currently under review (Huang Y-T, *Under Review*), focusing on this issue where we aim to develop a testing procedure with well-justified theoretical property.

The first stage analyses were performed for associations of CpG methylation with adult BMI due to prior discoveries in this and other datasets showing significant associations of methylation patterns with BMI (25). Since associations of SES with CpG are less understood, we only conducted the analyses for this association focusing on CpG sites significantly associated with BMI in the first stage analyses. The sequence of the strategy better used the existing knowledge and may minimize the agnostic analyses for the CpG-SES association. However sensitivity analyses were performed using association of SES with CpG associations in stage 1 instead of 2. The joint significance test-based mediation analyses were conducted for DNA methylation in blood and adipose tissue separately.

All analyses were adjusted for age, race, BMI at age 7, smoking status in adulthood. Age, race and smoking status are related to methylation patterns and were adjusted for as potential confounders. BMI at age 7 years was adjusted for so that the estimated indirect effects in the mediation analyses were in reference to the *change* in BMI from childhood to adulthood; this is important in the CPP sample because the socioeconomic gradient in *childhood* BMI was in the direction of lighter children among lower SES families (Gilman et al., *Under Review*) during the era in the United States when the children were born (1959–1966). In all multivariate adjusted analyses, age 7 SES and BMI were included as continuous variables.

While the genome-wide joint significance test strategy only identified CpG sites with significant p-values, it did not provide effect estimates. Therefore, we utilized product-based mediation analyses to quantify the direct and indirect effects for the candidate mediating CpG sites that were identified through the two stage process. For each of these CpG sites,

we fit two regression models using ordinary least square estimators. The first model regressed the methylation value on SES with adjustment of covariates: $E(M_i) = \alpha_X^T X_i + \alpha_S S_i$, and the second model regressed BMI on SES and methylation with adjustment of covariates: $E(Y_i) = \beta_X^T X_i + \beta_S S_i + \beta_M M_i + \beta_{SM} S_i M_i$, where S , M , Y and X are SES, methylation M-value, adult BMI and demographic covariates, respectively, and α 's and β 's are the regression coefficients for the methylation M-value and the adiposity outcome (i.e., adult BMI), respectively. The M-value is $\log_2 B/(1 - B)$ where B is the beta-value of DNA methylation. T is transpose operator in matrix algebra and E is the expectation operator for the random variable. Under the framework of causal inference (50) using potential outcomes (51), the point estimates of direct effect (DE) and indirect effect (IE, or mediation effect) can be expressed by the combinations of regression parameters of the above two models: $IE = (\beta_M \alpha_S + \beta_{SM} \alpha_S S_1)(S_1 - S_0)$ and $DE = (\beta_S + \beta_{SM}(\alpha_X^T X + \alpha_S S_0))(S_1 - S_0)$ (52). Their corresponding variance and confidence intervals were obtained using bootstrap procedures. Note that we conducted two different tests to identify mediation effects: we performed joint significance tests in epigenome-wide scan and directly testing IE from the above formula, termed as product significance tests for the candidate sites. It has been shown in the literature that the joint significance test is more powerful than the product significance test (41), and therefore, we only calculated the p-value from the product significance test for the candidate CpG sites. Proportion of mediation was calculated as: $IE/(DE + IE)$ to indicate the proportion of total effect of SES on BMI mediated through CpG methylation (53); note that the proportion of mediation has plausible interpretation only if both DE and IE are in the same direction.

RESULT

Unadjusted analyses demonstrated significant associations of age 7 SES with adulthood BMI in females ($p=0.004$) and not males ($p=0.29$) (Table 1). There were no significant associations of SES with other covariates, such as age, race/ethnicity, and smoking (Table 1).

In the first stage EWAS, adipose tissue DNA methylation at 110,452 CpG sites in males and 87,772 in females was associated with adult BMI at $FDR < 25\%$. Interestingly, no associations were observed in peripheral blood leukocytes at $FDR < 25\%$. Initial analyses showed an over-representation of DNA methylation-BMI associations at CpG sites in adipose tissue but not blood, for both males and females (Supplementary Figure 1). This suggests in initial analyses that methylation patterns are associated with body mass index in adipose tissue.

Of the 110,452 sites in males and 87,772 sites in females that were identified in the first stage EWAS of DNA methylation and adult BMI ($FDR < 25\%$), 91 sites in males and 71 sites in females showed suggestive p-values for SES-methylation associations ($P < 0.001$), after adjusting for age, race, sex, age 7 BMI, and smoking in the adipose tissue samples. There was no evidence of epigenetic mediation in peripheral blood leukocytes, as no CpG sites for either males or females passed the first stage of statistical testing ($FDR < 25\%$). Furthermore, there was minimal evidence of differential leukocyte admixture in relation to SES or BMI,

where proportions of B cells, CD4+ T cells, CD8+ T cells, granulocytes, monocytes, and Natural Killer cells were similar across tertiles of SES and BMI (Supplementary Figure 2).

The 20 CpG sites with the lowest FDR for the association of methylation with BMI (in adipose tissue) are shown in Table 2 (females) and Table 3 (males). The full lists of 71 and 91 CpG sites are displayed in Supplementary Table 1 (females) and Supplementary Table 2 (males). The candidate mediation CpG sites were different in analyses of females vs. males. R^2 values ranged from 0.000 to 0.297 (maximum possible: 1.000) in the association of the methylation sites with BMI (Tables 2 and 3, Supplementary Tables 1 and 2).

Among the 91 sites identified in males and 71 sites identified in females, there were several instances where multiple CpGs mapped to the same gene, including repeat hits in several genes that are biologically relevant for the development of obesity. These were fatty acid synthase (*FASN* – 2 sites), transmembrane protein 88 (*TMEM88* – 2 sites), signal transducer and activator of transcription 3 (*STAT3* – 2 sites) in females, and neuritin 1 (*NRN1* – 3 sites) in males (Tables 2 and 3; Supplementary Tables 1 and 2). These tables showed suggestive evidence of statistical mediation where the indirect effect p value was <0.05 for several CpG methylation sites. While the main effect between SES and body mass index is significant in females (Table 1), the direct effect became non-significant in mediation analyses, possibly due to indirect effects through the methylation patterns identified in Table 2 and Supplementary Table 1. Figure 1 shows a detailed examination of the methylation profile for the *FASN* gene in females, where 11 of 26 methylation sites showed mediation p-values <0.05 (indirect effect; shown by red line in Figure 1). In this figure, it can be seen that there are robust associations between SES and methylation at several CpG sites (blue line), as well as between methylation and BMI at several CpG sites (green line). Similar analyses in other genes showed mediation p-values <0.05 in 5 of 28 sites for *NRN1* in males (Figure 2), 3 of 11 sites for *TMEM88* in females (Supplementary Figure 3), and 1 of 17 sites for *STAT3* in females (Supplementary Figure 4).

Sensitivity analyses for associations of CpG site methylation with BMI in adipose tissue using a more restrictive $FDR < 10\%$ (instead of $< 25\%$) showed there were 21 sites in women and 35 sites in men identified as suggestive. Using $FDR < 5\%$, there were 2 sites in women and 16 sites in men identified. Further sensitivity analyses for associations of CpG site methylation with age 7 SES (instead of with adulthood BMI) at $FDR < 25\%$, showed 1 site in women and 0 sites in men as suggestive in adipose tissue. No sites in blood were identified as significant using this latter approach.

DISCUSSION

This study investigated the role of DNA methylation in the association between childhood SES and adult adiposity. Findings suggest that methylation of specific CpG sites in adipose tissue but not blood mediate the association between childhood SES and adulthood BMI. Specific gene sites identified were *FASN*, *STAT3* and *TMEM88* in females, and *NRN1* in males. These epigenetic mediation findings should be interpreted with caution, as this is the first study to report them, and reasonably liberal thresholds for detection were implemented. As they are novel, these epigenetic mediation findings require replication to confirm the

specific sites involved; more broadly, this study finds that early life conditions may be related to adult health partly through their influences on epigenetic mechanisms.

Prior Literature

Childhood socioeconomic disadvantage and adulthood adiposity—A systematic review demonstrated that childhood socioeconomic disadvantage is inversely associated with adulthood adiposity, with studies showing stronger associations in women than men. Specifically, inverse associations were found in 70% (14 of 20) of studies in women and 27% (4 of 15) of studies in men, where typical effect sizes for the differences in body mass index (BMI) between the highest and lowest SES were 1.0–2.0 kg/m² in women and 0.2–0.5 kg/m² in men (11). These findings are consistent with the current study, where associations of childhood socioeconomic disadvantage were statistically significantly associated with adulthood BMI in females and not males. Senese *et al.* have offered plausible explanations for these sex differences including parity and socioeconomic-patterned pressure for females to be slim (11).

Childhood socioeconomic disadvantage and methylation—Several previous studies have investigated associations of childhood disadvantage with DNA methylation patterns. To our knowledge, all studies to date used peripheral blood leukocytes or placental tissue. Our search of the literature revealed no studies of this issue that interrogated adipose tissue. With regard to studies in peripheral blood leukocytes, a genome-wide methylation study in 40 adult men from the 1958 British Birth Cohort showed associations of childhood SES with differential methylation of 1,252 gene promoters in whole blood DNA (26). Furthermore, a study in approximately 85 participants of the New York site of the Collaborative Perinatal Project showed associations of childhood SES with methylation of Sat2 repetitive elements in leukocyte DNA during adulthood, and no association with Alu and LINE-1 (27). Another study of 120 mother-offspring dyads showed that maternal education was significantly associated with methylation of *INSIGF* in blood at offspring age 17 months (28). A study, using purified monocytes, specifically examined 18 candidate genes related to stress reactivity and inflammation for 1,264 participants in the Multi-Ethnic Study of Atherosclerosis (MESA), demonstrating that low childhood SES was associated with adulthood DNA methylation in three stress-related genes (*AVP*, *FKBP5*, *OXTR*) and two inflammation-related genes (*CCL1*, *CD1D*) (29). A study of 92 adults showed substantial decreases in P-value distribution patterns for the relation of early life SES with methylation in peripheral blood mononuclear cells in samples that were corrected vs. not corrected for differential blood cells count (30). Most studies to date using leukocytes have not adjusted for cell admixture, consequently it is unclear if evidence of associations is due to DNA methylation or differential cell admixture. With regard to findings in placental tissue, one study showed significant associations of maternal education and maternal poverty status with *HSD11B2* methylation in males (n=215) and not females (n=230) (31). Overall, evidence suggests some indication of methylation patterns associated with childhood socioeconomic disadvantage, however replication efforts are needed to determine consistency between studies and tissue types.

Methylation and adiposity—Exploration of epigenetic underpinnings of adiposity is receiving substantial research attention. In the New England Family Study, we recently showed that adiposity phenotypes (e.g. body mass index, android fat mass) were associated with DNA methylation in several genes that are biologically relevant to the development of adiposity, such as *AOC3*, *LIPE*, *SOD3*, *AQP7*, *HIF3A*, and *CETP* (25). Blood DNA methylation profiles were not associated with adiposity, before or after adjustment for leukocyte cell mixture effects. Two studies in adipose tissue showed blood DNA methylation at 3 CpG sites located in the *HIF3A* gene to be associated with BMI (25,54). Several other studies investigated associations of DNA methylation with adiposity (14–23,54), primarily in leukocytes (14–21,23). Research on epigenetic determinants of adiposity is still in the fairly early discovery phase, and consequently, many CpG sites that are potentially effectors of this phenotype likely remain to be discovered and replicated.

Mediation analyses of methylation as a pathway of how childhood SES could influence adiposity—There has been little exploration of the possible nature of the relationship of alterations in methylation as it pertains to effecting the childhood SES/adult adiposity association. Here, we applied a formal mediation analyses to investigate potential mediating roles of epigenetic changes in translating early life socioeconomic disadvantage into adiposity later in life. However in a study of early life stress, a mediation analysis in 31 offspring of mothers who went through the 1998 Quebec ice storm showed that methylation levels of genes implicated in diabetes (e.g. *LTA*, *NFKBIA*, *PIK3CD*) showed significant mediation of the relation between prenatal maternal stress and offspring's central adiposity and BMI, where methylation was assessed in peripheral blood leukocytes (55). The current study's findings of 91 CpG sites in males and 71 CpG sites in females having evidence of mediation between early life socioeconomic disadvantage and adulthood adiposity appear to be very novel. Particular enrichment in the *FASN*, *TMEM88*, *STAT3*, and *NRN1* genes is biologically plausible. We reasoned that genes with multiple CpG sites showing significantly altered DNA methylation were most likely to have an important phenotypic change that significantly contributed to any SES-mediated epigenetic differences in adulthood. Each of the *STAT3*, *FASN*, *TMEM88* and *NRN1* genes showed alterations in DNA methylation in multiple CpG sites within each gene. Interestingly, these genes are all members of pathways that are known to be crucial in development. Members of the Nanog pathway that establishes and maintains pluripotency in embryonic stem cells include the *JAK/STAT3* transcription factor axis (56). This pathway has been reported to be epigenetically modified in numerous chronic diseases, with evidence that *STAT3* itself can be directly targeted by environmental exposures (57). The *STAT3* pathway is also activated by leptin, indicating that it plays a role in the metabolic pathways central to obesity (58). The *FASN* protein has long been associated with cellular and organismal metabolism. Dysregulated metabolism is a hallmark of inflammation and the gene itself has been identified as a potential therapeutic target in numerous cancers (59). It is also well established that fatty acids are critically important in the development of cardiovascular diseases (60). In mice, high dietary fat alter *FASN* expression and this change is more pronounced in female mice than males (61). In addition, *TMEM88* is a member of the WNT signaling pathway, which is now known to be critical in normal developmental patterning (62). Alterations in the pathway is associated with numerous adult degenerative diseases and cancers (63) and proteins with a similar

structure are well known to be important for adipocyte differentiation (64). Finally, *NRN1* has been reported to be expressed in high levels in adipose progenitor cells that give rise to white adipocytes (65) and, interestingly, it is selectively regulated by androgens in experiments conducted in-vitro (66). Hence, the sites that we identified as most likely to be phenotypically altered are all developmental mediators of pathways that play roles in chronic disease.

Mechanisms—With regard to how early life socioeconomic disadvantage could influence DNA methylation, there are a number of plausible mechanisms. One pathway may be through maternal and offspring diet related to socioeconomic disadvantage. It has been demonstrated that adults and children with lower socioeconomic status in the United States and other industrialized nations consume less of a number of methyl donor foods such as fruits and vegetables (67,68), as well as lower consumption of folate (68), compared to those with high SES. Socioeconomic disadvantage influences abilities to afford many of the sources of naturally occurring methylation donor such as citrus fruits and leafy greens (67,68). Furthermore, systematic reviews demonstrated that low childhood SES is predictive of adulthood obesity (11). Methylation patterns may be indicative of greater propagation of adipose cell number and size in the body, if the methylation influences genes involved with proliferation or enlargement of adipose cells. This could potentially be due in part to early life socioeconomic disadvantage. To the degree that socioeconomic disadvantage causes stress, stress has been shown to alter feeding behavior in animals and humans, where some respond with increased food consumption and others respond with decreased food consumption (69). It is important to note that stress can be dependent on the larger socioeconomic context in which participants are living. For example, parents and children living in poverty, with few employment opportunities, low education and inadequate support systems may be inherently more stressed (70), and more likely to express more adverse parenting styles, such as neglect, abuse and lower parental monitoring (71). There is evidence that obesity-related eating behaviors are enhanced when there is access to palatable food during periods of stress (69). Non-homeostatic eating has been suggested to activate the brain reward system (69). Early life environmental stressors such as disruption of mother-child relationships or abuse or neglect, have been shown to alter stress neurobiological outcomes and feeding behavior during adulthood (69). A prospective study by our group showed that elevated maternal cortisol levels during the gestational period were associated with increased coronary heart disease risk in female offspring 47 years later (72). These pathways, such as socioeconomic disadvantage-determined consumption of methyl-donor foods, likelihood of obesity, and stress-related consumption of palatable foods may be pathways through which early life socioeconomic disadvantage could influence methylation patterns in fat tissue, and eventually lead to greater adiposity.

Limitations and Strengths

Findings in this study should be interpreted with caution, as they have not been replicated in other studies. However, to our knowledge, there are no other studies that have sampled adipose tissue and have data on both individual estimates of early life SES as well as adulthood adiposity. Furthermore, in epigenome-wide methylation-BMI analyses, with 87,772 sites significant in females and 110,453 sites significant in males with FDR<25%,

and p-value <0.001 for SES-methylation associations, we would expect 88 CpG sites in females, and 110 CpG sites in males, to be significant mediators simply due to chance. This is greater than the observed number of significant methylation sites (91 sites in males and 71 sites in females), suggesting the mediation findings may be due to chance. However, examination of each of the CpG sites interrogated by the array in the *FASN* and *TMEM88* in females, and *NRN1* in males, showed substantial enrichment of significant methylation in these regions, suggesting they may be biologically important. Secondly, it was not possible to adjust for cell admixture in the adipose tissue samples due to the large number of degrees of freedom needed in current statistical approaches for adipose tissue (40,73), and the relatively modest sample size. Consequently, it is uncertain if observed methylation patterns are due to alterations in methylation patterns, or due to alterations in adipose tissue cellular composition, between individuals with low vs. high socioeconomic disadvantage. Either of these pathways are of interest and biologically relevant. Future studies with larger sample sizes should clarify the underlying biology. In addition, race/ethnicity-specific differences in epigenetic mechanisms could be present. In the current study, only 25 male and 23 female participants were non-white (including African Americans, Native Americans and others). With analyses in this study being mediation analyses, and performed sex-specific, power was insufficient to implement effect modification analyses for race/ethnicity. However, a recent paper by our group showed race-specific epigenetic methylation patterns in adipose vs. blood tissue (74), suggesting some mechanisms may be unique by race/ethnicity. Finally, this study, although prospective from early childhood through mean age 47 y, is observational in design, leading to some limitations in causal inference if there are underlying unmeasured confounders. Inferring that the mediation analyses reflect a chain of causal effects requires two sets of assumptions regarding confounding: no unmeasured SES-methylation confounders, and no unmeasured methylation-adiposity confounders.

Strengths of the study include that we were able to utilize prospective assessments of childhood SES with both parent's socioeconomic information, as well as direct prospective assessments of BMI at mean ages 7 years and 47 years. Furthermore, analyses were able to assess associations in both adipose tissue and blood instead of just blood as is usually performed. When exploring adiposity as an outcome, it is beneficial to assess the target tissue (i.e. adipose tissue), as shown in this study where all significant findings were in adipose tissue and not blood.

Conclusions

These findings provide evidence that the DNA methylation profile in adipose tissue at specific sites, including *FASN* and *TMEM88* in females, and *NRN1* in males, may explain part of the observed associations between childhood socioeconomic disadvantage and adulthood adiposity, particularly found in females (11). This adds to the small but growing literature on epigenetic etiologic explanations of social disparities in health. Plausible biological markers could one day serve as early biological indicators of socioeconomic intervention effectiveness, or as therapeutic targets. Findings are very early on in the discovery process, and it remains uncertain if DNA methylation could serve in this role.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Funding for this study was provided by NIH/NIA grants RC2AG036666 and R01AG048825 and by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

Conflicts of Interest and Source of Funding: This work was supported by NIH/NIA grants RC2AG036666 and R01AG048825, and by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

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Figure 1.

Formal mediation analyses of methylation patterns at 26 fatty acid synthase (FASN) CpG sites in female adipose tissue. Analyses evaluated whether FASN methylation patterns mediate the relation of perinatal socioeconomic status (SES) with age 47 body mass index (BMI). High vs. low for SEI and BMI was calculated using a median split.

Figure 2.

Formal mediation analyses of methylation patterns at 28 neuritin 1 (*NRN1*) CpG sites in male adipose tissue. Analyses evaluated whether *NRN1* methylation patterns mediate the relation of perinatal socioeconomic status (SES) with age 47 body mass index (BMI). High vs. low for SES and BMI was calculated using a median split.

Table 1.
Demographics showing associations of age 7 socioeconomic status (SES) with covariates, stratified by sex.

	Male			Female		
	Low SES	High SES	p	Low SES	High SES	p
n	34	33		38	36	
Age, mean years (SD)	47.1 (1.4)	47.0 (1.8)	0.94	46.9 (1.7)	46.7 (1.8)	0.72
Race/ethnicity, % white	55.9	69.7	0.36	57.9	80.6	0.064
Age 7 BMI, mean kg/m ² (SD)	16.8 (3.7)	16.2 (1.5)	0.39	16.1 (2.4)	16.2 (1.7)	0.84
Adult BMI, mean kg/m ² (SD)	31.2 (6.1)	32.7 (5.2)	0.29	34.1 (8.2)	28.3 (8.4)	0.0036
Current smoking, mean no. (SD)	10.8 (13.4)	9.7 (9.7)	0.71	13.4 (14.5)	8.3 (9.5)	0.078

No., number of cigarettes smoked per day; SD, standard deviation.

High vs. low for SES was calculated using a median split.

Table 2.

Female adipose tissue mediation effects, for methylation sites in the 20 CpG sites having the lowest false discovery rate (FDR) values.

CpG ID	Chrom	Gen Loc	Gene	pval(S->Mj)	pval(Mj->BMI)	FDR(Mj->BMI)	Indirect	95% CI	Pindirect	Direct	95% CI	Pdirect	R ²	Prop. of Mediation
cg21120176	6	6534797	LOC285780	<0.001	<0.001	0.036	-0.17	(-0.33,-0.06)	<0.001	-0.13	(-0.33,0.12)	0.350	0.264	0.57
cg222462726	3	184209261		0.001	0.001	0.046	-0.05	(-0.15,0.02)	0.136	-0.09	(-0.21,0.06)	0.220	0.276	0.38
cg11950105	17	80050646	FASN	<0.001	0.001	0.050	-0.29	(-0.58,-0.13)	<0.001	-0.21	(-0.55,0.20)	0.284	0.293	0.57
cg25490145	17	80358850	C17orf101	0.001	0.001	0.050	0.09	(0.02,0.19)	0.012	0.10	(-0.04,0.25)	0.164	0.295	0.47
cg26750548	10	88441152	LDB3	<0.001	0.001	0.052	-0.32	(-0.64,-0.10)	0.000	-0.28	(-0.73,0.21)	0.230	0.257	0.54
cg12930882	5	37825585	GDNF	0.001	0.003	0.060	-0.08	(-0.20,0.03)	0.224	-0.15	(-0.33,0.06)	0.168	0.210	0.33
cg17918937	15	64992780	OAZ2	<0.001	0.003	0.062	-0.11	(-0.27,0.00)	0.054	-0.24	(-0.49,0.03)	0.098	0.168	0.32
cg25840926	2	20647987	RHOB	0.001	0.003	0.062	-0.11	(-0.24,-0.02)	0.010	-0.16	(-0.33,0.04)	0.124	0.257	0.40
cg21170029	1	209799330	LAMB3	0.001	0.004	0.064	-0.02	(-0.13,0.05)	0.540	-0.13	(-0.28,0.03)	0.160	0.125	0.14
cg02422603	13	114890566	RASA3	<0.001	0.004	0.064	0.30	(0.05,0.64)	0.022	0.30	(-0.24,0.80)	0.268	0.229	0.50
cg18410680	17	7758397	TMEM88	0.001	0.004	0.066	-0.07	(-0.18,0.00)	0.060	-0.13	(-0.28,0.05)	0.202	0.272	0.37
cg27630153	16	88845038	FAM38A	0.001	0.005	0.071	-0.23	(-0.42,-0.08)	0.002	-0.21	(-0.56,0.14)	0.214	0.297	0.52
cg14917100	2	127528627		0.001	0.006	0.072	-0.14	(-0.29,-0.04)	0.006	-0.20	(-0.44,0.04)	0.100	0.132	0.40
cg07568841	7	30362781	ZNRF2	<0.001	0.006	0.073	0.21	(0.04,0.45)	0.020	0.28	(-0.10,0.63)	0.138	0.218	0.42
cg05522011	4	81122726	PRDM8	<0.001	0.006	0.074	-0.07	(-0.24,0.02)	0.182	-0.20	(-0.45,0.10)	0.246	0.215	0.26
cg16850945	3	52488229	TNNC1; NISCH	0.001	0.007	0.077	-0.10	(-0.21,-0.03)	0.008	-0.05	(-0.19,0.12)	0.614	0.246	0.69
cg06468133	11	3244441		0.001	0.008	0.079	0.26	(0.04,0.54)	0.020	0.26	(-0.19,0.70)	0.228	0.211	0.50
cg27296413	1	159037843	AIM2	0.001	0.008	0.079	0.15	(0.03,0.26)	0.026	0.07	(-0.11,0.33)	0.440	0.133	0.67
cg00278517	6	167070616	RPS6KA2	0.001	0.012	0.092	0.09	(-0.02,0.27)	0.124	0.18	(-0.10,0.41)	0.254	0.194	0.34
cg06608378	15	76016129	ODF3L1	<0.001	0.013	0.093	-0.08	(-0.22,0.00)	0.062	-0.15	(-0.34,0.11)	0.250	0.221	0.33

BMI, body mass index; Chrom, chromosome number; Direct, direct effect of methylation mediator in association of SEI with BMI; Gen Loc, gene location; FDR(Mj->BMI), false discovery rate for association of methylation mediator with BMI; Indirect, indirect effect of methylation mediator in association of SEI with BMI; Pdirect, p value for direct effect; Pindirect, p value for indirect effect; Prop. of Mediation, proportion of total association between SES and BMI explained by DNA methylation (calculated as indirect effect/[direct effect+indirect effect]); proportion of mediation has plausible interpretation only if both direct and indirect effects are in the same direction; "N/A" represents effects in opposite directions; R², percentage of the variance in BMI explained by DNA methylation, without adjusting for covariates (range 0.000–1.000); SEI, age 7 y socioeconomic index; pval(S->Mj), p value for association of SEI with methylation mediator; pval(Mj->BMI), p value for association of methylation mediator with BMI; 95% CI, 95% confidence intervals.

Table 3.

Male adipose tissue mediation effects, for methylation sites in the 20 CpG sites having the lowest false discovery rate (FDR) values.

CpG ID	Chrom	Gen Loc	Gene	pval(S->Mj)	pval(Mj->BMI)	FDR(Mj->BMI)	Indirect	95% CI	Pindirect	Direct	95% CI	Pdirect	R ²	Prop. of Mediation
cg21120176	6	6534797	LOC285780	<0.001	<0.001	0.036	-0.17	(-0.33,-0.06)	<0.001	-0.13	(-0.33,0.12)	0.350	0.264	0.57
cg222462726	3	184209261		0.001	0.001	0.046	-0.05	(-0.15,0.02)	0.136	-0.09	(-0.21,0.06)	0.220	0.276	0.38
cg11950105	17	80050646	FASN	<0.001	0.001	0.050	-0.29	(-0.58,-0.13)	<0.001	-0.21	(-0.55,0.20)	0.284	0.293	0.57
cg25490145	17	80358850	C17orf101	0.001	0.001	0.050	0.09	(0.02,0.19)	0.012	0.10	(-0.04,0.25)	0.164	0.295	0.47
cg26750548	10	88441152	LDB3	<0.001	0.001	0.052	-0.32	(-0.64,-0.10)	0.000	-0.28	(-0.73,0.21)	0.230	0.257	0.54
cg12930882	5	37825585	GDNF	0.001	0.003	0.060	-0.08	(-0.20,0.03)	0.224	-0.15	(-0.33,0.06)	0.168	0.210	0.33
cg17918937	15	64992780	OAZ2	<0.001	0.003	0.062	-0.11	(-0.27,0.00)	0.054	-0.24	(-0.49,0.03)	0.098	0.168	0.32
cg25840926	2	20647987	RHOB	0.001	0.003	0.062	-0.11	(-0.24,-0.02)	0.010	-0.16	(-0.33,0.04)	0.124	0.257	0.40
cg21170029	1	209799330	LAMB3	0.001	0.004	0.064	-0.02	(-0.13,0.05)	0.540	-0.13	(-0.28,0.03)	0.160	0.125	0.14
cg02422603	13	114890566	RASA3	<0.001	0.004	0.064	0.30	(0.05,0.64)	0.022	0.30	(-0.24,0.80)	0.268	0.229	0.50
cg18410680	17	7758397	TMEM88	0.001	0.004	0.066	-0.07	(-0.18,0.00)	0.060	-0.13	(-0.28,0.05)	0.202	0.272	0.37
cg27630153	16	88845038	FAM38A	0.001	0.005	0.071	-0.23	(-0.42,-0.08)	0.002	-0.21	(-0.56,0.14)	0.214	0.297	0.52
cg14917100	2	127528627		0.001	0.006	0.072	-0.14	(-0.29,-0.04)	0.006	-0.20	(-0.44,0.04)	0.100	0.132	0.40
cg07568841	7	30362781	ZNRF2	<0.001	0.006	0.073	0.21	(0.04,0.45)	0.020	0.28	(-0.10,0.63)	0.138	0.218	0.42
cg05522011	4	81122726	PRDM8	<0.001	0.006	0.074	-0.07	(-0.24,0.02)	0.182	-0.20	(-0.45,0.10)	0.246	0.215	0.26
cg16850945	3	52488229	TNNC1; NISCH	0.001	0.007	0.077	-0.10	(-0.21,-0.03)	0.008	-0.05	(-0.19,0.12)	0.614	0.246	0.69
cg06468133	11	3244441		0.001	0.008	0.079	0.26	(0.04,0.54)	0.020	0.26	(-0.19,0.70)	0.228	0.211	0.50
cg27296413	1	159037843	AIM2	0.001	0.008	0.079	0.15	(0.03,0.26)	0.026	0.07	(-0.11,0.33)	0.440	0.133	0.67
cg00278517	6	167070616	RPS6KA2	0.001	0.012	0.092	0.09	(-0.02,0.27)	0.124	0.18	(-0.10,0.41)	0.254	0.194	0.34
cg06608378	15	76016129	ODF3L1	<0.001	0.013	0.093	-0.08	(-0.22,0.00)	0.062	-0.15	(-0.34,0.11)	0.250	0.221	0.33

BMI, body mass index; Chrom, chromosome number; Direct, direct effect of methylation mediator in association of SEI with BMI; Gen Loc, gene location; FDR(Mj->BMI), false discovery rate for association of methylation mediator with BMI; Indirect, indirect effect of methylation mediator in association of SEI with BMI; Pdirect, p value for direct effect; Pindirect, p value for indirect effect; Prop. of Mediation, proportion of total association between SES and BMI explained by DNA methylation (calculated as indirect effect/(direct effect+indirect effect)); proportion of mediation has plausible interpretation only if both direct and indirect effects are in the same direction; "N/A" represents effects in opposite directions; R², percentage of the variance in BMI explained by DNA methylation, without adjusting for covariates (range 0.000–1.000); SEI, age 7 y socioeconomic index; pval(S->Mj), p value for association of SEI with methylation mediator; pval(Mj->BMI), p value for association of methylation mediator with BMI; 95% CI, 95% confidence intervals.