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Associations between prenatal physical activity, birth weight, and DNA methylation at genomically imprinted domains in a multiethnic newborn cohort

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Abbreviations: BW, birth weight; BMI, body mass index; DOHaD, developmental origins of health and disease; DMR, differentially methylated regions; HIV, human immunodeficiency virus; IGF, insulin-like growth factor; LMP, last menstrual period; LBW, low birth weight; MET, metabolic equivalent; NEST, Newborn Epigenetic Study; PA, physical activity; SE, standard error.

Birth weight is a commonly used indicator of the fetal environment and a predictor of future health outcomes. While the etiology of birth weight extremes is likely multifactorial, epidemiologic data suggest that prenatal physical activity (PA) may play an important role. The mechanisms underlying this association remain unresolved, although epigenetics has been proposed. This study aimed to estimate associations between prenatal PA, birth weight, and newborn DNA methylation levels at differentially methylated regions (DMRs) regulating 4 imprinted genes known to be important in fetal development. Study participants (N = 1281) were enrolled as part of the Newborn Epigenetics Study. Prenatal PA was ascertained using the Pregnancy Physical Activity Questionnaire, and birth weight data obtained from hospital records. Among 484 term mother-infant pairs, imprinted gene methylation levels were measured at DMRs using bisulfite pyrosequencing. Generalized linear and logistic regression models were used to estimate associations. After adjusting for preterm birth and race/ethnicity, we found that infants born to mothers in the highest quartile of total non-sedentary time had lower birth weight compared to infants of mothers in the lowest quartile ($\beta = -81.16$, SE = 42.02, $P = 0.05$). These associations appeared strongest among male infants ($\beta = -125.40$, SE = 58.10, $P = 0.03$). Methylation at the *PLAGL1* DMR was related to total non-sedentary time ($P < 0.05$). Our findings confirm that prenatal PA is associated with reduced birth weight, and is the first study to support a role for imprinted gene plasticity in these associations. Larger studies are required.

Introduction

Size at birth is a common indicator of the fetal environment, as well as a predictor of survival and future health outcomes.[1] Both low birth weight (LBW; <2500 g), due to prematurity or growth restriction, and macrosomia (>4500g) are associated with numerous deleterious health consequences in adulthood, such as cardiovascular disease,[2] type 2 diabetes (T2D),[3] obesity,[4] and some cancers.[5] While the proportion of LBW declined 3% between 2006 and 2012, the birth weight (BW) distribution has shifted slightly toward heavier infants.[6] There is urgent need for a more detailed understanding of factors contributing to the incidence of being born at the extreme tails of the BW distribution, and downstream health outcomes. Although small reductions in mean BW may be of relatively little significance for an individual newborn, these changes are potentially important on a population level.

Physical activity (PA) in pregnancy, a highly modifiable behavior, has been associated with both fetal growth and BW,[7] but the epidemiologic literature relating PA to BW remains equivocal to date.[8] There is evidence to suggest that maternal PA may reduce risk of macrosomia,[9] increasing the likelihood of delivering an appropriate-for-gestational age infant. PA may influence BW through its effects on maternal obesity, insulin sensitivity, placental blood flow and nutrient delivery.[10,11]

The developmental origin of health and disease (DOHaD) hypothesis posits that prenatal and early postnatal environments can induce changes in development that subsequently alter adult health and disease risk.[12,13] While mechanisms are still poorly understood,[14]epigenetics has been proposed to be one pathway

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through which maternal exercise influences fetal BW and subsequent health outcomes.[15,16] However, epigenetic targets for PA are largely unknown.

Genomically imprinted genes are involved in many disease processes,[17] and many are critical in fetal development, and could play an integral role in regulating early growth and development by allele-specific alterations in DNA methylation at differentially methylated regions (DMRs). The methylation patterns at imprinted DMRs are established before gastrulation, and are maintained in somatic tissues.[18] Therefore, any lack of fidelity in preserving these marks through epigenetic reprogramming may initiate changes that should be detectable in all tissues.[18] Further, because genomically imprinted genes may be networked,[19] information on dysregulation may inform changes in multiple genes.[20,21]

Thus far, no studies have examined the potential epigenetic effects of early prenatal exercise on birth outcomes. In these analyses, we examine the association between prenatal maternal PA, BW, and newborn DNA methylation status at 4 genomically imprinted gene DMRs known to be important in fetal growth and development.[22,23] Given that black women have higher risk of adverse pregnancy outcomes,[24] overweight or obesity before pregnancy is a risk factor for macrosomia,[25] and epigenetic perturbations may be sex-specific,[26] we examined potential modification of the maternal PA-BW association by these factors.

Results

Table 1 summarizes demographic characteristics of the 1281 Newborn Epigenetics Study (NEST) participants reporting PA. Total non-sedentary time varied by maternal age, race/ethnicity, marital status, parity, household income, education and smoking ($P < 0.01$). Participants were comparable with respect to gestational age at enrollment, folic acid supplementation, gestational weight gain and body mass index (BMI) at last menstrual period (LMP) ($P > 0.05$). Delivery outcomes (e.g., preterm birth, delivery mode, and infant sex) were not associated with quartiles of total non-sedentary time ($P > 0.05$).

Associations between PA and BW

The mean BW in this cohort was 3264 g (standard deviation = 603 g). Median time spent in light, moderate, intense, and total physical activities were 8 hours/day, 3 hours/day, ½ hour/day, and 6½ hours/day, respectively. Coefficient estimates (βs) and standard errors (SE) for the multivariate linear association between maternal PA and infant BW are shown in **Table 2**. Multivariate models were adjusted for race/ethnicity and preterm birth. With the exception of light PA, we observed inverse associations between all measures of maternal PA and BW. Associations were most pronounced for total non-sedentary time. We observed an inverse association between total non-sedentary time and infant BW when women in the highest quartile of total non-sedentary time were compared to women in the lowest quartile ($\beta = -81.16$, SE = 42.02, $P = 0.05$). Conversely, we found a positive association between low levels of light maternal PA and infant BW, which was attenuated with increasing light PA ($\beta_{\text{Quartile 2 vs. 1}} = 73.41$, $\beta_{\text{Quartile 3 vs. 1}} = 23.66$, $\beta_{\text{Quartile 4 vs. 1}} = 10.16$, P *trend* = 0.0678). Restricting these analyses to mother-infant pairs with ≥37 weeks gestation did not alter our findings.

We additionally explored associations between maternal PA and LBW/macrosomia. Adjusting for race/ethnicity and preterm birth we observed increased risk of both LBW and macrosomia at all levels of maternal light PA. In contrast, total non-sedentary time was associated with reduced risk of macrosomia. Associations between total non-sedentary time and LBW were approximately null (ORs~1.0) among infants born to women in quartiles 2 and 3, while an imprecise positive association was observed among infants born to mothers in the highest level of total non-sedentary time (**Table S2**).

Stratified associations between total non-sedentary time and BW by race/ethnicity, infant sex, and maternal BMI are presented in **Table 3**. We found more pronounced risk reductions among Whites in the highest quartile of total non-sedentary time compared to Blacks or Hispanics ($\beta = -106.58$, -79.58, -59.13, respectively). However, the cross-product term for PA quartiles and race/ethnicity exceeded α of 0.05. Stratifying by infant sex we observed striking difference by group. Male infants born to mothers in the highest quartile of total non-sedentary time experienced a large reduction in BW ($\beta = -125.40$, SE =58.10, $P = 0.03$), whereas the association among female infants was less pronounced and imprecise ($\beta = -31.28$, SE = 60.20, $P = 0.60$) (P for cross product term = 0.65). Finally, although the highest quartile of total non-sedentary time was inversely associated with BW at all levels of BMI, the association was more pronounced among women with BMI <25 kg/m^2 ($\beta = -112.55$, SE = 68.71, $P = 0.10$) compared to BMI ≥30 kg/m^2 ($\beta = -58.55$, SE = 75.24, $P = 0.44$) (P for cross product term = 0.55).

DNA methylation at DMRs regulating imprinted genes, prenatal PA, and BW

We also examined whether total non-sedentary time was associated with imprinted DMR methylation (*H19, MEG3, SGCE/PEG10*, and *PLAGL1*). After adjusting for race/ethnicity, gestational age at delivery, folic acid intake, and maternal smoking, we found that total non-sedentary time decreased DNA methylation at *PLAGL1* DMR and appeared to be consistent with a threshold effect ($\beta_{\text{Quartile 2 vs. 1}} = -2.15$, $\beta_{\text{Quartile 3 vs. 1}} = -1.77$, $\beta_{\text{Quartile 4 vs. 1}} = -1.57$, P *trend* = 0.0136) (**Table 4**). We subsequently examined whether inclusion of the *PLAGL1* DMR in the base model altered the association between total non-sedentary time and BW. After multivariate adjustment, including *PLAGL1* DMR methylation into the base model attenuated the association ($\beta = -11.10$, SE = 62.17, $P = 0.86$).

Discussion

We estimated the association between prenatal PA and BW, and subsequently examined the extent to which DNA

Table 1. Maternal characteristics by total non-sedentary time derived from self-reported recreational, household, occupational and transport activity: Newborn Epigenetic Study

	Quartile 1 (N = 311)	Quartile 2 (N = 337)	Quartile 3 (N = 314)	Quartile 4 (N = 319)	
Birthweight					
Low	23 (7%)	26 (8%)	26 (8%)	24 (8%)	
Average	280 (90%)	308 (91%)	285 (91%)	291 (91%)	
High	8 (3%)	3 (1%)	3 (1%)	4 (1%)	
Covariate	**N (%)**				***p*-value**
Age at delivery (years)					
20–29	174 (56%)	217 (64%)	161 (51%)	153 (48%)	0.0033
18-<20	16 (5%)	12 (4%)	9 (3%)	16 (5%)	
30–35	88 (28%)	80 (24%)	100 (32%)	103 (32%)	
36+	33 (11%)	28 (8%)	44 (14%)	47 (15%)	
Race					
Black	128 (46%)	166 (55%)	118 (41%)	98 (35%)	<0.0001
White	69 (25%)	75 (25%)	106 (37%)	115 (41%)	
Hispanic	79 (29%)	62 (20%)	63 (22%)	66 (24%)	
Preterm birth					
Term	281 (90%)	305 (91%)	283 (90%)	297 (93%)	0.5316
Preterm	30 (10%)	31 (9%)	31 (10%)	22 (7%)	
Gestational age at enrollment					
≤10 weeks	101 (33%)	106 (32%)	134 (43%)	122 (38%)	0.0557
11–20 weeks	180 (58%)	198 (59%)	160 (51%)	174 (55%)	
>20 weeks	29 (9%)	31 (9%)	20 (6%)	22 (7%)	
Marital status					
Married	111 (36%)	110 (33%)	151 (49%)	161 (51%)	<0.0001
Never married	92 (30%)	123 (37%)	71 (23%)	67 (21%)	
Living with partner	87 (28%)	81 (24%)	68 (22%)	73 (23%)	
Widowed/divorced/separated	10 (3%)	7 (2%)	16 (5%)	9 (3%)	
Other	8 (3%)	11 (3%)	4 (1%)	5 (2%)	
Parity at enrollment					
Multiparous	234 (75%)	221 (66%)	159 (51%)	211 (66%)	<0.0001
Nulliparous	77 (25%)	116 (34%)	155 (49%)	108 (34%)	
Household income					
<$25,000	130 (54%)	162 (59%)	101 (40%)	123 (46%)	<0.0001
$25,000-$49,999	46 (19%)	43 (16%)	37 (15%)	52 (19%)	
$50,000–$100,000	44 (18%)	45 (17%)	70 (27%)	60 (22%)	
>$100,000	21 (9%)	22 (8%)	47 (18%)	34 (13%)	
Education					
College graduate	75 (25%)	82 (25%)	135 (43%)	114 (36%)	<0.0001
High school/GED	63 (20%)	78 (23%)	59 (19%)	59 (19%)	
Less than high school	103 (34%)	106 (32%)	66 (21%)	89 (28%)	
Some college	66 (22%)	66 (20%)	52 (17%)	56 (18%)	
Smok status					
No smoking	215 (71%)	253 (67%)	239 (77%)	233 (74%)	0.0028
Smoking prior to pregnancy	37 (12%)	29 (9%)	34 (11%)	35 (11%)	
Smoking during pregnancy	51 (17%)	81 (24%)	37 (12%)	48 (15%)	
Folic acid supplementation					
Yes	275 (92%)	300 (92%)	270 (89%)	285 (90%)	0.4257
No	25 (8%)	26 (8%)	35 (11%)	31 (10%)	
Type 2 diabetes					
No	302 (98%)	327 (98%)	308 (99%)	314 (99%)	0.7180
Yes	6 (2%)	6 (2%)	3 (1%)	4 (1%)	
Gestational diabetes					
No	287 (93%)	312 (94%)	290 (94%)	296 (95%)	0.9389
Yes	20 (7%)	20 (6%)	20 (6%)	17 (5%)	
Gestational weight gain					
Adequate	82 (26%)	64 (19%)	87 (28%)	78 (25%)	0.1954
Less than adequate	77 (25%)	86 (26%)	70 (22%)	84 (27%)	
Excessive	149 (48%)	180 (55%)	154 (50%)	151 (48%)	
Vaginal delivery					
Yes	214 (69%)	229 (68%)	199 (63%)	229 (72%)	0.1508
No	97 (31%)	106 (32%)	115 (37%)	90 (28%)	

(*Continued on next page*)

Table 1. Maternal characteristics by total non-sedentary time derived from self-reported recreational, household, occupational and transport activity: Newborn Epigenetic Study *(Continued)*

	Quartile 1 (N = 311)	Quartile 2 (N = 337)	Quartile 3 (N = 314)	Quartile 4 (N = 319)	
BMI (kg/m^2)					
18.5–24.99	107 (36%)	115 (36%)	129 (43%)	128 (42%)	0.2198
25-29.99	87 (29%)	102 (32%)	80 (27%)	73 (24%)	
>29.99	103 (35%)	106 (33%)	90 (30%)	102 (34%)	
Infant sex					
Male	164 (52%)	164 (49%)	176 (56%)	167 (52%)	0.3109
Female	147 (47%)	173 (51%)	138 (44%)	152 (48%)	

methylation differences for DMRs regulating growth and development accounted for this association. We found that infants born to mothers in the highest quartile of total non-sedentary time had lower BW compared to infants of mothers in the lowest quartile of total non-sedentary time. Associations appeared to be strongest among male infants. DMR methylation at *PLAGL1* was associated with total non-sedentary time, and may, in part, account for the association between PA and BW.

There is accumulating evidence that regular PA during pregnancy may increase the odds of delivering an appropriate-for-gestational age infant by reducing the risk of babies born at the extreme ends of the BW range,[27] although results are not entirely consistent.[28,29] Further data suggest inverse associations between PA and mean BW,[30] but the differences are generally small, and few reach statistical significance. Previous investigations have largely focused on recreational activities in late pregnancy. Our finding that total non-sedentary time during early pregnancy was associated with small reductions in mean BW is therefore consistent with most previous reports. Exploratory analyses examining the association between maternal PA and BW extremes suggest that total non-sedentary time may reduce the risk of macrosomia. Further, among women in the lowest quartiles of total non-sedentary time (Quartiles 1–3) we found null associations with LBW. We observed elevated risk of LBW among women in the highest quartile of total non-sedentary time, which was primarily comprised of moderate to vigorous activity. High levels of PA have been shown to: (1) increase the formation of reactive oxygen and nitrogen species;[31] (2) induce lipid peroxidation; and (3) cause DNA damage. These biomarkers

Table 2. Crude and adjusteda regression coefficients and standard errors for the association between maternal physical activity and birthweight: Newborn Epigenetic Study

	Crude (N = 1281)	Adjusted (N = 1144[b])
	β coefficient, Standard Error, *p*-value	
Light physical activity (median = 8 hours/day)		
Quartile 1	Reference	Reference
Quartile 2	114.34, 46.28, 0.01	73.41, 39.90, 0.07
Quartile 3	79.13, 48.19, 0.10	23.66, 42.04, 0.57
Quartile 4	56.49, 47.40, 0.23	10.16, 41.43, 0.81
		p trend = 0.0678
Moderate physical activity (median = 3 hours/day)		
Quartile 1	Reference	Reference
Quartile 2	−70.86, 47.42, 0.14	−65.44, 40.64, 0.11
Quartile 3	−16.87, 50.00, 0.74	−50.86, 42.91, 0.24
Quartile 4	−21.87, 48.85, 0.65	−18.63, 42.54, 0.66
		p trend = 0.0773
Intense physical activity (median = ½ hour/day)		
None	Reference	Reference
≤ Median	47.98, 44.23, 0.28	−17.71, 38.18, 0.64
> Median	68.15, 49.88, 0.17	−0.73, 42.79, 0.99
		p trend = 0.6428
Total non-sedentary time (median = 6.5 hours/day)		
Quartile 1	Reference	Reference
Quartile 2	−72.28, 47.47, 0.13	−43.63, 41.02, 0.29
Quartile 3	−47.28, 48.29, 0.33	−51.93, 41.59, 0.21
Quartile 4	−19.96, 48.11, 0.68	−81.16, 42.02, 0.05
		p trend = 0.4342

[a]Adjusted for maternal race/ethnicity and preterm birth.
[b]N = 137 women were missing covariate values.

Table 3. Adjusted regression coefficients and standard errors for the association between total maternal physical activity and birthweight within strata of maternal race, infant sex and maternal body size: Newborn Epigenetic Study

	Total non-sedentary time				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	
	β coefficient, Standard Error, *p*-value				
Maternal race [a]					*p* trend
Blacks (N = 510)	Reference	−62.75, 58.50, 0.28	−46.72, 63.50, 0.46	−79.58, 66.79, 0.23	0.4098
Whites (N = 364)	Reference	−86.40, 83.65, 0.30	−76.66, 77.40, 0.32	−106.58, 76.17, 0.16	0.3781
Hispanics (N = 270)	Reference	45.05, 80.29, 0.58	−41.95, 80.01, 0.60	−59.13, 79.13, 0.46	0.5505
Infant sex [b]					
Male Infants (N = 602)	Reference	−53.18, 57.74, 0.36	−96.60, 56.98, 0.09	−125.40, 58.10, 0.03	0.4737
Female Infants (N = 542)	Reference	−21.26, 57.70, 0.71	−6.13, 60.31, 0.92	−31.28, 60.20, 0.60	0.8334
Maternal body mass index [b]					
18.5–24.99 kg/m^2 (N = 415)	Reference	−75.23, 69.05, 0.28	−105.91, 67.43, 0.12	−112.55, 68.71, 0.10	0.3124
25–29.99 kg/m^2 (N = 301)	Reference	−62.67, 75.85, 0.41	−104.62, 80.52, 0.19	−25.47, 85.03, 0.76	0.2735
≥30 kg/m^2 \(N = 374)	Reference	−31.06, 73.90, 0.67	74.11, 75.86, 0.33	−58.55, 75.24, 0.44	0.8663

[a]Adjusted for birth.
[b]Adjusted for maternal race/ethnicity and preterm birth.

have been associated with poor birth outcomes, including LBW.[32,33] However, these analyses were based on small numbers and should be interpreted with caution. If confirmed, our collective data could indicate that increased PA could be a useful strategy for reducing infant BW.

In our population, reductions in mean BW from total non-sedentary activity was primarily limited to White, non-Hispanic women and women with BMI <25 kg/m^2. To date, there is limited data examining PA-BW associations by both race/ethnicity and maternal BMI. Although we observed modest reductions in BW among Black participants in the highest quartile of total non-sedentary time, they were less in magnitude than that of Whites, consistent with other studies in this demographic.[34] While we observed risk reductions among obese women in the highest quartile of total non-sedentary time, these data are imprecise. Our findings are in agreement with one study[35] that found overweight/obesity was associated with higher BW only among women with no or low PA, but are contradictory to the findings by Lof et al.,[36] who showed that PA did not reduce the risk of high BW infants in women who were overweight. Further research on the effect of PA within strata of both race and maternal BMI will be important in uncovering whether PA may attenuate adverse consequences of race and/or excess maternal body weight on infant BW.

Although mechanisms by which maternal PA could reduce BW are unknown, it has been suggested that PA during pregnancy may influence fetal growth by improving maternal insulin sensitivity and downstream regulation of fetal nutrient supply.[30] Data show that moderate increases in glucose are associated with augmented levels of fetal insulin, insulin-like growth factor (IGF)-1 and even increased incidence of macrosomia.[8,37] PA has been associated with decreased maternal blood glucose immediately following PA,[38,39] as well as reduced cord blood concentrations of IGF-1 and IGF-2.[30,40] Maternal exercise thereby influences fetal growth, in part, through endocrine regulation. Another potential regulator of fetal growth is the delivery of oxygen and nutrients, via the placenta, to the developing fetus.[8] PA has been shown to influence parameters of placental development, which have been correlated with offspring size at birth.[30,37] Specifically, data suggest that maternal activity during pregnancy is associated with greater placental volume and function.[41,42] Acute

Table 4. Adjusted a regression coefficients and standard errors for the association between total maternal physical activity and offspring DMR methylation (among term infants) at 4 regions: Newborn Epigenetic Study

mean methylation % (standard deviation)	**H19 DMR 47.85 (3.82)**	**MEG3 DMR 72.40 (5.49)**	**SGCE/PEG10 DMR 45.13 (5.45)**	**PLAGL1 DMR 57.54 (6.12)**
	β coefficient, Standard Error, *p*-value			
Total non-sedentary time				
Quartile 1	Reference	Reference	Reference	Reference
Quartile 2	0.37, 0.56, 0.52	−0.16, 0.80, 0.84	−1.38, 0.75, 0.07	−2.15, 0.84, 0.01
Quartile 3	0.65, 0.60, 0.28	−0.88, 0.82, 0.28	−0.18, 0.79, 0.82	−1.77, 0.89, 0.04
Quartile 4	0.35, 0.58, 0.54	−0.45, 0.80, 0.57	0.23, 0.77, 0.76	−1.57, 0.86, 0.07
p trend	0.4385	0.7209	0.0570	0.0136

[a]Adjusted for gestational age, race/ethnicity, smoking and folic acid supplementation.

activity is likely to cause intermittent fluctuations in oxygen and nutrient delivery to the placenta, which, if performed regularly, may stimulate placental growth.[37] Fetal growth patterns stimulated by recurrent acute stimuli, such as regular PA, are unlike those produced by chronic depressions in oxygen delivery, which may cause fetal hypoxia and growth restriction. Discriminating among these possibilities will require larger studies with serial specimens collected during pregnancy.

While the benefits of prenatal PA on BW outcomes are well documented, and epigenetics has been hypothesized to mediate these relationships,[43] there are a paucity of studies examining the potential epigenetic programming effects of exercise during pregnancy. To the best of our knowledge, this is the first study to examine the role of maternal exercise on DNA methylation of regulatory sequences of genomically imprinted genes, and its potential effects on BW outcomes. Certain epigenetic targets, including *IGF2, MEG3, SGCE/PEG10*, and *PLAGL1* have been identified as key regulators of placental and fetal growth and have been associated with BW in our study population.[44] This study found PA-associated methylation differences at *PLAGL1* and suggests that alterations at this DMR may partially mediate the association between maternal PA and BW. *PLAGL1*, widely expressed in many human tissues during fetal development and throughout life, is part of a network of co-regulated imprinted genes involved in the control of embryonic growth. Methylation of the *PLAGL1* DMR has been positively associated with fetal[45] and post-natal weight and is known to correlate with maternal BMI.[46] The results from our investigation may suggest that maternal PA during the prenatal period favorably alters offspring methylation of the *PLAGL1* regulatory DMR, resulting in reduced BW. Further, *PLAGL1* is a candidate tumor-suppressor gene known to regulate apoptosis and cellular growth.[47] While the longer term effects of prenatal exposure on *PLAGL1* and chronic diseases are still unknown, we posit that PA-induced loss of methyl groups at this imprinted domain may increase mRNA expression of *PLAGL1*. Loss of expression has been observed in a number of adult cancers; maternal PA may provide one avenue through which epigenetic programming alter cancer risk.

Our findings, that maternal PA may influence DNA methylation markers in offspring, are biologically plausible and supported by recent intervention and clinical studies. Among men exposed to regular exercise, genome-wide epigenetic modifications were induced in skeletal muscle[48] and adipose tissue,[49] and altered micro RNA expression was observed at both sites. Many of the genes identified in these studies affect glucose and lipid metabolism and are candidates for T2D. The findings of this study supports the DOHaD hypothesis, which suggests that impaired preadipocyte maturation, due to aberrant DNA methylation among LBW individuals, may contribute to the increased risk of T2D in adulthood.

Although it has been hypothesized,[43] we present one of the first studies to examine epigenetic targets in relation to maternal PA and BW. Strengths of our study include its population-based design, relatively large sample size, and detailed exposure assessment. However, our study is limited by the assessment of maternal PA at a single point in time point during early pregnancy. Data have shown that exercise levels tend to decline across pregnancy.[50] While our assessment may not reflect all the appropriate exposure windows in pregnancy for effects on timing of BW, it is an appropriate time window for understanding the effects on epigenetic regulation of imprinted genes. While we made every effort to correct for potential over-reporting of maternal PA by study participants, we were unable to re-contact women to verify that our adjustment was valid. However, given our unbiased approach, based on empirical data from national surveys, we were likely successful in ranking women for comparisons. Further, restriction of these analyses to plausible respondents did not substantially alter data interpretation. Another potential limitation of these analyses is that we used infant BW to evaluate fetal growth. Although BW is a crude estimation of overall fetal development, it is easily measured in large studies, tends to reflect the in utero environment, and has been associated with later postnatal health outcomes.[8] Unfortunately, due to the low proportion of LBW and marcrosomic infants our analyses were exploratory in nature and estimates imprecise. The imprint regulatory network contains other DMRs that were not evaluated in this study. Higher resolution genome-wide studies in diverse populations with improved exposure assessment are necessary to better characterize these epigenetic networks, potential regulators, and their interactions.

Data from our study support those of other previous investigations: maternal PA may reduce infant BW, particularly in White, non-overweight populations, and male infants. However, our understanding of potential mechanisms underlying this association is lacking. We found that methylation at the *PLAGL1* DMR was associated with both maternal PA and BW, and perturbations in this gene may partially account for this association. Further research on the long term effects of changes at the *PLAGL1* DMR may be important for understanding the extent to which prenatal PA may modify subsequent health effects. If replicated, these findings could provide insights into the mechanisms underlying associations between maternal PA and BW and provide epigenetic targets for surveillance or intervention.

Materials and Methods

Study participants

Study participants were enrolled as part of the Newborn Epigenetics Study (NEST), a prospective study of women and their offspring. Methods for enrollment have been detailed elsewhere.[51] Briefly, between 2009 and 2011, English or Spanish speaking pregnant women aged 18 y and older were identified from clinic logs of 5 prenatal clinics in Durham County, NC, USA. Women who intended to relinquish custody of the child, planned to move from the area in the subsequent 3 years, or did not intend to use one of the participating obstetric facilities for delivery were excluded from the study. Additionally, women with confirmed human immunodeficiency virus (HIV) infection were excluded due to uncertainty about how HIV positivity and treatment may alter methylation profiles. Of 2548 women who

met eligibility criteria, 1700 (66.7%) consented and were enrolled. Women who declined study participation were more likely to be of Asian and Native American descent ($P < 0.001$), but were similar to enrolled participants with respect to age ($P = 0.70$). Of the 1700 women who consented, we excluded 36 women who delivered multiple gestation. An additional 383 were excluded due to infant deaths before, during, or soon after birth; illiteracy; being underage; subsequent refusal; delivery at a non-participating hospital (precluding collection of parturition data); or missing PA data, such that 1281 (75.4%) women remained in this analysis. The study protocol was approved by the Duke University and Durham Regional Hospital Institutional Review Boards.

Data collection

A self-administered questionnaire was completed by all participants at enrollment, and queried women on their sociodemographic, reproductive and lifestyle characteristics in the 6 months prior, including periconception. Upon delivery, medical records were abstracted to collect information on birth outcomes and infant cord blood specimens were obtained.

Assessment of maternal PA

As part of the NEST baseline questionnaire, participants were asked about their involvement in PA since LMP using a modification of the Pregnancy Physical Activity Questionnaire, which has been previously validated.[52] Specifically, participants were asked to report how much time they spent in 32 activities using a 5-point scale: none, <½ hour/day, ½ to <1 hour/day, 1 to <2 hours/day, 2 to <3 hours/day, and ≥3 hours/ day. The questions covered 4 modes of activity including household/caregiving (N = 13 activities), recreational (N = 8 activities), occupational (N = 4 activities), and transportation (N = 2 activities) (**Table S1**). The questionnaire also included 5 sedentary activities (excluded from these analyses).

For these analyses, we were interested in exploring associations with BW and methylation status by intensity level, regardless of the mode. Metabolic equivalent (MET) values were assigned to each reported activity according to the Compendium of Physical Activities.[53] Using the standard cutoffs[54] for light (<3.0 METs), moderate (3–6 METs), and intense (>6 METs) activities, we summarized total non-sedentary PA by intensity category. For example, if a woman reported walking slowly for exercise (3.8 METs) 1 hour/day, playing with children (4.0 METs) for ½ hour/day, and gardening (4.5 METs) for 1 hour/day, her cumulative moderate activity would be 2½ hours/day. We similarly calculated total light and intense activity for each participant. Finally, we summed the duration of all non-sedentary activities to obtain each participants total non-sedentary time per day.

We found that NEST participants had a high degree of over-reporting compared to other studies of prenatal PA[55,56] which may suggest that some women misread the questionnaire response as hours/week rather than hours/day. Our sensitivity analyses showed that 3%, 30%, 4%, and 40% of respondents had implausible values for light (>16 hours/day), moderate (>8 hours/day) intense (>2 hours/day) and total non-sedentary (>16 hours/day) activities, respectively based on data among women from the Bureau of Labor Statistics.[57] We identified no demographic differences among participants who over-reported compared to those with plausible values. We therefore rescaled women exceeding the aforementioned cutoffs by dividing their total or intensity-specific activity contribution by 7. We also conducted analyses to confirm that excluding women with rescaled data did not meaningfully influence our results.

Our final analysis included 4 mutually exclusive PA variables, 3 of which account for both intensity and duration (light, moderate and intense hours/day), and a summary variable (total non-sedentary time), which accounts for duration only. Light activity contributed to 64%, 60%, 56%, and 48% of total non-sedentary time for quartiles 1–4, respectively.

Assessment of birth outcomes

Parturition data, abstracted via medical records by trained personnel after delivery, included BW, gestational age at birth, infant sex, and delivery mode. Infant BW (measured in grams g) showed no evidence of departure from normality and was analyzed as both a continuous and categorical variable. Trichotomous categories included LBW (<2500 g), normal weight (2500–4500 g), and macrosomia/high birth weight (>4500 g). Given the low proportion of BW extremes, however, these analyses were exploratory in nature.

Assessment of covariates and effect measure modifiers

Maternal age at delivery, race/ethnicity, marital status, parity, diabetes, and weight and height at LMP were self-reported and then verified with abstracted medical records. Household income, maternal education, cigarette smoking, and folic acid supplementation were self-reported via questionnaire.

A priori we considered race/ethnicity, infant sex and maternal BMI as potential modifiers of the association between PA and BW. Race/ethnic categories were assigned based on women's self-identification as Black/African American, Non-Hispanic White, or Hispanic White. Infant sex was abstracted from medical records as previously described. Maternal BMI was calculated from self-reported weight and height at LMP, converted to kilograms (kg) and meters (m) and expressed as kg/m^2.

DNA methylation analysis

Maternal peripheral blood samples were collected at enrollment and infant cord blood specimens were collected at birth. Samples were collected in EDTA-treated tubes and centrifuged using standard protocols to allow for collection of plasma and buffy coat for DNA extraction (Qiagen, Valencia, CA, USA); samples were stored at −80°C prior to use. DNA was extracted using Puregene reagents according to the manufacturer's protocol (Qiagen), and quantity and quality assessed using a Nanodrop 1000 Spectrophotometer (Thermo Scientific; Wilmington, DE, USA). Maternal and infant genomic DNA (800 ng) was modified by treatment with sodium bisulfite using the Zymo EZ DNA Methylation kit (Zymo Research; Irvine, CA, USA). Pyrosequencing was performed using a Pyromark Q96 MD pyrosequencer (Qiagen) to measure DNA methylation at 9 imprint

regulatory regions in mothers and newborns. Four DMRs previously shown to be associated with BW in this study were included in these analyses including: the *H19* DMR regulating the *IGF2/H19* domain, the *MEG3* DMR regulating the *DLK1/MEG3* domain, the *SGCE/PEG10* DMR positioned between Epsilon Sarcoglycan and Paternally Expressed Gene 10 and the *PLAGL1* DMR.[18] Assays were designed to query established imprinted gene DMRs using the Pyromark Assay Design Software (Qiagen). Polymerase chain reaction conditions were optimized to produce a single, robust amplification product by adjusting annealing temperature and magnesium chloride concentrations. Defined mixtures of fully methylated and unmethylated control DNAs were used to show a linear increase in detection of methylation values as the level of input DNA methylation increased (Pearson r > 0.99 for all DMRs). Once optimal conditions were defined, each DMR was analyzed using the same amount of input DNA from each specimen (40 ng, assuming complete recovery following bisulfite modification), keeping the thermocycler and pyrosequencer constant. Percent methylation for each CpG cytosine was determined using Pyro Q-CpG Software (Qiagen). We interrogated between 3 and 10 CpG sites per DMR: 4 for *H19*, 8 for *MEG3*, 6 for *SGCE/PEG10*, and 6 for *PLAGL1*.

Statistical analysis

Chi-squared tests[58] were used to compare the distribution of demographic and obstetric descriptors across quartiles of total non-sedentary time. Variables were selected based on their known or suspected associations with either infant BW or maternal PA. We used generalized linear models to estimate associations between maternal PA and mean BW[59] (a priori $P \leq 0.05$). Logistic regression models were used to estimate associations between maternal PA and BW extremes.[60] In all models, we considered adjustment for maternal age (<20, 20–29, 30–35, and >35 years); race/ethnicity (Black, White, Hispanic); preterm birth (yes/no); gestational age at enrollment (≤10, 11–20, and >20 weeks); marital status (married, never married, living with partner, widowed/divorced/separated, and other); parity upon enrollment (nulliparous and multiparous); household income (<$25000, $25000–$49999, $50000–$100000, and >$100000); maternal education (less than high school, high school/GED, some college, college graduate); maternal smoking (no smoking prior to pregnancy and smoking during pregnancy); type 2 diabetes (yes/no); gestational diabetes (yes/no); and vaginal delivery (yes/no). BMI and total energy were not considered as confounders in these analyses because they are likely on the causal pathway between PA and BW. The refined list of confounders was selected based on directed acyclic graphs[60] and backward elimination.[61] Only race/ethnicity and preterm birth remained in our final main effects models. Refined models adjusted for race/ethnicity and preterm birth were used to examine potential effect modification of BMI at LMP (BMI18.5–24.99, 25–29.99, ≥30 kg/m^2) and infant sex (male/female). Race/ethnic stratified models (Black, White, and Hispanic) were adjusted only for preterm birth.

Among the 484 term (≥37 weeks gestation) mother-infant pairs where methylation data for at least one of four DMRs (*H19, MEG3, SGCE/PEG10*, and *PLAGL1*) was available, we examined associations with maternal PA. Mother-infant pairs with DMR data available were similar to those without DNA methylation data with respect to maternal age ($P = 0.59$), total activity ($P = 0.30$), and BW ($P = 0.52$). DNA methylation fractions at two DMRs (*SGCE/PEG10* and *PLAGL1* DMRs) were not normally distributed as assessed via Kolmogorov-Smirnov tests.[51] Additionally, Cronbach's alphas for all DMRs considered were >0.89. We therefore used mean methylation level for each DMR in our models.[51] We used F-tests,[61] for parametric analyses, and Wilcoxon rank-sum tests[58] to examine DNA methylation levels by PA, adjusting for factors previously shown to influence DNA methylation[62] (a priori $P \leq 0.05$). The subset of DMRs associated with both maternal PA and BW were then included in refined models to determine the extent to which the main effects were attenuated. All statistical analyses were conducted in SAS v9.3 (SAS Institute, Cary, NC, USA).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website

Ethics Statement

IRB approval was obtained for all participating institutions.

References

1. Calkins K, Devaskar SU. Fetal origins of adult disease. Curr Probl Pediatr Adolesc Health Care 2011; 41:158-76; PMID:21684471; http://dx.doi.org/10.1016/j.cppeds.2011.01.001
2. Drake AJ, Walker BR. The intergenerational effects of fetal programming: non-genomic mechanisms for the inheritance of low birth weight and cardiovascular risk. J Endocrinol 2004; 180:1-16; PMID:14709139; http://dx.doi.org/10.1677/joe.0.1800001
3. Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, Barrett-Connor E, Bhargava SK, Birgisdottir BE, Carlsson S, et al. Birth weight and risk of type 2 diabetes: a systematic review. JAMA 2008;

300:2886-97; PMID:19109117; http://dx.doi.org/10.1001/jama.2008.886
4. Yu ZB, Han SP, Zhu GZ, Zhu C, Wang XJ, Cao XG, Guo XR. Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. Obes Rev 2011; 12:525-42; PMID:21438992; http://dx.doi.org/10.1111/j.1467-789X.2011.00867.x
5. McCormack VA, dos Santos Silva I, Koupil I, Leon DA, Lithell HO. Birth characteristics and adult cancer incidence: swedish cohort of over 11,000 men and women. Int J Cancer 2005; 115:611-7; PMID:15700315; http://dx.doi.org/10.1002/ijc.20915
6. Martin JA, Hamilton BE, Osterman JK, Curtin SC, Mathews TJ. Births: Final Data for 2012. National Vital Statistics Reports. Hyattsville, MD: National Center for Health Statistics 2013; 62:9.
7. Leiferman JA, Evenson KR. The effect of regular leisure physical activity on birth outcomes. Matern Child Health J 2003; 7:59-64; PMID:12710801; http://dx.doi.org/10.1023/A:1022545718786
8. Hopkins SA, Cutfield WS. Exercise in pregnancy: weighing up the long-term impact on the next generation. Exerc Sport Sci Rev 2011; 39:120-7; PMID:21519301; http://dx.doi.org/10.1097/JES.0b013e31821a5527
9. Owe KM, Nystad W, Bo K. Association between regular exercise and excessive newborn birth weight. Obstet Gynecol 2009; 114:770-6; PMID:19888034; http://dx.doi.org/10.1097/AOG.0b013e3181b6c105
10. Clapp JF 3rd. The effects of maternal exercise on fetal oxygenation and feto-placental growth. Eur J Obstet Gynecol Reprod Biol 2003; 110:S80-85; PMID:12965094; http://dx.doi.org/10.1016/S0301-2115(03)00176-3
11. Nelson SM, Matthews P, Poston L. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. Hum Reprod Update 2010; 16:255-75; PMID:19966268; http://dx.doi.org/10.1093/humupd/dmp050
12. Langley-Evans SC, McMullen S. Developmental origins of adult disease. Med Princ Pract 2010; 19:87-98; PMID:20134170; http://dx.doi.org/10.1159/000273066
13. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature 2007; 447:433-40; PMID:17522677; http://dx.doi.org/10.1038/nature05919
14. Hanson M, Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. Prog Biophys Mol Biol 2011; 106:272-80; PMID:21219925; http://dx.doi.org/10.1016/j.pbiomolbio.2010.12.008
15. Pareja-Galeano H, Sanchis-Gomar F, Garcia-Gimenez JL. Physical exercise and epigenetic modulation: elucidating intricate mechanisms. Sports Med 2014; 44:429-36; PMID:24399634; http://dx.doi.org/10.1007/s40279-013-0138-6
16. Ling C, Ronn T. Epigenetic adaptation to regular exercise in humans. Drug Discov Today 2014; 19:1015-8; PMID:24632002; http://dx.doi.org/10.1016/j.drudis.2014.03.006
17. Das R, Hampton DD, Jirtle RL. Imprinting evolution and human health. Mamm Genome 2009; 20:563-72; PMID:19830403; http://dx.doi.org/10.1007/s00335-009-9229-y
18. Murphy SK, Huang Z, Hoyo C. Differentially methylated regions of imprinted genes in prenatal, perinatal and postnatal human tissues. PLoS One 2012; 7:e40924; PMID:22808284; http://dx.doi.org/10.1371/journal.pone.0040924
19. Murphy SK, Huang Z, Wen Y, Spillman MA, Whitaker RS, Simel LR, Nichols TD, Marks JR, Berchuck A. Frequent IGF2/H19 domain epigenetic alterations and elevated IGF2 expression in epithelial ovarian cancer. Mol Cancer Res 2006; 4:283-92; PMID:16603642; http://dx.doi.org/10.1158/1541-7786.MCR-05-0138
20. Skaar DA, Li Y, Bernal AJ, Hoyo C, Murphy SK, Jirtle RL. The human imprintome: regulatory mechanisms, methods of ascertainment, and roles in disease susceptibility. ILAR J 2012; 53:341-58; PMID:23744971; http://dx.doi.org/10.1093/ilar.53.3-4.341
21. Jirtle RL. Epigenome: the program for human health and disease. Epigenomics 2009; 1:13-6; PMID:22122631; http://dx.doi.org/10.2217/epi.09.16
22. Murphy SK, Jirtle RL. Imprinting evolution and the price of silence. Bioessays 2003; 25:577-88; PMID:12766947; http://dx.doi.org/10.1002/bies.10277
23. Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, Forman MR, Iversen ES, Kurtzberg J, Overcash F, Huang Z, et al. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. Epigenetics 2011; 6:928-36; PMID:21636975; http://dx.doi.org/10.4161/epi.6.7.16263
24. Blackmore CA, Ferre CD, Rowley DL, Hogue CJ, Gaiter J, Atrash H. Is race a risk factor or a risk marker for preterm delivery? Ethn Dis 1993; 3:372-7; PMID:7888988
25. Frederick IO, Williams MA, Sales AE, Martin DP, Killien M. Pre-pregnancy body mass index, gestational weight gain, and other maternal characteristics in relation to infant birth weight. Matern Child Health J 2008; 12:557-67; PMID:17713848; http://dx.doi.org/10.1007/s10995-007-0276-2
26. Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 2009; 18:4046-53; PMID:19656776; http://dx.doi.org/10.1093/hmg/ddp353
27. Juhl M, Olsen J, Andersen PK, Nohr EA, Andersen AM. Physical exercise during pregnancy and fetal growth measures: a study within the danish national birth cohort. Am J Obstet Gynecol 2010; 202:63.e1-63.e8; PMID:19800601
28. Jukic AM, Evenson KR, Daniels JL, Herring AH, Wilcox AJ, Hartmann KE. A prospective study of the association between vigorous physical activity during pregnancy and length of gestation and birthweight. Matern Child Health J 2012; 16:1031-44; PMID:21674218; http://dx.doi.org/10.1007/s10995-011-0831-8
29. Hegaard HK, Petersson K, Hedegaard M, Ottesen B, Dykes AK, Henriksen TB, Damm P. Sports and leisure-time physical activity in pregnancy and birth weight: a population-based study. Scand J Med Sci Sports 2010; 20:e96-102; PMID:19422639; http://dx.doi.org/10.1111/j.1600-0838.2009.00918.x
30. Hopkins SA, Baldi JC, Cutfield WS, McCowan L, Hofman PL. Exercise training in pregnancy reduces offspring size without changes in maternal insulin sensitivity. J Clin Endocrinol Metab 2010; 95:2080-8; PMID:20335449; http://dx.doi.org/10.1210/jc.2009-2255
31. Clarkson PM, Thompson HS. Antioxidants: what role do they play in physical activity and health? Am J Clin Nutr 2000; 72:637S-47S; PMID:10919970
32. Tabacova S, Baird DD, Balabaeva L. Exposure to oxidized nitrogen: lipid peroxidation and neonatal health risk. Arch Environ Health 1998; 53:214-21; PMID:9814718; http://dx.doi.org/10.1080/00039899809605698
33. Negi R, Pande D, Kumar A, Khanna RS, Khanna HD. In vivo oxidative DNA damage and lipid peroxidation as a biomarker of oxidative stress in preterm low-birthweight infants. J Trop Pediatr 2012; 58:326-8; PMID:21930665; http://dx.doi.org/10.1093/tropej/fmr078
34. Orr ST, James SA, Garry J, Prince CB, Newton ER. Exercise and pregnancy outcome among urban, low-income, black women. Ethn Dis 2006; 16:933-7; PMID:17061749
35. Krogsgaard S, Gudmundsdottir SL, Nilsen TI. Pre-pregnancy physical activity in relation to offspring birth weight: a prospective population-based study in norway-the HUNT study. J Pregnancy 2013; 2013:780180; PMID:23431451; http://dx.doi.org/10.1155/2013/780180
36. Lof M, Hilakivi-Clarke L, Sandin S, Weiderpass E. Effects of pre-pregnancy physical activity and maternal BMI on gestational weight gain and birth weight. Acta Obstet Gynecol Scand 2008; 87:524-30; PMID:18446535; http://dx.doi.org/10.1080/00016340802012288
37. Clapp JF. Influence of endurance exercise and diet on human placental development and fetal growth. Placenta 2006; 27:527-34; PMID:16165206; http://dx.doi.org/10.1016/j.placenta.2005.07.010
38. Bonen A, Campagna PD, Gilchrist L, Beresford P. Substrate and hormonal responses during exercise classes at selected stages of pregnancy. Can J Appl Physiol 1995; 20:440-51; PMID:8563676; http://dx.doi.org/10.1139/h95-035
39. McMurray RG, Hackney AC, Guion WK, Katz VL. Metabolic and hormonal responses to low-impact aerobic dance during pregnancy. Med Sci Sports Exerc 1996; 28:41-6; PMID:8775353; http://dx.doi.org/10.1097/00005768-199601000-00012
40. Hopkins SA, Baldi JC, Cutfield WS, McCowan L, Hofman PL. Effects of exercise training on maternal hormonal changes in pregnancy. Clin Endocrinol (Oxf) 2011; 74:495-500; PMID:21198740; http://dx.doi.org/10.1111/j.1365-2265.2010.03964.x
41. Bergmann A, Zygmunt M, Clapp JF 3rd. Running throughout pregnancy: effect on placental villous vascular volume and cell proliferation. Placenta 2004; 25:694-8; PMID:15450386; http://dx.doi.org/10.1016/j.placenta.2004.02.005
42. Clapp JF 3rd, Kim H, Burciu B, Schmidt S, Petry K, Lopez B. Continuing regular exercise during pregnancy: effect of exercise volume on fetoplacental growth. Am J Obstet Gynecol 2002; 186:142-7; PMID:11810100; http://dx.doi.org/10.1067/mob.2002.119109
43. Donovan EL, Miller BF. Exercise during pregnancy: developmental origins of disease prevention? Exerc Sport Sci Rev 2011; 39:111; PMID:21701279; http://dx.doi.org/10.1097/JES.0b013e31821f7d78
44. Hoyo C, Daltveit AK, Iversen E, Benjamin-Neelon SE, Fuemmeler B, Schildkraut J, Murtha AP, Overcash F, Vidal AC, Wang F, et al. Erythrocyte folate concentrations, CpG methylation at genomically imprinted domains, and birth weight in a multiethnic newborn cohort. Epigenetics 2014; 9:1120-30; PMID:24874916; http://dx.doi.org/10.4161/epi.29332
45. Liu Y, Hoyo C, Murphy S, Huang Z, Overcash F, Thompson J, Brown H, Murtha AP. DNA methylation at imprint regulatory regions in preterm birth and infection. Am J Obstet Gynecol 2013; 208:395.e391-397; PMID:23477525
46. Azzi S, Sas TC, Koudou Y, Le Bouc Y, Souberbielle JC, Dargent-Molina P, Netchine I, Charles MA. Degree of methylation of ZAC1 (PLAGL1) is associated with prenatal and post-natal growth in healthy infants of the EDEN mother child cohort. Epigenetics 2014; 9:338-45; PMID:24316753; http://dx.doi.org/10.4161/epi.27387
47. Valleley EM, Cordery SF, Bonthron DT. Tissue-specific imprinting of the ZAC/PLAGL1 tumour suppressor gene results from variable utilization of monoallelic and biallelic promoters. Human Mol Gen 2007; 16:972-81; PMID:17341487; http://dx.doi.org/10.1093/hmg/ddm041
48. Nitert MD, Dayeh T, Volkov P, Elgzyri T, Hall E, Nilsson E, Yang BT, Lang S, Parikh H, Wessman Y, et al. Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of

patients with type 2 diabetes. Diabetes 2012; 61: 3322-32; PMID:23028138; http://dx.doi.org/10.2337/db11-1653
49. Rönn T, Volkov P, Davegårdh C, Dayeh T, Hall E, Olsson AH, Nilsson E, Tornberg A, Dekker Nitert M, Eriksson KF, et al. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. PLoS Genet 2013; 9: e1003572.
50. Owe KM, Nystad W, Bo K. Correlates of regular exercise during pregnancy: the Norwegian mother and child cohort study. Scand J Med Sci Sports 2009; 19:637-45; PMID:18627550; http://dx.doi.org/10.1111/j.1600-0838.2008.00840.x
51. Liu Y, Murphy SK, Murtha AP, Fuemmeler BF, Schildkraut J, Huang Z, Overcash F, Kurtzberg J, Jirtle R, Iversen ES, et al. Depression in pregnancy, infant birth weight and DNA methylation of imprint regulatory elements. Epigenetics 2012; 7:735-46; PMID:22677950; http://dx.doi.org/10.4161/epi.20734
52. Chasan-Taber L, Schmidt MD, Roberts DE, Hosmer D, Markenson G, Freedson PS. Development and validation of a pregnancy physical activity questionnaire. Med Sci Sports Exerc 2004; 36:1750-60; PMID:15595297; http://dx.doi.org/10.1249/01.MSS.0000142303.49306.0D
53. Ainsworth BE, Haskell WL, Leon AS, Jacobs DR Jr, Montoye HJ, Sallis JF, Paffenbarger RS Jr. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc 1993; 25:71-80; PMID:8292105; http://dx.doi.org/10.1249/00005768-199301000-00011
54. Global strategy on diet, physical activity, and health. Programmes and Projects. World Health Organization 2014. Accessed December 11, 2014. http://www.who.int/dietphysicalactivity/physical_activity_intensity/en/
55. Melzer K, Schutz Y, Boulvain M, Kayser B. Pregnancy-related changes in activity energy expenditure and resting metabolic rate in switzerland. Eur J Clin Nutr 2009; 63:1185-91; PMID:19550432; http://dx.doi.org/10.1038/ejcn.2009.49
56. Haakstad LA, Gundersen I, Bo K. Self-reporting compared to motion monitor in the measurement of physical activity during pregnancy. Acta Obstet Gynecol Scand 2010; 89:749-56; PMID:20450260; http://dx.doi.org/10.3109/00016349.2010.484482
57. USBoLS. Table 1. Time spent in primary activities (1) and percent of the civilian population engaging in each activity, averages per day by sex, 2013 annual averages. Economic News Realease. U.S. Bureau of Labor Statistics 2014. Accessed December 11, 2014. http://www.bls.gov/news.release/atus.t01.htm
58. Corder GW, Foreman DI. Nonparametric Statistics for Non-Statisticians: A Step-By-Step Approach, 1st edn. Hoboken, NJ: John Wiley & Songs, Inc, 2009.
59. Madsen H, Thyregod P. Introduction to general and generalized linear models. Int Stat Rev 2012. 80:183-4
60. Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health 1989; 79:340-9; PMID:2916724; http://dx.doi.org/10.2105/AJPH.79.3.340
61. Harrell FE. Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York. NY: Springer-Verlag, 2001.
62. Hoyo C, Murtha AP, Schildkraut JM, Forman MR, Calingaert B, Demark-Wahnefried W, Kurtzberg J, Jirtle RL, Murphy SK. Folic acid supplementation before and during pregnancy in the Newborn Epigenetics STudy (NEST). BMC Public Health 2011; 11:46; PMID:21255390; http://dx.doi.org/10.1186/1471-2458-11-46