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PROSPECTIVE EVALUATION OF ASSOCIATIONS BETWEEN PRENATAL CORTISOL AND ADULTHOOD CORONARY HEART DISEASE RISK: THE NEW ENGLAND FAMILY STUDY

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Abstract

OBJECTIVE—Increasing evidence suggests that early life factors may influence coronary heart disease (CHD) risk, however little is known about contributions of prenatal cortisol. Objectives were to prospectively assess associations of maternal cortisol levels during pregnancy with offspring's 10-year CHD risk during middle-age.

METHODS—Participants were 262 mother-offspring dyads from the New England Family Study. Maternal free cortisol was assessed in third trimester maternal serum samples. Ten-year CHD risk was calculated in offspring at mean age 42 years, using the validated Framingham risk algorithm incorporating diabetes, systolic and diastolic blood pressure, total and HDL cholesterol, smoking, age and sex.

RESULTS—In multivariable-adjusted linear regression analyses adjusted for age and race/ethnicity, high vs. low maternal cortisol tertile was associated with 36.7% (95% CI: 8.4%, 72.5%) greater mean 10-year CHD risk score in females. There was no association in males (–2.8%, 95% CI: –23.8%, 24.0%). Further adjustment for *in utero* socioeconomic position showed 25.9% (95% CI: –1.0%, 60.0%) greater CHD risk in females. Adjustment for maternal age and size for gestational age had little effect on findings.

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CONCLUSIONS—Maternal prenatal cortisol levels were positively associated with 10-year CHD risk among female, and not male, offspring. Adjusting for socioeconomic position during pregnancy reduced effect size in females, suggesting it may be a common prior factor in both maternal cortisol and CHD risk. These findings provide evidence that targeting mothers who have elevated prenatal cortisol levels, including elevated cortisol in the setting of low socioeconomic position, may potentially reduce long-term CHD risk in their offspring.

Keywords

cortisol; stress; coronary heart disease; prospective; life course; prenatal

INTRODUCTION

Coronary heart disease (CHD) remains a major cause of mortality world-wide (1). There is increasing evidence that early life factors (e.g. birth weight, socioeconomic position) may contribute to development of CHD risk factors such as obesity, blood pressure and cholesterol in adulthood (2). The “fetal origins hypothesis” suggests that adverse insults *in utero* permanently program fetal organs and tissues for a postnatal environment, leading to physiological dysfunction and disease later in life (3). Humans respond to stress through a cascade of biological responses, which mobilize energy stores and promote vigilance (4). One of the cascades of biological responses to stress is mediated by the hypothalamic-pituitary-adrenal (HPA) axis and results in the release of glucocorticoids such as cortisol from the adrenal glands (4). Cortisol, being a stress hormone, is often used as a biological marker of participant stress levels, including during pregnancy (4–6). Cortisol levels at different times of day have been shown to be elevated in late pregnancy for those with greater vs. fewer stressful life events (5,6), suggesting it may be a reasonable biological measure of stress during pregnancy.

Although a recent meta-analysis suggested a possible link between stress during adulthood and incident CHD (7), there is little known about associations of prenatal stress with adulthood CHD risk. However, preliminary findings are intriguing. For example, participants conceived during the 1944–1945 Dutch Famine (which involved both physical stress via undernutrition as well as emotional stress) had double the risk of developing CHD compared to those not exposed to the famine (8). Furthermore, several studies support associations between biological markers of maternal stress and individual risk factors for CHD (9–11).

With regard to mechanisms through which maternal cortisol could influence offspring’s CHD risk, elevated prenatal cortisol levels may contribute in sex-specific ways by influencing the development of common regulatory pathways, such as the autonomic nervous system, limbic brain areas associated with stress and anxiety-related behaviors, and/or vascular development within brain areas central to HPA control, as reviewed elsewhere and later in this manuscript (12).

The relation between maternal cortisol and offsprings’ overall risk for CHD has not been evaluated to our knowledge. Consequently, the objectives of this study were to prospectively examine sex-specific associations between maternal serum cortisol collected during the third trimester of pregnancy and 10-year calculated CHD risk (using the Framingham algorithm)

in middle-aged offspring of the New England Family Study birth cohort. Further objectives were to evaluate whether maternal cortisol was associated with individual CHD risk factors in offspring, including smoking, total cholesterol, HDL cholesterol, and blood pressure.

METHODS

Study Sample

Study participants were from the New England Family Study (NEFS), which is comprised of 17,921 offspring of pregnant women in the Collaborative Perinatal Project (CPP) at the Providence, Rhode Island and Boston, Massachusetts sites (United States) born between 1959 and 1966 (13). Selection and sampling for 1674 NEFS participants has been previously described (14–16). Participants in the present study were mother-offspring pairs enrolled in the NEFS from live, singleton births who had available maternal prenatal serum sampled between 31 and 36 weeks gestation (1086 of 1674; 65%). Of these 1086 participants, 289 were also part of a different NEFS sub-study called the EdHealth Study that performed direct physiologic assessments, described in more detail elsewhere (17). Of the 289 participants, 27 were missing covariate data (e.g. *in utero* socioeconomic index, birth weight), resulting in an analytic sample size of 262 participants. Included offspring participants were more likely to be younger (42.3 vs. 42.8 y), have higher *in utero* socioeconomic index (56.9 vs. 51.1 units), less likely to be small for gestational age (7.0% vs. 13.9%) and more likely to be large for gestational age (10.9% vs. 6.4%) (all $p < 0.05$). There were no significant differences between included and excluded participants for other covariates. All NEFS participants provided written informed consent. The study protocol was approved by institutional review boards at the Harvard School of Public Health, Brown University, and Miriam Hospital.

Cortisol Measurement

Non-fasting maternal blood was collected at each prenatal visit in the CPP between 1959 and 1966. Late third-trimester serum samples for this study were obtained from the CPP central repository in Bethesda, MD, and assayed for cortisol and cortisol binding globulin (CBG; for determining free cortisol levels). For each mother, a single serum sample was selected from the repository for analysis. As described in prior studies by our group (9,18,19), only samples drawn between 31 and 36 weeks following the last menstrual period and at least 14 days prior to the infant's birth date were selected. We excluded samples taken during the final 2 weeks of pregnancy because of known effects of labor and delivery on steroid hormone levels (20–22). Weeks 31–36 were selected because: (i) these weeks provided a relatively tight window within the third trimester to examine hormone levels; (ii) these weeks included the greatest number of participants with available serum samples; and (iii) in addition to other periods of gestation, stress during the third trimester of pregnancy has been associated with offspring neurobehavioural outcomes in prior literature (23–26). If mothers had more than one blood draw during weeks 31–36, the latest draw within this window was selected. The average length of storage of these samples was 42.5 years (SD. 1.7). The time of day at which samples were obtained was not recorded. Serum samples were assayed for total cortisol and CBG levels using enzyme-linked immunoassay and radioimmunoassay, respectively (www.ibl-hamburg.com, laboratory of C. Kirschbaum).

Inter/intra-assay coefficients of variability ranged from 3 to 10%. For further details of sample collection, storage and analysis, see Stroud *et al.* (19). Validity of cortisol and CBG levels after decades of storage has been described in detail elsewhere, where cortisol and CBG assessed during the third trimester showed generally consistent findings with studies of fresh samples that were collected and assayed for cortisol and CBG during the third trimester (19).

In serum samples, 80% of cortisol is bound to CBG, making it biologically inactive and unable to affect the fetus (27). Free cortisol was examined in this study to provide a better estimate of fetal exposure to maternal cortisol. The amount of free cortisol in a given serum sample was estimated using a formula developed by Coolens *et al.* (28), which takes into account both total cortisol and CBG levels. In order to examine dose response of cortisol and allow for threshold effects, maternal free cortisol was categorized into sex-specific tertiles, where the ranges of cortisol levels for low, medium and high tertiles were 4.7–15.5 (n=52), 15.8–24.8 (n=50), and 24.8–91.4 (n=52) nmol/L, respectively, in females, and 6.1–14.4 (n=36), 14.5–22.9 (n=37), and 23.6–90.7 (n=35) nmol/L, respectively, in males.

10-year Coronary Heart Disease (CHD) Risk

Ten-year CHD risk was calculated as a percentage, using the validated Framingham risk algorithm (29). This algorithm uses Cox regression models that incorporate diabetes status, total and HDL cholesterol, systolic and diastolic blood pressure, smoking, age and sex, described in detail elsewhere (29). The c-statistic for prediction of CHD events in the Framingham Heart Study is 0.74 in men and 0.77 in women, suggesting good predictive validity (29). External validity tests on white and black participants perform reasonably well (30).

Current smoking was based on self-report to the question “Do you smoke cigarettes now?”. Lipids were measured in non-fasting plasma samples at CERLab (Harvard Medical School, Boston, MA) using a Hitachi 911 analyzer, and participating in the CDC/NHLBI Lipid Standardization Program. Total cholesterol (CV=1.7%) and HDL cholesterol (CV=3.3%) were measured enzymatically, described elsewhere (31,32). Presence of diabetes was based on: HbA1c greater than 6.5% (33) and/or under treatment with insulin or oral hypoglycemic agents (29). The HbA1c assay was based on turbidimetric immunoinhibition using packed red cells (Roche Diagnostics, Indianapolis, IN). The assay is approved by the U.S. Food and Drug Administration for clinical use and is certified by the National Glycohemoglobin Standardization Program. The day-to-day variability at %HbA1c values of 5.5 and 9.1 are 1.9% and 3.0%, respectively. Five systolic and diastolic blood pressure measurements were obtained over one-minute intervals in participants seated, after 5 minutes rest, in the right arm at heart level, using automated blood pressure monitors (VSMedTech BpTru, Coquitlam, BC, Canada) demonstrated to have good validity and reliability compared with the auscultation method (34). Systolic and diastolic blood pressure values were calculated as the mean of the lowest three systolic or diastolic blood pressure readings, excluding the first recorded blood pressure.

Covariates

Age was directly assessed as the difference from the date of birth (assessed during CPP) to the date of the exam. Race/ethnicity was obtained via self-report. *In utero* socioeconomic index (SEI) was calculated using a weighted percentile based from information on both parents' educational attainment, occupation, and income relative to the US population prenatally (35). Specifically, analyses utilized head of the household education level, family income, and occupation of the head of the household or the chief wage earner (35). Maternal age was assessed at the prenatal registration. Birth weight was recorded by nurse observers at delivery, and reported in grams. Participants were categorized as small for gestational age (SGA), normal for gestational age (NGA), or large for gestational age (LGS) if they were 10th percentile, >10th to <90th percentile, or 90th percentile, respectively, for their gestational age within the Boston and Providence CPP study sites, consistent with prior studies (36,37).

Statistical Analyses

Descriptive characteristics were generated for dependent variables and covariates in males and females, according to tertiles of maternal cortisol. Multivariable-adjusted linear regression analyses assessed associations between maternal cortisol tertiles and calculated 10-year CHD risk. Based on prior work showing associations of prenatal cortisol with cognitive outcomes (18), we hypothesized that only high levels of cortisol exposure would be related to CVD risk. Modeling tertiles of exposure allowed analyses to evaluate the possibility of threshold effects, while maintaining adequate statistical power.

Sex-specific analyses adjusted for offspring age and race/ethnicity in the base models. Models further adjusted for *in utero* SEI, maternal age, and size for gestational age. Associations between ordinal tertiles of maternal cortisol and individual CHD risk factor components were assessed using multivariable-adjusted linear regression (for systolic and diastolic blood pressures, total cholesterol, HDL, HbA1c) and logistic regression (for smoking). Diabetes was not analyzed separately due to low prevalence and inadequate statistical power for multivariable-adjusted analyses. Parental mental illness was not included as a potential covariate due to low prevalence of the only available measure, specifically history of psychiatric hospitalization (n=1). Formal tests for statistical interaction with sex were not performed due to sex being a component of the primary outcome (i.e. 10-year CHD risk score, calculated using the Framingham risk algorithm). Analyses were completed using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Of the study participants (n=262), 59% were female and 82% were white race/ethnicity, with mean age of 42 (range 39–46) years. In descriptive analyses, for females (n=154), maternal free cortisol was positively associated with offspring's smoking, being small for gestational age, and 10-year CHD risk score, and negatively associated with maternal age and *in utero* socioeconomic index (Table 1). In males (n=108), there were no associations of maternal cortisol levels with offspring's 10-year CHD risk score or covariates.

In multivariable-adjusted analyses, for females, elevated (highest tertile) maternal cortisol was associated with 36.7% (95% CI: 8.4%, 72.5%) increase in offspring's mean risk for CHD in the next 10 years, compared to those in the lowest tertile of maternal cortisol, after adjusting for participant's age and race/ethnicity (Table 2). Further adjustment for *in utero* SEI reduced the effect size somewhat to a 26.1% (95% CI: -0.5%, 59.9%; *p* for trend=0.05) increase in mean risk for CHD, while adjustment for maternal age and size for gestational age had little effect (Table 2). In males, there were no significant associations between maternal cortisol and offspring's 10-year CHD risk.

In an effort to evaluate which specific CHD risk factors may be driving associations between maternal cortisol and 10-year CHD risk, analyses were performed on associations of maternal cortisol with each of the modifiable CHD risk factor components of the Framingham algorithm. In females, regression analyses demonstrated trends towards elevated maternal cortisol being associated with higher systolic blood pressure, diastolic blood pressure, total cholesterol and smoking, as well as lower HDL cholesterol (Table 3). However, none of the comparisons reached statistical significance, suggesting each factor may have contributed somewhat to the CHD risk score, leading to an overall significant effect demonstrated between maternal cortisol and CHD risk score which took into account many CHD risk factors. In males, associations were non-significant and mixed in direction of effects (Table 3).

DISCUSSION

Overall, findings demonstrated that higher prenatal levels of maternal cortisol were positively associated with calculated 10-year CHD risk among middle-aged female, but not male, offspring in this 40-year prospective study. Further adjustment for prenatal socioeconomic position reduced the effect size by 29% in females, suggesting it may be a prior common cause to maternal cortisol and CHD risk. Analyses demonstrated positive association trends of maternal cortisol with riskier levels of all components of the Framingham risk algorithm (i.e. systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, HbA1c, smoking). These results provide evidence consistent with the hypothesis that CHD risk may have fetal origins in maternal cortisol levels, particularly for female offspring.

Prior Literature

Little is known about associations between prenatal maternal cortisol and offspring's CHD risk. A previous study of 1,086 participants in the New England Family Study (from which the current study participants are drawn from in a sub-study of 262 participants that had direct cardiovascular biomarker assessments) demonstrated positive associations between third trimester maternal cortisol with female, but not male, offspring nicotine dependence, similar to associations found with smoking in the current study (9). Third trimester maternal cortisol was positively associated with systemic vascular resistance and BMI in 130 Brazilian children, ages 5–7 years (10). In the United States-based Project Viva cohort, umbilical cord cortisol was associated with higher systolic blood pressure in 286 participants at age 3 years (11). There were no observed sex differences in the former study (10) and sex

differences were not examined in the latter study (11). With regard to other CHD risk factors, fetal sheep who were administered prenatal glucocorticoids exhibited enhanced endothelin-induced vasoconstriction and attenuated endothelium-dependent vasorelaxation in adulthood, thus suggesting endothelial dysfunction (38). Similarly in humans, late pregnancy maternal cortisol was associated with systemic vascular resistance measured via pulse wave analysis, in children aged 5–7 years (10).

Some studies have evaluated associations of synthetic glucocorticoid administration with blood pressure. Specifically, animal studies typically demonstrate that administering synthetic glucocorticoids *in utero* is associated with adult offspring blood pressure and development of hypertension (39,40). Evidence in humans on associations of prenatal administration of synthetic glucocorticoids (such as betamethasone) with offspring blood pressure is mixed (11,41–43).

It is important to note that maternal stress is dependent on the larger context in which expectant mothers are living. For example, parents living in poverty, with few employment opportunities, low education and inadequate support systems may be inherently more stressed (44), and more likely to express more adverse parenting styles, such as neglect, abuse and lower parental monitoring (45). Efforts to address some these potential upstream fundamental determinants of health may help to break the intergenerational cycles that tend to link early life adversity with later life disease. If maternal prenatal stress does indeed affect risk for CHD, there is substantial literature on interventions to help prevent or mitigate the effects on children, described in more detail elsewhere (45–47). A number of specific programs such as the Nurse-Family Partnership, Perry Preschool, and Triple P programs have been evaluated for providing a nurturing environment to children and their families to improve developmental and health outcomes, described in further detail elsewhere (45–47). Adjusting for prenatal socioeconomic position in the current analyses reduced the effect size, suggesting that the prenatal socioeconomic position may be an explanatory mechanism that could lead to maternal stress and elevated cortisol, thereby resulting in CHD risk.

There are a number of mechanisms through which maternal cortisol could influence offspring's CHD risk, including sex difference as shown in the current study. Specifically, fetal cortisol exposure is buffered from maternal cortisol by placental 11- β -hydroxysteroid dehydrogenase type 2 (11- β -HSD-2). This enzyme metabolizes and inactivates glucocorticoids (48), and increases in activity during the gestational period (49,50). Maternal cortisol promotes release of placental corticotrophin-releasing hormone (CRH), which further activates the fetal HPA axis (51,52). Studies have demonstrated that maternal cortisol levels in the third trimester are directly associated with infant negative reactivity (23), mental and motor delays (24), and childhood intelligence (18). This provides supportive evidence that exposure to maternal cortisol may influence offspring's developmental outcomes. Developmental outcomes such as intelligence and literacy are in turn associated with CHD risk (53,54). Furthermore, it has been proposed that there may be sex-specific associations between exposure to excess prenatal maternal glucocorticoids and CHD, due to sex-dependent effects of glucocorticoids on fetal brain development, as well as neuronal and vascular development in HPA circuitry (12). Many of the brain regions implicated in the stress response circuitry are morphologically or functionally sexually dimorphic and

involved in autonomic nervous system (ANS) regulation (55). ANS dysregulation, often assessed via heart rate variability, has evidence to be a CHD risk factor (56). It is a plausible mechanism for sex differences in associations of *in utero* cortisol exposure with later life CHD risk. Another potential mechanism is depression, which is a risk marker for CHD (57). Depression is influenced by early life stress and adversity, and is twice as prevalent in females than males (57). Sex differences in depression prevalence could be another potential contributor to sex differences in associations of *in utero* cortisol exposure with adulthood CHD risk. Maternal cortisol programming of CHD risk could be mediated by intrauterine growth. For example, preterm birth was moderately associated with increased blood pressure in adult offspring after prenatal exposure to synthetic glucocorticoids (58). However, adjustment for size for gestational age had little impact on study findings in the current analyses, suggesting intrauterine growth may not be an important explanatory mechanism. Overall, elevated prenatal cortisol levels may contribute to sex differences in CHD by influencing the development of common regulatory pathways, such as the ANS, limbic brain areas associated with stress and anxiety-related behaviors, and/or vascular development within brain areas central to HPA control, as reviewed elsewhere (12). However, the relation between maternal cortisol and offsprings' overall risk for CHD has not been evaluated to our knowledge. Future studies that perform mediation analyses evaluating roles of factors such as depression, autonomic nervous system activity, brain morphology, and timing of menopause (59) will provide further information on reasons for sex differences. Furthermore, replication of sex-specific effects are needed to evaluate whether sex-specific findings in the current study are consistent across studies.

Strengths and Weaknesses

Strengths of the study include longitudinal follow-up, which allowed for direct assessment of third trimester maternal free cortisol levels and adulthood clinical assessments in middle-aged offspring approximately 42 years later. Third trimester free cortisol levels were advantageous due to the narrow duration prior to delivery and critical period for maturation of fetal physiological systems and organs (e.g. cardiovascular system, pulmonary, renal, overall fetal growth) (60). Study protocols enabled accurate measurement of CHD risk factors (e.g. cholesterol, blood pressure and BMI), using substantial internal and external quality control protocols.

This study was limited by the small sample size (n=154 females and 108 males), which reduced statistical power. For example, after adjustment for childhood socioeconomic position, effect sizes for associations between maternal cortisol and 10-year CHD risk went from being large and highly significant (36.7% [95% CI: 8.4%, 72.5%; p for trend 0.008]), to being fairly large and borderline significant (26.1% [95% CI: -0.5%, 59.9; p for trend=0.05]) in females, as shown in Table 2. Given the relatively small sample size (n=154 females), the borderline statistical significance may be due to limited statistical power, as the effect size remained high. Further larger studies will shed additional information on statistical significance and independence of cortisol-CHD risk associations, in relation to childhood socioeconomic position. A second limitation is that cortisol was assessed at only one time point. It has been shown that cortisol levels peak near the time of awakening, after which cortisol levels decrease until bedtime with the exception of a small spike at lunchtime

(61,62). Maternal serum was collected in the CPP without recording the time of day or other factors (e.g. caffeine, food intake) that may affect cortisol levels. If these factors are assumed to be random across prenatal visits (18), variation in hormone levels due to time of day, or other aforementioned factors, should introduce greater error variance, but not bias, into observed associations between maternal cortisol and offspring CHD risk. Finally, this study may suffer from unmeasured confounders, specifically factors that could influence both maternal cortisol levels and offspring CHD risk. There may be yet identified common genetic or environmental factors that influence both of these variables (Figure S1, Supplemental Digital Content 1).

CONCLUSIONS

Prenatal exposure to elevated maternal cortisol levels was associated with increased 10-year calculated CHD risk score among adult female, but not male, offspring. Further adjustment for prenatal socioeconomic position somewhat reduced effect size, suggesting it may be a potential upstream mechanism. These results are suggestive that CHD may have fetal origins in maternal cortisol, particularly in females. These findings provide evidence that targeting mothers who have experienced elevated prenatal cortisol levels, including those caused by low socioeconomic position, may have some potential to reduce cardiovascular risk in offspring (63).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

11-β-HSD-2	11-β-hydroxysteroid dehydrogenase type 2
ANS	autonomic nervous system
BMI	body mass index
CBG	cortisol binding globulin
CHD	Coronary Heart Disease
CHR	corticotrophin-releasing hormone
CPP	Collaborative Perinatal Project

HDL	high density lipoprotein
HPA	hypothalamus pituitary adrenal axis
NEFS	New England Family Study
SD	standard deviation

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Table 1

Characteristics of participants, according to sex-specific tertiles of third trimester maternal free cortisol levels.

	Females (n=154)			Males (n=108)		
	Low (n=52)	Medium (n=50)	High (n=52)	Low (n=36)	Medium (n=37)	High (n=35)
Age, years	42.1 (41.6, 42.5)	41.8 (41.3, 42.2)	41.8 (41.3, 42.3)	42.3 (41.8, 42.8)	42.4 (41.9, 42.9)	42.3 (41.8, 42.8)
White race/ethnicity, %	80.8 (67.7, 89.4)	74.0 (60.1, 84.3)	90.4 (78.8, 96.0)	86.1 (70.5, 94.2)	73.0 (56.4, 84.9)	80.0 (63.4, 90.2)
<i>In utero</i> socio-economic index	62.9 (57.7, 68.1)	56.9 (51.6, 62.1)	52.4 (47.2, 57.6)	58.8 (52.1, 65.4)	52.8 (46.2, 59.4)	53.1 (46.4, 59.9)
Maternal age, years	26.7 (25.3, 28.2)	24.9 (23.4, 26.3)	24.5 (23.1, 25.9)	26.0 (24.2, 27.8)	25.1 (23.3, 26.9)	24.4 (22.6, 26.2)
Small for gestational age, %	5.8 (1.9, 16.5)	10.0 (4.2, 22.0)	13.5 (6.5, 25.8)	8.3 (2.7, 23.1)	2.7 (0.4, 17.2)	8.6 (2.8, 23.7)
10-yr CHD Framingham risk score, %	1.62 (1.36, 1.93)	1.78 (1.49, 2.13)	2.09 (1.76, 2.50)	5.97 (5.02, 7.10)	6.32 (5.32, 7.49)	5.73 (4.77, 6.83)
Total cholesterol, mg/dL	186 (177, 195)	187 (177, 196)	190 (181, 199)	209 (195, 223)	192 (178, 206)	208 (194, 222)
HDL cholesterol, mg/dL	57.3 (52.6, 61.9)	61.0 (56.3, 65.8)	52.0 (47.3, 56.6)	44.2 (40.2, 48.3)	37.7 (33.7, 41.7)	44.2 (40.1, 48.3)
Systolic blood pressure, mmHg	110 (106, 114)	113 (109, 117)	110 (106, 115)	122 (117, 128)	121 (116, 126)	119 (114, 124)
Diastolic blood pressure, mmHg	72.4 (69.5, 75.2)	75.2 (72.3, 78.1)	72.4 (69.6, 75.3)	82.2 (77.4, 84.9)	79.2 (75.5, 82.9)	78.4 (74.6, 82.1)
Diabetes, %	1.9 (0.3, 12.6)	0	5.8 (1.9, 16.5)	2.8 (0.4, 17.6)	8.1 (2.6, 22.6)	5.7 (1.4, 20.4)
Current smoker, %	15.4 (7.8, 28.0)	32.0 (20.5, 46.1)	32.7 (21.3, 46.6)	22.2 (11.4, 38.7)	29.7 (17.2, 46.3)	22.9 (11.8, 39.7)
HbA1c, %	5.0 (4.9, 5.1)	5.0 (4.9, 5.1)	5.1 (5.0, 5.1)	5.1 (4.9, 5.3)	5.3 (5.1, 5.6)	5.1 (4.9, 5.3)

Point estimates reflect means and percentages (95% confidence intervals).

Bolded text represents point estimates for which 95% confidence intervals do not overlap.

Multivariable-adjusted linear regression analyses demonstrating associations of maternal free cortisol tertiles with 10-year coronary heart disease risk, stratified by sex.

Table 2

		Model Adjustment					
		Age, Race/Ethnicity	Age, Race/Ethnicity, <i>In Utero</i> SEI	Age, Race/Ethnicity, Maternal Age	Age, Race/Ethnicity, Size for Gestational Age		
Cortisol Tertile		β	95% CI	β	95% CI	β	95% CI
Females							
Low		0.0	0	0.0		0.0	
Medium		13.0	-10.6, 42.8	9.5	-13.1, 38.1	13.8	-9.9, 43.8
High		36.7	8.4, 72.5	26.1	-0.5, 59.9	39.1	10.2, 75.6
Males							
Low		0.0	0.0	0.0		0.0	
Medium		7.7	-15.5, 37.2	6.7	-16.4, 36.1	11.6	-12.2, 41.8
High		-2.8	-23.8, 24.0	-3.8	-24.8, 22.9	-2.4	-23.2, 24.0

Effect sizes represent the percent change in mean risk for coronary heart disease over the next 10 years, for cortisol tertiles of interest. Low cortisol tertile is the referent category. CI, confidence interval; SEI, socioeconomic index.

Table 3

Linear and logistic regression analyses demonstrating associations of maternal free cortisol tertile with specific coronary heart disease (CHD) risk factors, adjusted for age and race/ethnicity.

CHD Risk Factor	Females			Males		
	Cortisol Tertile	PE	95% CI	Cortisol Tertile	PE	95% CI
Systolic Blood Pressure, mmHg	Low	0.00		Low	0.00	
	Medium	3.17	-2.46, 8.81	Medium	-1.99	-9.25, 5.28
	High	1.88	-3.70, 7.47	High	-3.29	-10.61, 4.03
Diastolic Blood Pressure, mmHg	Low	0.00		Low	0.00	
	Medium	2.68	-1.23, 6.59	Medium	-1.71	-6.99, 3.57
	High	0.94	-2.94, 4.82	High	-2.63	-7.94, 2.69
Total Cholesterol, mg/dL	Low	0.00		Low	0.00	
	Medium	2.06	-11.22, 15.33	Medium	-14.25	-33.84, 5.33
	High	3.05	-10.11, 16.21	High	-0.01	-19.73, 19.71
HDL Cholesterol, mg/dL	Low	0.00		Low	0.00	
	Medium	4.49	-2.13, 11.10	Medium	-7.02	-12.77, -1.27
	High	-5.91	-12.47, 0.65	High	-0.29	-6.08, 5.50
HbA1c, %	Low	0.00		Low	0.00	
	Medium	-0.05	-0.16, 0.06	Medium	0.21	-0.11, 0.54
	High	0.06	-0.05, 0.17	High	0.01	-0.32, 0.33
Smoking	Low	1.00		Low	1.00	
	Medium	2.52	0.94, 6.74	Medium	1.46	0.50, 4.24
	High	2.57	0.98, 6.78	High	1.03	0.34, 3.15

Point Estimates (PE) represent either odds ratios (for smoking, where odds ratios represent change in odds of smoking per each tertile compared to lowest tertile) or regression coefficients (for all other dependent variables, representing increase in dependent variable for each cortisol tertile, compared to low cortisol tertile as referent category).