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Prenatal stress, methylation in inflammation-related genes, and adiposity measures in early childhood: the PROGRESS cohort study

Shaowei Wu, PhD¹, Chris Gennings, PhD², Rosalind J. Wright, MD^{3,4}, Ander Wilson, PhD⁵, Heather H. Burris, MD, MPH^{1,6}, Allan C. Just, PhD⁷, Joseph M. Braun, PhD⁸, Katherine Svensson, MS⁷, Jia Zhong, ScD⁹, Kasey J.M. Brennan, BS⁹, Alexandra Dereix, BA⁹, Alejandra Cantoral, MS¹⁰, Lourdes Schnaas, PhD¹¹, Martha Maria Téllez-Rojo, ScD¹⁰, Robert O. Wright, MD⁷, and Andrea A. Baccarelli, MD, PhD⁹

¹Department of Occupational and Environmental Health Sciences, School of Public Health, Peking University, Beijing, China

²Population Health and Science Policy, Mount Sinai Hospital, New York, NY, USA

³Department of Pediatrics, Kravis Children's Hospital, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁴The Mindich Child Health & Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁵Department of Statistics, Colorado State University, Fort Collins, CO, USA

⁶Department of Neonatology and Obstetrics, Gynecology and Reproductive Biology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

⁷Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA

⁸Department of Epidemiology, School of Public Health, Brown University, Providence, RI, USA

⁹Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, USA

¹⁰Center for Research in Nutrition and Health, National Institute of Public Health, Cuernavaca, Morelos, Mexico

¹¹Division of Research on Community Interventions, National Institute of Perinatology, Mexico City, Mexico

Abstract

Correspondence: Shaowei Wu, Ph.D., Department of Occupational and Environmental Health Sciences, School of Public Health, Peking University, No. 38 Xueyuan Road, Beijing 100191, China, Phone: 86-10-82805296, Fax: 86-10-82805779 shaowei_wu@bjmu.edu.cn OR Andrea A Baccarelli, M.D., Ph.D., Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, USA, Phone: 1-212-305-3959, andrea.baccarelli@columbia.edu.

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List of Supplemental Digital Content:

A supplemental word file for this manuscript is attached below.

OBJECTIVE—Maternal stress during pregnancy may influence childhood growth and adiposity, possibly through immune/inflammatory programming. We investigated whether exposure to prenatal stress and methylation in inflammation-related genes were associated with childhood adiposity in 424 mother-child pairs in Mexico City, Mexico.

METHODS—A stress index was created based on four prenatally administered stress-related scales (Exposure to Violence, Crisis in Family Systems, State-Trait Anxiety Inventory, and Edinburgh Postnatal Depression Scale). We measured weight, height, body fat mass (BFM), percentage body fat (PBF) and waist circumference in early childhood (age range 4–6 years). Body mass index (BMI) z-scores were calculated according to World Health Organization standards. DNA methylation in gene promoters of tumor necrosis factor- α , interleukin 8 and interleukin 6 (*IL6*) in umbilical cord blood were determined by pyrosequencing.

RESULTS—An interquartile range (IQR) increase in stress index (27.3) was associated with decreases of 0.14 unit in BMI z-score (95% CI: $-0.28, -0.005$), 5.6% in BFM (95% CI: $-9.7, -1.4$), 3.5% in PBF (95% CI: $-6.3, -0.5$), and 1.2% in waist circumference (95% CI: $-2.4, -0.04$) in multivariable-adjusted models. An IQR increase in *IL6* methylation (3.9%) was associated with increases of 0.23 unit in BMI z-score (95% CI: $0.06, 0.40$), 8.1% (95% CI: $2.3, 14.3$) in BFM, 5.5% (95% CI: $1.7, 9.5$) in PBF and 1.7% (95% CI: $0.2, 3.3$) in waist circumference.

CONCLUSIONS—Prenatal stress was associated with decreased childhood adiposity, whereas cord blood *IL6* methylation was associated with increased childhood adiposity in Mexican children.

Keywords

adiposity; body mass index; inflammation; interleukin 6; methylation; prenatal stress

Introduction

Obesity is a leading cause of mortality and lost disability-adjusted life years worldwide(1), and underweight is also associated with substantial disease burden, especially in underdeveloped countries(2). Epidemiological evidence suggests that both overweight and underweight predict long-term adverse health outcomes, such as mortality and incidence of adult metabolic syndrome in children(3, 4). Modern lifestyles have created an increasing burden of chronic psychosocial stress, which may affect the development of metabolic complications(5).

Pregnancy is a potential key life stage for developmental programming, and intrauterine stress exposure may represent an underlying mechanism programming body composition, energy balance homeostasis, and metabolic function. In particular, immune/inflammatory processes may be a candidate pathway underling the long-term effects of intrauterine stress(6). The relationship between stress and weight gain in early life is complex. Animal experiments have demonstrated that prenatal stress increases the susceptibility to diet-induced obesity in rat offspring(7), but chronic stress can also induce anxiety- and depression-like behaviors and significantly reduce body weight gain and food intake in rats in their early life(8). Several studies have reported an association between exposure to prenatal stress and increased body mass index (BMI) or a higher risk of overweight/obesity

in childhood or adolescence(9–12), whereas others have also reported a positive association between prenatal stress and increased risk of being underweight in children(13). These studies only focused on one stressor such as maternal bereavement, natural disaster, or trauma experience. However, exposure to multiple stressors is prevalent, and individuals may be at increased risk of adverse health outcomes when exposed to the cumulative effects of multiple stressors(14). Specifically, low-income women are more likely to experience exposure to violence, financial crisis, anxiety and depression symptoms(15, 16). Furthermore, both epidemiological and animal studies have shown that males and females may respond to stress differently, suggesting that sex is an important modifier of the stress-related response(17–21). To date, however, no study has investigated the cumulative effects of multiple prenatal psychosocial stressors on childhood adiposity and whether these effects may be different among males and females.

Prenatal stress has been associated with altered immune function and inflammation in pregnant women (22, 23), which may be a mechanism linking prenatal stress and offspring obesity via the maternal-placental-fetal neuroendocrine axis (e.g., by placental corticotropin-releasing hormone) (24). Maternal stress-related hormones may cross the placenta and induce epigenetic changes in newborns(25). Epigenetics refer to changes in gene expression and phenotype without DNA sequence alteration. DNA methylation is a type of epigenetic changes that is particularly sensitive to fetal exposures due to the reprogramming of genome-wide methylation profile after fertilization(26). When located in a gene promoter, DNA methylation typically acts to repress gene expression(27). Once established, epigenetic marks may persist over time and affect the health status of offspring over the lifecourse. Given the above body of evidence, the roles of epigenetic changes in key genes of immune function and inflammation such as interleukin 6 (*IL6*), interleukin 8 (*IL8*) and tumor necrosis factor-alpha (*TNF-α*) in offspring development are of particular interest. In support of this notion, interleukin 6 (*IL6*) deficiency has been shown to cause obesity in mice(28, 29). However, it is unclear whether prenatal stress may program the long-term development of adiposity in offspring through the epigenetic modification of immune or inflammatory pathways with involvement of these important genes.

In this study, we investigated whether exposure to prenatal maternal stress, summarized as an index for multiple stressors, was associated with childhood adiposity measures and whether this association was different among males and females and may be mediated or modified by methylation in inflammation-related genes in umbilical cord blood in a prospective birth cohort in Mexico City, Mexico.

Materials and Methods

Study population

We used data from a prospective birth cohort study, Programming Research in Obesity, GRowth Environment and Social Stress (PROGRESS), which enrolled an original sample of 1054 pregnant women from Mexico City, Mexico between 2007 and 2011. Details of participant enrollment have been published elsewhere(30). Briefly, eligible pregnant women receiving prenatal care through the Mexican Social Security System in Mexico City were enrolled prior to 20 weeks gestation after they provided written informed consent for

themselves and their children. Among those enrolled, 948 (90%) women gave birth to a live born infant and at the time of this study (April 2015) 504 (53%) had been followed-up until early childhood (age range: 4–6 years). Of the 504 children, 80 were excluded due to missing information in either BMI z-score (n=20), prenatal stress exposure (n=46) or major covariates (see below, n=14), leaving a sample of 424 children. All study protocols were approved by the Institutional Review Boards of the participating institutions.

Prenatal maternal stress assessment

Trained psychologists confidentially administered a battery of stress scales described below among women in their second or third trimester. Four stress scales that covered the major parts of stress, including violence, negative life events, depression and anxiety, were selected for this analysis (see Supplemental Digital Content for details).

Exposure to Violence—The Exposure to Violence (ETV) questionnaire, validated in Spanish(31), assessed hearing gunshots, witnessing and/or experiencing fights, knife attacks, and/or shootings in their neighborhood (in the past year) as well as frequency of events. The multi-item survey was summarized into a continuous scale using Rasch modeling generalized to calculate conditional probabilities for each “yes” response, given event severity and accounting for frequency(32). Higher scores indicate greater severity and/or frequency of events.

Prenatal Negative Life Events—The Crisis in Family Systems-Revised (CRISYS) survey, validated in Spanish, assessed other life events experienced over the past six months and judged by the subject as having a negative impact and/or “some or very difficult” to go through(33). The survey includes 11 domains (e.g., financial, legal, relationships, safety in the home, etc.), with multiple items in each domain. The number of domains with one or more negative endorsed events was summed to create a negative life events domain score.

Edinburgh Postnatal Depression Scale—The 10-item Edinburgh Postnatal Depression Scale (EPDS), validated in Spanish, has been shown to be valid to assess depressive symptoms in the past week both during pregnancy and in the postpartum periods (34, 35). The EPDS has a high degree of internal consistency (Cronbach’s $\alpha=0.914$) in pregnant women (36). Two items assessing positive symptoms were reversely coded before being summed with the rest of the items for a total score.

State-Trait Anxiety Inventory—Prenatal maternal anxiety was quantified via the 10-item trait anxiety scale of the Spielberger State-Trait Anxiety Inventory (STAI), which is validated in Spanish and has a range of 0.86 to 0.95 for internal consistency coefficients (37). Each item is scored on a 4-point rating scale and summed for a total score.

Composite stress index—Stress was conceptualized as a single-dimension construct where higher scores on each scale (continuous) indicated greater stress and/or more negative psychological impact. A composite stress index was constructed by: (1) converting each of the stress scales to the percentile scale based on the empirical distribution of stress scores in the PROGRESS cohort; (2) estimating the empirical weights for each of the four stress

scales that maximized association with the primary outcome (BMI z-score) using Weighted Quantile Sum (WQS) regression(38); and (3) taking a linear combination of the four stress scales using the estimated weights. Thus, the composite stress index is high (close to 100) for subjects with high levels of stress; and low (close to 0) for subjects with low levels of stress relative to their peers. For detailed methods on the construction of the stress index, see Supplemental Digital Content and Table S1.

Child anthropometry

Birth length and weight were recorded at delivery or abstracted from the infants' hospital chart. At later study visits, length/height and weight were measured using the Infant-o-meter (Health O Meter Inc.) for children 1–12 months old and the Health-o-meter (Health O Meter Inc.) for children older than 12 months. BMI (kg/m^2) and BMI z-scores were calculated based on the World Health Organization (WHO) reference data(39). Tetrapolar bioelectrical impedance was measured using the InBody 370 or 230 (Biospace Co., Ltd.) to estimate body fat mass (BFM) and percentage body fat (PBF). Because values on these two instruments differed systematically for children, we used a robust linear model fit on a calibration set of 36 children with concurrent measures to adjust the values (R^2 of 0.99 and 0.96 for BFM and PBF, respectively). Waist circumference was measured above the iliac crests using a SECA measuring tape. BMI z-score was set as the primary outcome and the other three adiposity measures including BFM, PBF and waist circumference were set as the secondary outcomes. All adiposity measures were treated as continuous variables without categorization.

DNA methylation analysis

We obtained 554 umbilical cord blood samples at delivery and analyzed DNA methylation in promoters of three candidate inflammation genes, *TNF- α* , *IL6* and *IL8*. Details about sample extraction, DNA bisulfite treatment, and pyrosequencing assay of these samples have been previously described(40) and could be found in the Supplemental Digital Content. Among the 424 children included in the analysis, 247 had methylation data in *TNF- α* and *IL6*, and 239 had methylation data in *IL8*.

Covariates

We obtained information on major covariates from baseline questionnaires, hospital records or field measurements. Major covariates include maternal pre-pregnancy BMI (calculated as BMI during 2nd trimester minus 2 kg/m^2), parity, socioeconomic status, birth weight, child sex, and child total energy intake and percent fat intake of total energy at 4–6 years of age. A full description on the assessment of covariates is provided in the Supplemental Digital Content.

Statistical Analysis

Normality test and histogram inspection suggested that BMI z-score and DNA methylation values appeared normally-distributed, whereas BFM, PBF and waist circumference were $\log_{10}$ -transformed to improve data normality. We performed regression analyses for three exposure-outcome pairs: stress index-adiposity measures, stress index-DNA methylation, and DNA methylation-adiposity measures.

Unadjusted and adjusted regression models were constructed to evaluate the consistency of exposure-outcome associations before and after accounting for covariates. For the analysis between the stress index, methylation and adiposity measures, we adjusted for maternal pre-pregnancy BMI, parity, socioeconomic status and child sex in the first-level multivariable-adjusted model, and additionally adjusted for birth weight, child total energy intake and percent fat intake of total energy at 4–6 years of age in the second-level multivariable-adjusted model (i.e., fully-adjusted model). Child sex was not adjusted for in the models for BMI z-score because BMI z-score has been standardized according to child sex. We tested the potential interaction between the stress index and DNA methylation and between the stress index and other adjusted covariates, and performed analyses stratified by child sex and birth weight that showed evidence of interaction ($p_{\text{interaction}} = 0.10$) with the stress index. In sensitivity analyses, we further adjusted for other potential confounders, including maternal age at delivery, maternal second hand smoking exposure, breast feeding, gestational age, child age in days, and percentages of major leukocyte cell types (lymphocytes and neutrophils) in cord blood, to test the robustness of exposure-outcome associations. Results were reported as estimated changes with 95% confidence intervals (CIs) in outcome variables associated with interquartile range (IQR) increases in exposure variables. All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC), and the statistical significant level was set at $p\text{-value} < 0.05$ (2-sided).

Results

Table 1 summarizes participant characteristics. The stress index had an average of 50.6 ± 19.3 and a range of 6.6–98.7. Thirty-one (7.3%) infants had low birth weight (< 2.5 kg) and 62 (14.6%) infants were small [birth weight]-for-gestational age (SGA) babies ($< 10^{\text{th}}$ Fenton percentile). Average child BMI z-score at 4–6 years of age (mean $\pm$ SD: 0.2 ± 1.0) was similar to the WHO international reference population (39). Average child BFM was 4.3 ± 1.8 kg, accounting for $23.7 \pm 5.8\%$ of the body weight. There were no significant differences in major baseline characteristics between mother-infant pairs who were included and not included in the study (results not shown). For the children included in the analysis, there was a significant difference in the proportion of infants with low birth weight between genders (3.3% males vs. 11.4 females, $p = 0.001$), but no difference was found for baseline characteristics between children with ($n = 247$) and without ($n = 177$) methylation data in three inflammation genes (data not shown).

The four stress scales had estimated weights ranging from 0.09 (EPDS) to 0.41 (STAI) (Table S1). Increasing scores of the four stress scales and the stress index were associated with decreasing child BMI z-score at 4–6 years of age after covariate adjustment (Figure 1). Regression results in unadjusted models and multivariable-adjusted models were generally consistent with each other, and an IQR increase in stress index (27.3) was associated with decreases of 0.14 unit [95% confidence interval (CI): $-0.28, -0.005$] in BMI z-score, 5.6% (95% CI: $-9.7, -1.4$) in BFM, 3.5% (95% CI: $-6.3, -0.5$) in PBF, and 1.2% (95% CI: $-2.4, -0.04$) in waist circumference in fully-adjusted models (Table 2). These associations appeared stronger among girls than among boys. In particular, there were a significant 9.0% decrease in BFM and a significant 6.4% decrease in PBF in girls, compared to the insignificant decreases of 2.6% and 1.4% in boys, respectively ($p_{\text{interaction}}$ for stress index

and sex were 0.10 for BFM and 0.053 for PBF). We found generally consistent evidence for interactions between the stress index and birth weight on offspring adiposity measures. The negative associations between stress index and major offspring adiposity measures (BMI z-score, BFM and PBF) appeared greater among children with birth weight lower than the median (3.1kg) than among children with birth weight above or equal to the median (Table S2). Similar results of stratified analyses were also found for individual stress scales of ETV, STAI and EPDS (Table S2). Sensitivity analyses with further adjustments for other covariates yielded similar effect estimates (results not shown).

The stress index and individual stress scales were generally not associated with methylation in *TNF- α* , *IL6* or *IL8*, except that EPDS was associated with a significant increase in *IL6* methylation (Table S3). However, *IL6* methylation was consistently associated with increases in offspring adiposity measures at 4–6 years of age. An IQR increase in *IL6* methylation (3.9%) was associated with increases of 0.23 unit (95% CI: 0.06, 0.40) in BMI z-score, 8.1% (95% CI: 2.3, 14.3) in BFM, 5.5% (95% CI: 1.7, 9.5) in PBF, and 1.7% (95% CI: 0.2, 3.3) in waist circumference in fully-adjusted models (Table 3). Interestingly, the association for *IL6* methylation and BMI z-score was stronger among girls than among boys ($P_{\text{interaction}}=0.030$). In contrast, methylation in *TNF- α* and *IL8* was not associated with BMI z-score or other adiposity measures, and DNA methylation did not mediate the associations between stress index and adiposity measures (results not shown). Sensitivity analyses with adjustments for lymphocyte and neutrophil percentages and methylation batch showed similar results as detailed above (results not shown).

Discussion

Contrary to our hypothesis, we found that prenatal maternal stress was associated with reduced rather than increased adiposity in early childhood. Furthermore, while *IL6* DNA methylation in cord blood was predictive of increased childhood adiposity, it was not associated with prenatal maternal stress and did not mediate the association between prenatal stress and childhood adiposity. Epidemiological studies have yielded conflicting results with regards to prenatal maternal stress and offspring adiposity(9–13). Mixed findings may reflect the fact that these studies had measured different dimensions of stress, or the possibility that some important covariates (e.g., birth weight) varied substantially across the study populations. Interestingly, the potential adverse down-regulatory effect of psychological stress on children's growth has been demonstrated in an experiment with two orphanages, which showed that harsh and unsympathetic handling may seriously curtail growth rates in children under poor conditions(41). Our study used a sample from a low-income country, with 14.8% children born as SGA. It is therefore plausible that prenatal maternal stress may, in combination with other poor conditions (e.g., lower birth weight), account for the decreased adiposity measures in children from low-income families (Table S2).

Interestingly, the negative associations between prenatal stress and two adiposity measures (BFM and PBF) appeared greater among girls than boys. These results suggest that prenatal stress may primarily affect weight gain in girls. Sex-specific differences in stress-related responses have been documented in previous studies, and heightened stress sensitivity may

be a risk factor for onset of affective disorder and susceptibility(42). Animal experiments in rodents have demonstrated that females typically respond to stress with a greater release of stress regulatory hormones compared to males and show a more prolonged pattern of heightened plasma levels of these hormones following the stress, suggesting a greater resistance to recovery(20). Pending further investigation, our study suggests that females may be more susceptible to prenatal stress exposures than males.

It is well known that obesity predisposes to metabolic inflammation, but whether a state of low-grade inflammation can predispose to obesity is unclear. Our study showed that high methylation in *IL6* promoter in cord blood DNA was associated with increased offspring adiposity measures, independent of prenatal stress and other covariates. When located in a gene promoter, DNA methylation typically acts to repress gene expression(27). Therefore, our findings suggest that high *IL6* methylation (or low *IL6* expression) in cord blood may predispose to childhood obesity, which is in line with the evidence from animal experiments that *IL6*-deficient mice develop obesity, and *IL6* replacement at low doses can reverse the obesity when given centrally rather than peripherally(28, 29). Interestingly, the association between *IL6* methylation and child BMI z-score appeared stronger in girls than in boys, and no association with adiposity was found for methylation in the other two inflammation-related genes *TNF- α* and *IL8*. These results are partially supported by a recent study showing that increased methylation in *IL6*, but not in *TNF- α* , was associated with obesity in women(43).

Our study has a number of strengths, including its prospective and hypothesis-driven design, the use of a summary stress index to account for cumulative effects of multiple stressors, inclusion of various adiposity measures and detailed information on covariates. Cumulative environmental stressors may contribute to increased health risk more than each stressor considered separately(44), and low-income women are more likely to expose to multiple stressors, including violence, financial crisis, anxiety and depression symptoms(15, 16). Therefore, the use of a summary stress index allowed us to capture the effect of prenatal stress in an aggregated manner, which may better reflect the overall impact of prenatal stress. It is plausible that the conflicting results on prenatal stress and offspring adiposity reported by previous studies may be due to the fact that these studies measured different dimensions of the stress rather than accounted for the overall influence of cumulative stress.

There are also several study limitations. First, our study sample comprised mother-child pairs from a Mexican, low-income population, and generalizability of the study findings to other ethnicities and high-income group may be limited. However, the more homogenous background of our study participants reduces confounding due to cultural- or ethnic-specific risk factors and thereby increases internal validity. Second, we investigated the potential programming consequences of prenatal stress, whereas it is plausible that postpartum stress may also contribute to the observed association, although not investigated in the study. Third, we investigated a limited number of CpG sites within candidate genes, and thus cannot determine whether DNA methylation in other regulatory regions of these genes may be differentially associated with stress or adiposity. However, other DNA methylation marks in proximity to one another are generally highly correlated(45). Cord blood DNA may not be the target tissue for some long-term health outcomes and is often considered a surrogate

tissue. Fourth, we were not able to examine the expression levels of IL6 to testify our hypothesis that high *IL6* methylation in cord blood was linked to low IL6 expression in this study. Whether DNA methylation in cord blood captures the systemic epigenetic programming effects and whether methylation levels in cord blood are correlated with gene expression levels in early childhood need further investigation. Finally, our study suffers from the issues of missing data, loss to follow-up and residual confounding of measured or unmeasured factors that are common to epidemiological studies. It is plausible that our results could have been biased to some extent due to missing data and loss to follow-up, and therefore the results need to be interpreted with caution.

In summary, we observed a negative association between prenatal maternal stress and adiposity in children at 4–6 years of age in this cohort. We also documented a positive association between methylation in *IL6* promoter in cord blood leukocyte DNA and adiposity in early childhood, which was independent of prenatal stress. Pending further investigation, these findings highlight the importance of investigating the programming effects of prenatal factors on children's development in early life, which may be useful for preventing the adverse health effects associated with prenatal exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviation list

BFM	body fat mass
BMI	body mass index
CI	confidence interval
CRISYS	Crisis in Family Systems
EPDS	Edinburgh Postnatal Depression Scale
ETV	Exposure to Violence
<i>IL6</i>	interleukin 6
<i>IL8</i>	interleukin 8
IQR	interquartile range

PBF	percentage body fat
PROGRESS	Programming Research in Obesity, GRowth Environment and Social Stress
SGA	small [birth weight]-for-gestational age
STAI	State-Trait Anxiety Inventory
TNF-α	tumor necrosis factor-alpha
WQS	Weighted Quantile Sum
WHO	World Health Organization

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Figure 1.

Association of four individual prenatal stress-related scales (A: Exposure to violence; B: CRISIS-sum of negative life events; C: State-Trait Anxiety Inventory; D: Edinburgh Postnatal Depression Scale) and the estimated stress index (E) with adjusted child BMI z-score at 4–6 years of age in the PROGRESS longitudinal cohort in Mexico City, Mexico. A weighted quantile sum (WQS) regression model was built for predicting child BMI z-score adjusted for maternal BMI, parity, socioeconomic status, birth weight, child total energy intake and percent fat intake of total energy. An adjusted BMI z-score variable was created from the residuals of this model with the mean BMI z-score added. The associations between the adjusted child BMI z-score and the estimated WQS stress index and four individual stress scales were depicted graphically. Details of the stress index development are shown in the Supplemental Material.

Table 1

Maternal and child characteristics in the PROGRESS longitudinal cohort in Mexico City, Mexico (n=424).

Characteristic	n	Percent, mean $\pm$ SD, or range
Maternal characteristics		
Maternal age (years)		
<20	24	5.7
20–29	271	63.9
30–39	121	28.5
40	8	1.9
BMI during 2 nd trimester (kg/m ²)		
<20.5	12	2.8
20.5–26.9	229	54.0
27.0–31.9	138	32.6
32.0	45	10.6
Socioeconomic status		
Low	226	53.3
Medium	158	37.3
High	40	9.4
Parity		
0	189	44.6
1	172	40.6
2	63	14.9
Second hand smoking exposure during pregnancy		
Yes	141	33.3
No	283	66.7
Breast feeding status		
No	47	11.6
Yes	359	88.4
Prenatal stress scale scores		
Exposure to violence	424	−0.03 $\pm$ 0.32
Range		−0.40–1.06
CRISYS-sum of negative life events	424	3.1 $\pm$ 2.0
Range		0–11
State-Trait Anxiety Inventory	424	19.2 $\pm$ 5.2
Range		10–34
Edinburgh Postnatal Depression Scale	424	8.6 $\pm$ 5.7
Range		0–28
Stress index	424	50.6 $\pm$ 19.3
Range		6.6–98.7
Child characteristic at birth		
Sex		
Boy	214	50.5

Characteristic	n	Percent, mean $\pm$ SD, or range
Girl	210	49.5
Gestational age (weeks)	424	38.4 $\pm$ 1.6
Preterm (<37 weeks)		
Yes	41	9.7
No	383	90.3
Birth weight (kg)	424	3.1 $\pm$ 0.4
SGA (<10 th Fenton percentile)		
Yes	62	14.6
No	362	85.4
Average gene methylation level in umbilical cord blood		
<i>TNF-α</i>	247	23.1 $\pm$ 5.2
Range		3.1–34.3
<i>IL8</i>	239	10.9 $\pm$ 4.2
Range		0–25.7
<i>IL6</i>	247	7.3 $\pm$ 2.9
Range		0.4–16.8
Child characteristic at 4–6 years of age		
Age at visit (years)	424	4.8 $\pm$ 0.5
Range		4–6
Height (cm)	424	105.5 $\pm$ 5.2
Weight (kg)	424	17.5 $\pm$ 3.0
BMI (kg/m ²)	424	15.6 $\pm$ 1.7
BMI z-score	424	0.2 $\pm$ 1.0
Body fat mass (kg)	420	4.3 $\pm$ 1.8
Percentage body fat	417	23.7 $\pm$ 5.8
Waist circumference (cm)	424	52.3 $\pm$ 5.1
Total energy intake (cal/100g)	424	1676 $\pm$ 639
Percent fat intake of total energy (%)	424	33.4 $\pm$ 5.4

Note: BMI, body mass index; CRISYS, Crisis in Family Systems; IL-6, interleukin 6; IL-8, interleukin 8; TNF- α , tumor necrosis factor-alpha; SD, standard deviation; SGA, small [birth weight]-for-gestational age.

Table 2

Association between an IQR increase in prenatal stress index (27.3) and offspring adiposity measures at 4–6 years of age in the PROGRESS longitudinal cohort, Mexico City, Mexico

Outcome measure	n	Unadjusted estimate	Adjusted estimate ^a	Fully-adjusted estimate ^b	p-value for interaction ^c
All children					
BMI z-score [β (95% CI)]	424	-0.18 (-0.32, -0.04)	-0.13 (-0.26, 0.01)	-0.14 (-0.28, -0.005)	0.78
Body fat mass [% (95% CI)]	420	-6.9 (-11.1, -2.6)	-5.1 (-9.2, -0.8)	-5.6 (-9.7, -1.3)	0.10
Percentage body fat [% (95% CI)]	417	-4.3 (-7.2, -1.3)	-3.1 (-6.0, -0.2)	-3.5 (-6.3, -0.5)	0.053
Waist circumference [% (95% CI)]	424	-1.5 (-2.7, -0.2)	-1.0 (-2.2, 0.2)	-1.2 (-2.4, -0.04)	0.59
Boys					
BMI z-score [β (95% CI)]	214	-0.17 (-0.36, 0.02)	-0.11 (-0.30, 0.08)	-0.14 (-0.33, 0.06)	
Body fat mass [% (95% CI)]	210	-3.5 (-8.9, 2.2)	-1.6 (-7.1, 4.2)	-2.6 (-8.3, 3.4)	
Percentage body fat [% (95% CI)]	208	-1.0 (-5.0, 3.2)	0.1 (-4.1, 4.4)	-1.4 (-5.7, 3.0)	
Waist circumference [% (95% CI)]	214	-1.2 (-2.5, 0.2)	-0.7 (-2.1, 0.7)	-0.8 (-2.2, 0.7)	
Girls					
BMI z-score [β (95% CI)]	210	-0.19 (-0.40, 0.02)	-0.12 (-0.32, 0.08)	-0.15 (-0.36, 0.05)	
Body fat mass [% (95% CI)]	210	-9.8 (-15.8, -3.4)	-7.7 (-13.7, -1.3)	-9.0 (-14.8, -2.7)	
Percentage body fat [% (95% CI)]	209	-7.0 (-10.9, -2.9)	-5.7 (-9.7, -1.6)	-6.4 (-10.3, -2.3)	
Waist circumference [% (95% CI)]	210	-1.7 (-3.7, 0.3)	-1.2 (-3.2, 0.8)	-1.5 (-3.4, 0.5)	

Note: BMI, body mass index; CI, confidence interval; IQR, interquartile range.

^aLinear regression models were adjusted for maternal pre-pregnancy BMI (continuous), parity (0, 1, or ≥ 2) and socioeconomic status (low, medium, or high). Analyses for body fat mass, percentage body fat and waist circumference were additionally adjusted for child sex.

^bLinear regression models were adjusted for maternal pre-pregnancy BMI (continuous), parity (0, 1, or ≥ 2), socioeconomic status (low, medium, or high), birth weight (continuous), and child total energy intake (continuous) and percent fat intake of total energy (continuous). Analyses for body fat mass, percentage body fat and waist circumference were additionally adjusted for child sex.

^cp-value for interaction between stress index and child sex based on the fully-adjusted models.

Table 3

Association between an IQR increase in *IL6* methylation (3.9%) in umbilical cord blood and offspring adiposity measures at 4–6 years of age in the PROGRESS longitudinal cohort, Mexico City, Mexico

Outcome measure	n	Unadjusted estimate	Adjusted estimate ^a	Fully-adjusted estimate ^b	p-value for interaction ^c
All children					
BMI z-score [β (95% CI)]	247	0.29 (0.11, 0.46)	0.27 (0.10, 0.44)	0.23 (0.06, 0.40)	0.03
Body fat mass [% (95% CI)]	245	8.8 (2.7, 15.4)	9.3 (3.6, 15.4)	8.1 (2.3, 14.3)	0.79
Percentage body fat [% (95% CI)]	243	5.4 (1.3, 9.6)	5.7 (1.9, 9.6)	5.5 (1.6, 9.5)	0.90
Waist circumference [% (95% CI)]	247	1.8 (0.2, 3.4)	2.0 (0.5, 3.5)	1.7 (0.2, 3.3)	0.33
Boys					
BMI z-score [β (95% CI)]	133	0.20 (−0.03, 0.43)	0.17 (−0.05, 0.39)	0.18 (−0.04, 0.41)	
Body fat mass [% (95% CI)]	131	8.3 (1.4, 15.7)	7.4 (0.8, 14.4)	7.5 (0.6, 14.9)	
Percentage body fat [% (95% CI)]	130	6.1 (1.1, 11.4)	5.6 (0.7, 10.7)	5.9 (0.8, 11.2)	
Waist circumference [% (95% CI)]	133	1.0 (−0.7, 2.8)	0.9 (−0.7, 2.6)	1.0 (−0.6, 2.7)	
Girls					
BMI z-score [β (95% CI)]	114	0.40 (0.12, 0.67)	0.39 (0.13, 0.66)	0.33 (0.05, 0.61)	
Body fat mass [% (95% CI)]	114	11.0 (1.0, 22.0)	11.7 (2.1, 22.2)	10.9 (0.8, 22.0)	
Percentage body fat [% (95% CI)]	113	5.7 (−0.3, 12.1)	5.9 (0.1, 12.1)	5.8 (−0.3, 12.4)	
Waist circumference [% (95% CI)]	114	3.0 (0.3, 5.8)	3.1 (0.5, 5.9)	3.3 (0.4, 6.2)	

Note: BMI, body mass index; CI, confidence interval; IL-6, interleukin 6; IQR, interquartile range.

^aLinear regression models were adjusted for maternal pre-pregnancy BMI (continuous), parity (0, 1, or ≥ 2) and socioeconomic status (low, medium, or high). Analyses for body fat mass, percentage body fat and waist circumference were additionally adjusted for child sex.

^bLinear regression models were adjusted for maternal pre-pregnancy BMI (continuous), parity (0, 1, or ≥ 2), socioeconomic status (low, medium, or high), birth weight (continuous), and child total energy intake (continuous) and percent fat intake of total energy (continuous). Analyses for body fat mass, percentage body fat and waist circumference were additionally adjusted for child sex.

^c p-value for interaction between *IL6* methylation and child sex.