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Maternal cortisol output in pregnancy and newborn telomere length: Evidence for sex-specific effects

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ABSTRACT

Newborn telomere length is a potential biomarker of the effects of maternal-fetal processes on offspring long-term health. A number of maternal psychosocial and environmental factors in pregnancy (e.g., stress, health, socioeconomic status) have been associated with shortened telomere length at birth. The physiological mechanisms responsible for potential effects of maternal factors on newborn telomere length have yet to be identified. Indirect evidence suggests that disruptions in maternal hypothalamic-pituitary-adrenal (HPA) axis functioning in pregnancy may be involved. Studies are needed that test whether maternal HPA axis functioning in pregnancy is associated with newborn telomere length. This study examined whether maternal HPA axis functioning across pregnancy, reflected in hair cortisol collected within one week after delivery, predicted newborn telomere length assessed from leukocyte cord blood collected at birth among 93 sociodemographically diverse mother-infant dyads. We further tested whether associations between maternal hair cortisol and newborn telomere length differed by infant sex, given documented sex differences in prenatal environmental exposure effects on offspring health, patterns of cortisol exposure during gestation, and telomere biology across the lifespan. In a multi-group structural equation modeling analysis that accounted for cortisol exposures across trimesters, maternal cortisol levels in pregnancy were not associated with newborn telomere length in the sample as a whole. However, significant sex differences emerged, with a significant positive association among females and a lack of a significant association among males. In addition, analyses revealed that cortisol levels were higher across trimesters among mothers of male infants than mothers of female infants. The results suggest that functioning of the maternal HPA axis in pregnancy may differ by fetal sex and have sex-specific effects on newborn telomere biology. These findings have implications for understanding the mechanisms by which maternal psychosocial and environmental exposures influence newborn telomere length and for elucidating mechanisms contributing to sex disparities in health.

1. Introduction

Telomeres are repeating nucleotide sequences of variable number that protect against chromosome deterioration and regulate cellular and tissue function (Blackburn and Gall, 1978). Shorter telomere length has been associated with chromosomal instability and is predictive of decreased immunocompetence, development of chronic disease throughout life (e.g., cardiovascular disease, diabetes, obesity, inflammatory diseases, depression), abnormalities in brain structure and functioning, and earlier mortality (Baragetti et al., 2015; Factor-Litvak et al., 2016; Geronimus et al., 2015; Hochstrasser et al., 2012; Mundstock et al., 2015; Rode et al., 2015). Telomere length at birth provides an individual's initial telomere length setting, such that telomere length at any given time point is determined by newborn telomere

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length and subsequent attrition (Factor-Litvak et al., 2016; Martens et al., 2016). Thus, newborn telomere length may be a powerful biomarker of lifetime disease risk. Consequently, identifying mechanisms that contribute to shortened telomere length at birth may inform our understanding of prenatal processes that contribute to long-term health (Entringer et al., 2015).

Limited research has explored determinants of newborn telomere length. Research among adults suggests high heritability, with a large meta-analysis estimating telomere length heritability as 0.70 (Broer et al., 2013). However, many biological variables show increased heritability with advancing age (Sillanpaa et al., 2017), such that heritability of telomere length is likely lower among newborns than adults. Moreover, data suggest that telomere dynamics during early development are largely determinative of relative telomere length for life and that experiential/environmental factors have major impact on telomere length at birth (Hjelmborg et al., 2015). To date, a number of maternal prenatal health and exposure variables have been implicated as potential factors that may accelerate telomere attrition during fetal development. For example, data suggest that maternal smoking, increased body mass index (BMI), lower socioeconomic status, heightened stress, and depression during pregnancy are each associated with shorter newborn telomere length (Bosquet Enlow et al., 2018; Drury et al., 2015; Entringer et al., 2011, 2013; Factor-Litvak et al., 2016; Marchetto et al., 2016; Martens et al., 2016; Send et al., 2017). However, the underlying biological mechanisms responsible for driving the effects of these maternal exposures on offspring telomere length erosion currently are unknown.

Data suggest that one possible mechanism that may be responsible for maternal risk factor effects on newborn telomere length is increased fetal exposure to cortisol due to increased maternal cortisol production that crosses the placenta to the fetus. Many of the maternal risk factors associated with shortened newborn telomere length have been associated with disruptions to functioning of the hypothalamic-pituitary-adrenal axis (HPA) axis, including during pregnancy (Davis and Sandman, 2010; Entringer et al., 2015; Geronimus et al., 2015; Kalra et al., 2007; Lopez and Seng, 2014; Suglia et al., 2010; Van den Bergh et al., 2017). Moreover, in adults, elevated cortisol has been found to dampen telomerase activity, which regulates telomere length (Choi et al., 2008; Epel et al., 2006; Tomiyama et al., 2012). Further, dysregulation of the HPA axis, including HPA axis hypoactivity, flatter diurnal slopes, and greater cortisol responses to stress, has been associated with shorter telomere length in adults (Tomiyama et al., 2012; Wikgren et al., 2012). In a cross-sectional study of 5- to 6-year-old children, a pattern of high cortisol reactivity, along with increased sympathetic activity and parasympathetic withdrawal, was associated with shorter buccal cell telomere length (Kroenke et al., 2011). A recent study found that increases in infant cortisol stress reactivity and reductions in cortisol recovery between 6 and 12 months of age predicted shorter salivary telomere length at 18 months of age (Nelson et al., 2018). Studies are needed that test whether maternal HPA axis functioning in pregnancy is associated with newborn telomere length. This is a critical step in determining if disrupted maternal HPA axis activity is mechanistically involved in the establishment of newborn telomere length and is responsible for documented associations between maternal psychosocial and health factors (e.g., stress, BMI) and shortened newborn telomere length.

A number of findings highlight the need to consider potential sex differences in prenatal cortisol exposure effects on newborn telomere biology. First, maternal stress exposures and stress reactivity during pregnancy appear to have sex-specific effects on various aspects of fetal development, with males and females showing varying vulnerability, depending on the nature and timing of the exposure and the developmental outcome of interest (Davis et al., 2013; Doyle et al., 2015; Gabory et al., 2009; Ostlund et al., 2016; Van den Bergh et al., 2017). Some have suggested that female fetuses are more responsive to a range of in utero exposures, whereas others posit that males are more vulnerable to maternal distress in pregnancy (Doyle et al., 2015). Additionally, fetal sex appears specifically to moderate the production of maternal cortisol during pregnancy, with one study suggesting that both males and females are exposed to increasing levels of cortisol from mid- to late pregnancy but that males are exposed to relatively higher levels in mid-pregnancy and females to relatively higher levels in late pregnancy (DiPietro et al., 2011). Thus, fetal characteristics, in addition to maternal factors, may influence maternal HPA axis functioning and, consequently, fetal cortisol exposure. Changes in epigenetic regulation of factors affecting fetal cortisol exposure also show sex effects (Gabory et al., 2009; Ostlund et al., 2016). For example, data suggest that maternal stress/depression during pregnancy is associated with epigenetic modification of the fetal glucocorticoid receptor gene *NR3C1*, which modulates sustained HPA axis activity, with some studies finding effects only among females and others only among males (Braithwaite et al., 2015; Ostlund et al., 2016). Further, data suggest that male and female fetuses show different strategies for adapting to exposure to stress hormones that result in sex differences across a range of outcomes (Davis et al., 2013). Importantly, sex differences in the effects of various risk factors on telomere length have been documented, albeit primarily in adults, with males more susceptible in many, but not all, studies (Drury et al., 2015; Enokido et al., 2014; Moller et al., 2009; Shalev et al., 2014; Zalli et al., 2014). Notably, our group recently has shown sex differences in the effects of a variety of maternal exposures on newborn telomere length, with only males showing shortened telomeres in the context of maternal smoking, heightened BMI, lower socioeconomic status, and elevated depressive symptoms in pregnancy and maternal sexual abuse and decreased familial support in childhood (Bosquet Enlow et al., 2018). Finally, animal studies have documented associations between heightened corticosterone exposure and shortened telomere length, with some studies finding this association specifically among males, depending on species and context (Angelier et al., 2018). Together, these data suggest that male and female fetuses may experience or even evoke different patterns of cortisol exposure in utero and that in utero cortisol exposure may have sex-specific effects on newborn telomere length.

The current study had two main aims: (1) to test whether maternal cortisol output during pregnancy is associated with newborn telomere length and (2) to examine whether there are sex-specific effects of maternal HPA axis activity in pregnancy on newborn telomere length. We hypothesized that increased exposure to maternal cortisol in pregnancy is associated with shorter offspring telomere length at birth. We further hypothesized that increased maternal cortisol output in pregnancy has greater impact on male than female newborn telomere length. Additionally, we hypothesized that mothers of male fetuses demonstrate a different pattern of cortisol output than mothers of female fetuses.

2. Materials and methods

2.1. Participants

Participants were pregnant women enrolled in the PRogramming of Intergenerational Stress Mechanisms (PRISM) study, a prospective pregnancy cohort originally designed to recruit $N = 276$ mother-child dyads to examine the role of maternal and child stress exposures on child development. Between July 2011 and November 2013, pregnant women were recruited from prenatal clinics in urban hospitals and community health centers in the Northeast of the United States. Eligibility criteria included: 1) English- or Spanish-speaking, 2) age $\geq$ 18 years at enrollment, and 3) single gestation birth. Exclusion criteria included: 1) maternal endorsement of drinking $\geq$ 7 alcoholic drinks/week during pregnancy and 2) maternal positive HIV status, which would influence/confound biomarkers of interest. Based on screening data, there were no differences in race/ethnicity, education, or income between women who enrolled in PRISM and those who declined.

Subsequent to study initiation, funding was obtained to collect maternal hair samples, utilized to assess prenatal cortisol levels, and cord blood at delivery, utilized to assess newborn telomere length. Among women enrolled in PRISM who had not yet delivered when this additional funding was obtained, 93 provided usable hair samples and cord blood. Exclusion criteria for the current analyses included use of oral steroids in the prior year, due to its potential impact on hair cortisol levels (Braig et al., 2015); none of the 93 women met this criterion. Within the PRISM cohort, compared to mothers who were not included in the current analyses, mothers who were included were more likely to be White, Black, or other race/ethnicity, and less likely to be Hispanic and had on average higher educational attainment and annual income during pregnancy, *ps* ≤ .002. There were no differences between families who did and did not participate with respect to maternal age at enrollment or infant sex, gestational age, birthweight, or birthweight adjusted for gestational age, *ps* > .05.

2.2. Measures

2.2.1. Hair cortisol in pregnancy

Hair cortisol was assessed from participants' scalp hair, collected within one week after delivery. Hair cortisol measures allow for characterization of HPA axis activity across pregnancy and are not affected by factors that influence salivary and serum cortisol protocols (e.g., nonadherence, circadian patterns, invasiveness, stress of sampling) (D'Anna-Hernandez et al., 2011; Kalra et al., 2007).

A hair strand roughly 3 mm in diameter was cut with scissors as close to the scalp as possible from the posterior vertex, the suggested standard position for hair cortisol collection, given that this region has the most uniform rate of growth, lowest inter-individual variability, and lowest proportion of resting phase in the hair follicle (Sauve et al., 2007; Stalder and Kirschbaum, 2012). Hair samples were cut into three 3-cm segments, length permitting, with each 3-cm segment corresponding to one trimester based on a hair growth rate of approximately 1 cm/month (D'Anna-Hernandez et al., 2011). As previously described (Braig et al., 2015), the 3-cm segment closest to the scalp reflected cortisol levels during the third trimester, and the next two 3-cm segments reflected second and first trimester levels, respectively. Data support the use of cortisol measured from hair collected postpartum as an indicator of maternal prenatal HPA axis activity (Braig et al., 2015; D'Anna-Hernandez et al., 2011).

Hair was stored in manila envelopes at room temperature out of direct sunlight until shipment for analysis. Hair samples were analyzed in the Kirschbaum laboratory at the Technical University of Dresden, Germany. Washing and steroid extraction followed an established protocol (Stalder et al., 2013). Hair was washed in isopropanol, and cortisol was extracted from 7.5 mg of whole nonpulverized hair using methanol in the presence of internal standards. Samples were centrifuged at 15,200 x *g*, and the supernatant was collected; alcohol was evaporated under a stream of nitrogen and reconstituted with double-distilled water and then injected into a Shimadzu HPLC-tandem mass spectrometry system (Shimadzu, Canby, Oregon) coupled to an AB Sciex API 5000 Turbo-ion-spray triple quadrupole tandem mass spectrometer (AB Sciex, Foster City, CA), with purification by on-line solid-phase extraction (Gao et al., 2013). Lower limits of quantification were 0.1 pg/mg; inter- and intra-assay variabilities were 3.7–8.8%.

2.2.2. Cord blood telomere length

Newborn telomere length was assessed from banked cord blood leukocyte DNA, a valid index of newborn telomere length (Entringer et al., 2013; Okuda et al., 2002). Cord blood was drawn from the umbilical cord at delivery after the clinician collected the blood gas immediately after clamping the cord. Cord blood samples were collected in EDTA-tubes, centrifuged to obtain buffy coat fraction, and stored at −80 °C until DNA extraction. DNA extraction was conducted using the Promega Wizard DNA extraction system (Madison, WI, USA) according to the manufacturer's recommended protocol. DNA quantity and quality were assessed using an Implen NanoPhotometer Pearl (Westlake Village, CA) to ensure that the optical density ratios were within expected ranges; values were found to be within acceptable ranges.

Samples were assayed for telomere length at the University of Milan (PI Bollati). Telomere length was determined using real-time polymerase chain reaction (PCR), which requires a small amount of DNA. A recent meta-analysis determined that this method is a valid technique for quantifying telomere length (Ridout et al., 2017). Telomere length was measured using the quantitative real-time method described by Cawthon (Cawthon, 2002, 2009). This method measures the relative telomere length by determining the ratio of telomeric repeat copy number (T) to a nuclear single copy gene (S, human beta-globin gene) copy number (T/S ratio) in a given sample relative to a reference pooled DNA used to generate a standard curve, which is inserted in each PCR run. Details regarding the telomere assaying procedure are provided in the Supplementary Material, Cord Blood Telomere Length Assaying Procedures. Because PCR provides a relative measure of telomere length, the acronym rTL (for "relative telomere length") is utilized when describing the current analyses.

2.2.3. Perinatal health variables

Maternal and infant perinatal health variables that prior literature has associated with cortisol production and/or newborn telomere length were considered as potential covariates. Maternal perinatal health indicators considered were maternal smoking in pregnancy; maternal pre-pregnancy BMI; use of inhaled corticosteroids or topical steroids in the past year; and mode of delivery. Smoking in pregnancy was categorized as yes/no based on maternal self-report of smoking at baseline and/or in the third trimester. BMI was calculated by dividing maternal self-reported pre-pregnancy weight (kg) by height squared (meter). Use of inhaled corticosteroids or topical steroids over the prior year were each categorized as yes/no based on self-report and/or medical records. Mode of delivery was categorized as vaginal or cesarean delivery, based on medical records. Infant perinatal health indicators included gestational age, birthweight, and birthweight adjusted for gestational age. Gestational age and birthweight were extracted from medical records. Sex-specific Fenton birthweight for gestational age z-scores were calculated (Fenton and Kim, 2013). These infant variables were considered as continuous variables.

2.2.4. Hair cortisol covariates

Variables that prior literature has associated with variation in hair cortisol levels were considered as potential covariates. These variables included maternal educational attainment, season of delivery, hair treatment, and hair shampooing frequency (Braig et al., 2015). Maternal educational attainment was based on self-report and categorized as high school diploma/GED or less, some college, college degree, or graduate degree. Season of delivery was categorized as winter (December-February), spring (March-May), summer (June-August), or autumn (September-November). Three dichotomous hair treatment variables were derived on the basis of self-report: (a) hair artificially dyed (yes/no); (b) hair chemically straightened and/or permed in the prior 12 months (yes/no); (c) hair shampooing frequency (five times or more per week or less than five times per week).

2.3. Procedures

Maternal sociodemographics were assessed via in-person interviews shortly following recruitment in pregnancy (M = 19.5 weeks of gestation, SD = 8.5 weeks of gestation). Leukocyte cord blood samples were collected at delivery. Within one week after delivery, staff collected maternal hair samples. Study procedures were approved by the relevant institutions' human studies ethics committees (Brigham and Women's Hospital/Partners HealthCare, Beth Israel Deaconess Medical Center). Mothers provided written informed consent in their preferred language.

2.4. Data analytic plan

Hair cortisol values were log-transformed to reduce skewness, as recommended (Braig et al., 2015). Descriptive statistics were calculated to describe the sample. Next, differences between male and female infants on the study variables were tested via *t*-test and chi-square analyses. Correlational analyses then tested associations of the maternal and infant perinatal health variables and potential hair cortisol covariates with the maternal hair cortisol variables and with leukocyte cord blood rTL; perinatal health variables and hair cortisol covariates that were significantly associated with both maternal hair cortisol and leukocyte cord blood rTL were considered as covariates in subsequent analyses.

To test for the association between maternal hair cortisol in pregnancy and leukocyte cord blood rTL, a multi-group structural equation modeling (SEM) approach was utilized. To maximize use of all available data, pairwise deletion was employed. The available hair cortisol data from each trimester comprised a latent variable with three indicators (i.e., hair cortisol levels during 1st, 2nd, and 3rd trimesters). To test the structural relationship between maternal hair cortisol levels and leukocyte cord blood rTL by infant sex, the measurement of maternal hair cortisol between male and female infants needed to be equivalent in both function (factor loadings) and level (intercepts). Thus, the factor loadings of these three indicators were constrained to be equivalent across male and female infants, as any comparisons regarding this latent variable for hair cortisol would be rendered meaningless in the absence of measurement invariance (i.e., construct equivalence in how hair cortisol was operationalized). The association between maternal hair cortisol in pregnancy and leukocyte cord blood rTL was then simultaneously tested for male and female infants, and a test of significance was constructed to test for differences in the correlation coefficient between sexes. Several steps were taken to ensure proper model evaluation, stability of the estimated parameters, and the model's power to fit the measurement model and the structural model on the equivalence of the relationship between maternal hair cortisol and leukocyte cord blood rTL across infant sex. Power of the SEM was assessed via a Monte Carlo Simulation. The population model parameters involved a measurement model with one latent dimension and factor loadings equal to 0.8, item residual variances equal to 0.36, a factor mean equal to zero, and a factor variance equal to 1 (for identification). The structural part of the model involved a medium-sized correlational path between hair cortisol and leukocyte cord blood rTL equal to 0.30 (Cohen, 1992). Results using 1000 simulated samples of $N = 93$ participants indicated power levels equal to 99% for the significance of the factor loadings, mean values of the chi-square test equal to 9.363 for an expected critical value of 9.488, suggesting proper rejections of discrepant models, and power levels equal to 75% to identify as significant the correlation between hair cortisol and leukocyte cord blood rTL when it was equal to 0.30 (coverage of the correlation coefficient was 95.7% from the 1000 samples). The simulation findings agreed with earlier findings suggesting proper levels of power and stability of the estimated parameters with sample sizes greater than 70 participants (Sideridis et al., 2014). Thus, the sample size of 93 participants should suffice to fit a proper measurement model and identify as significant a correlation coefficient that exceeds a medium effect size (i.e., 0.30). To increase confidence regarding the stability of the estimated correlations between the latent cortisol variable and leukocyte cord blood rTL for male and female infants, and specifically, the difference in correlation estimates by infant sex, 95% confidence intervals were constructed using 1000 replicated samples from the original sample, assuming normality of the difference correlation coefficient (Efron, 1979). A finding that zero is not included in the 95% confidence interval of the simulated population distribution of difference correlation coefficients strengthens support that the difference correlation coefficient is truly different from zero. In addition, the bootstrapping procedure was repeated without assuming a known distribution of the difference correlation coefficient by simulating difference correlation coefficients from 10,000 samples of $N = 93$ participants. Again, 95% confidence intervals were constructed to ensure that, in the presence of a significant difference correlation value, the confidence intervals did not include the value of zero. Finally, SEM model fit was evaluated utilizing several means, including the chi-square test and several descriptive fit indices, as recommended (Hu and Bentler, 1999). Specifically, we relied on the Comparative Fit Index (CFI), the Tucker-Lewis Index (TLI), and the Root Mean Square Error of Approximation (RMSEA), with values greater than 0.95 on the fit indices and values between 5%–8% for the RMSEA being indicative of acceptable model fit. Because prior literature has documented differences among racial/ethnic groups in HPA axis activity, including during pregnancy, telomere length at birth, and many of the covariates associated with these variables (Drury et al., 2015; Schreier et al., 2016; Suglia et al., 2010), the SEM analyses were then repeated, adjusted for race/ethnicity. Analyses were conducted using SPSS version 23, R, and Mplus version 8.

3. Results

3.1. Descriptive data

Table 1 details the sample characteristics for the whole sample and by infant sex. The sample was sociodemographically diverse in terms of maternal and child race/ethnicity, maternal educational attainment, annual household income, and maternal marital status. The infants were primarily of normal birthweight (95% born greater than 2500 g) and born full-term (93% born 37 weeks or later). Due to varied hair length, valid cortisol data were available for 56 women during the first trimester, 76 during the second trimester, and 90[1] during the third trimester. Hair cortisol levels were highly correlated across trimesters: first and second trimester $r = 0.97$; second and third trimester $r = 0.92$; first and third trimester $r = 0.87$. The mean leukocyte cord blood rTL for the full sample was 2.52 (SD = 0.76); the values were normally distributed with no outliers.

Compared to mothers of females, mothers of males had higher hair cortisol levels during all three trimesters: 1st trimester, $t(54) = 2.43$, $p = .019$; 2nd trimester, $t(74) = 3.23$, $p = .002$; 3rd trimester, $t(88) = 2.01$, $p = .047$. Leukocyte cord blood rTL did not differ between male and female infants, $t(91) = 1.11$, $p = .269$. There also were no significant differences between male and female infants on any of the maternal or infant perinatal health or sociodemographic characteristics, all $ps > .10$.

Among the perinatal health variables, maternal pre-pregnancy BMI was significantly associated with hair cortisol during the 2nd trimester, $r = 0.25$, $p = .030$, and 3rd trimester, $r = 0.27$, $p = .010$, but not with leukocyte cord blood rTL, $r = -0.07$, $p = .514$. Maternal inhaled corticosteroid use was associated with leukocyte cord blood rTL, $r = -0.26$, $p = .014$, but not with any of the hair cortisol measures, $ps > .20$. None of the remaining perinatal health variables (maternal smoking in pregnancy, maternal topical steroid use, mode of delivery, infant gestational age, infant birthweight, infant birthweight adjusted for gestational age) were associated with any of the maternal hair cortisol measures or with leukocyte cord blood rTL, $ps > .08$. Among the potential hair cortisol covariates, maternal education was significantly associated with hair cortisol during the 3rd trimester, $r_s = -0.30$, $p = .004$, but not with hair cortisol levels during the 1st or 2nd trimesters or with leukocyte cord blood rTL, $ps > .09$. None of the remaining potential hair cortisol covariates (season of delivery, hair dyed, hair

[1] Three mothers had hair cortisol data that were measurable for the first and/or second trimester but not for the third trimester, producing the final sample size of $N = 93$ dyads with maternal hair cortisol data during any trimester and leukocyte cord blood rTL data.

Table 1
Sample characteristics for entire sample (N = 93) and by male infants (n = 48) and female infants (n = 45).

	Entire Sample				*Males*				*Females*			
	N[a]	%	*M*	*SD*	*n*	%	*M*	*SD*	*n*	%	*M*	*SD*
Maternal age (years)			31.83	5.11			31.75	5.39			31.90	4.87
Maternal race/ethnicity												
White	49	53			22	46			27	60		
Black	27	29			16	33			11	24		
Hispanic	8	9			4	8			4	9		
Other[b]	9	10			6	13			3	7		
Child race/ethnicity												
White	41	44			17	35			24	53		
Black	27	29			15	31			12	27		
Hispanic	12	13			8	17			4	9		
Other[b]	13	14			8	17			5	11		
Maternal relationship status												
Married	68	73			31	65			37	82		
Living together	10	11			8	17			2	4		
Other[c]	14	15			8	17			6	13		
Maternal education												
High school diploma/GED or less	11	12			4	8			7	16		
Some college	18	19			12	25			6	13		
College degree	25	27			10	21			15	33		
Graduate degree	38	41			21	44			17	38		
Annual household income												
< $20,000	14	15			6	13			8	18		
$20,000-$34,999	7	8			5	10			2	4		
$35,000-$49,999	6	7			2	4			4	9		
$50,000-$69,999	8	9			6	13			2	4		
$70,000-$99,999	15	16			10	21			5	11		
$100,000+	37	40			13	27			24	53		
Maternal pre-pregnancy BMI			25.48	6.06			26.31	6.11			24.55	5.93
Maternal inhaled corticosteroid use	7	8			3	6			4	9		
Maternal topical steroid use	5	5			4	8			1	2		
Maternal smoking in pregnancy	21	23			12	25			9	20		
Mode of delivery (% cesarean)	23	25			10	21			13	29		
Infant birthweight (grams)			3399	503			3412	516			3386	493
Infant gestational age (weeks)			39.1	1.6			39.1	1.8			39.1	1.4
Infant birthweight adjusted for gestational age (z-score)			0.03	0.92			0.00	0.97			0.06	0.88
Maternal hair cortisol[d], 1st trimester			0.55	0.76			0.79	0.89			0.32	0.52
Maternal hair cortisol[d], 2nd trimester			0.50	0.70			0.74	0.76			0.25	0.54
Maternal hair cortisol[d], 3rd trimester			0.74	0.79			0.90	0.74			0.57	0.81
Leukocyte cord blood telomere length (T/S ratio)[e]			2.52	0.76			2.43	0.76			2.61	0.75

Note. [a]Data were missing for 0 to 2 participants across all but hair cortisol variables; hair cortisol data were available for $n = 56$ in the 1st trimester, $n = 76$ in the 2nd trimester, and $n = 90$ in the 3rd trimester. [b]The majority categorized as "other" race/ethnicity were identified by self/mother as Asian or multi-racial. [c]Other included never married, divorced, separated, and other relationship status. [d]Values were log-transformed to reduce skewness. [e]Telomere length is represented by the ratio between the average of three values obtained from telomere amplification and from β-globin amplification (T/S ratio). BMI = body mass index.

chemically straightened/permed, shampooing frequency) were associated with any of the maternal hair cortisol measures or with leukocyte cord blood rTL, *ps* > .05. Because none of the perinatal health or hair cortisol covariate variables under consideration were associated with both hair cortisol and leukocyte cord blood rTL values, for the sake of parsimony, they were not considered further in analyses.

3.2. Maternal cortisol output in pregnancy and newborn telomere length

The SEM model fit to the full dataset showed good model fit, with a non-significant chi-square test, $\chi^2(2) = 4.220$, $p = .121$, suggesting "exact fit" of the data to the model. Furthermore, the descriptive fit indices were excellent (CFI = .986, TLI = .958), with a borderline RMSEA index (.109). Within the sample as a whole, the correlation between the maternal hair cortisol construct and leukocyte cord blood rTL was not significant, $r = .004$, $p = .996$.

Subsequent tests fit the data of male and female infants simultaneously (Fig. 1a) to evaluate the structural relationship between maternal hair cortisol levels and leukocyte cord blood rTL for each sex and to test the difference between the two correlation coefficients. As shown in Fig. 1a, the model was fit to the data with the factor loadings between males and females constrained to be equivalent to allow for comparison of means. Following application of the protocol of measurement invariance developed by Randall and Engelhard (2010), results indicated that constraining the factor loadings to be equivalent between sexes did not result in inferior model fit, as indicated by a delta chi-square test [$\chi^2(2) = 3.311$, $p = .191$], thus, pointing to the presence of metric invariance of hair cortisol. Similarly, in addition to constraining slopes, intercepts were posited to be equivalent between sexes. Again, the constrained model (equivalent slopes and equivalent intercepts) was not associated with inferior model fit in relation to both the configural model [$\chi^2(4) = 4.187$, $p = .381$] and the metric model [$\chi^2(2) = 0.805$, $p = .669$]. Consequently, both metric and scalar invariance were satisfied, and examination of the relationship between maternal hair cortisol and newborn telomere length by infant sex was possible. SEM results indicated excellent model fit, with a non-significant chi-square test, $\chi^2(9) = 9.561$, $p = .387$, excellent fit indices (CFI = .997, TLI = 0.996), and the unstandardized residuals well below acceptable levels (RMSEA = 0.037). The correlation between the maternal hair cortisol construct and leukocyte cord blood rTL was $r = -0.117$, $p = .336$ for males and $r = 0.171$, $p = .037$ for females. The difference correlation coefficient was significant, $r = -.288$, $p = .046$, indicating that males and females differed in their relationship between the latent maternal hair cortisol factor and leukocyte cord blood rTL.

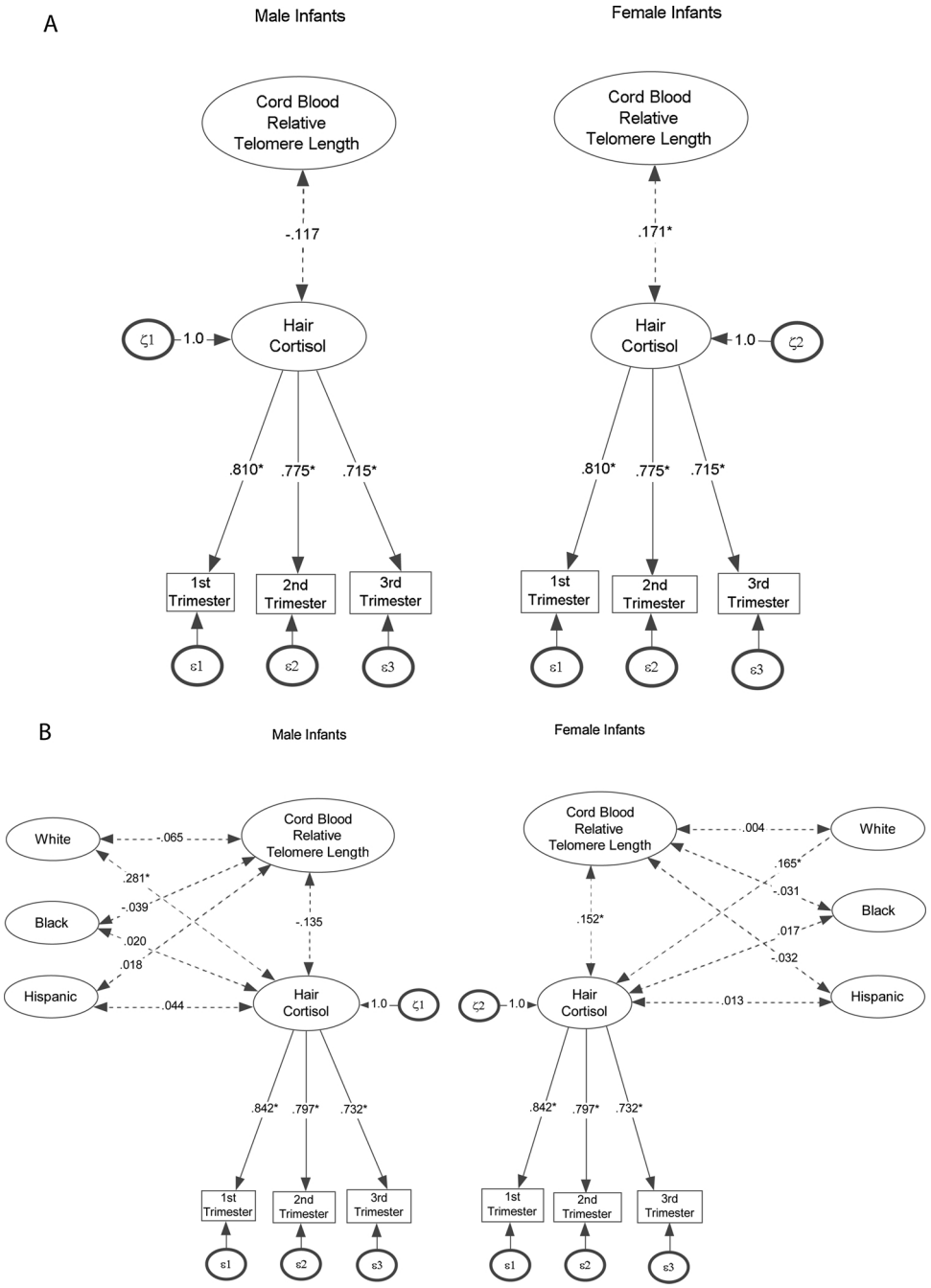

Fig. 1. Structural equation model testing associations between maternal hair cortisol levels across trimesters (latent variable with three indicators) and leukocyte cord blood relative telomere length by infant sex. The Greek letter ζ refers to the variance of the latent factors that is fixed to unity for identification. Solid lines represent factor loadings and dashed bi-arrow lines correlations. Letter ε denotes the error variances of the items. Factor loadings are equivalent for male and female infants as per metric/scalar invariance. Asterisks (*) indicate significance at $p < .05$. Fig. 1a is the unadjusted model, and Fig. 1b is adjusted for maternal race/ethnicity.

Further analyses simulated the distribution of *r*-difference values to estimate the population distribution and assess 95% confidence intervals that assumed a normal and a non-normal distribution. When simulating the population distribution of *r* values assuming normality, results indicated that the 95% confidence interval of that population distribution did not include zero (lower confidence limit = -0.570, upper confidence limit = -0.005). Consequently, the observed value of $r = -0.288$ was deemed to be significantly different from zero. Results were replicated when simulating *r*-difference values without assuming normality of that distribution, with a mean difference correlation value of r = -0.2875, almost identical to the original sample estimate. Zero was not included, and, consequently, a conclusion of a non-zero difference correlation coefficient was again supported. Thus, the present findings are likely robust and reflective of true population effects. For visualization purposes, Fig. 2 displays the scatterplot of leukocyte cord blood rTL values by maternal hair cortisol level for each trimester for male and female infants.

These analyses were repeated with the addition of controls for the effects of maternal race/ethnicity on both the hair cortisol latent construct and on leukocyte cord blood rTL. The same pattern of results was found after controlling for maternal race/ethnicity (Fig. 1b), and the 95% confidence interval of the population distribution did not include

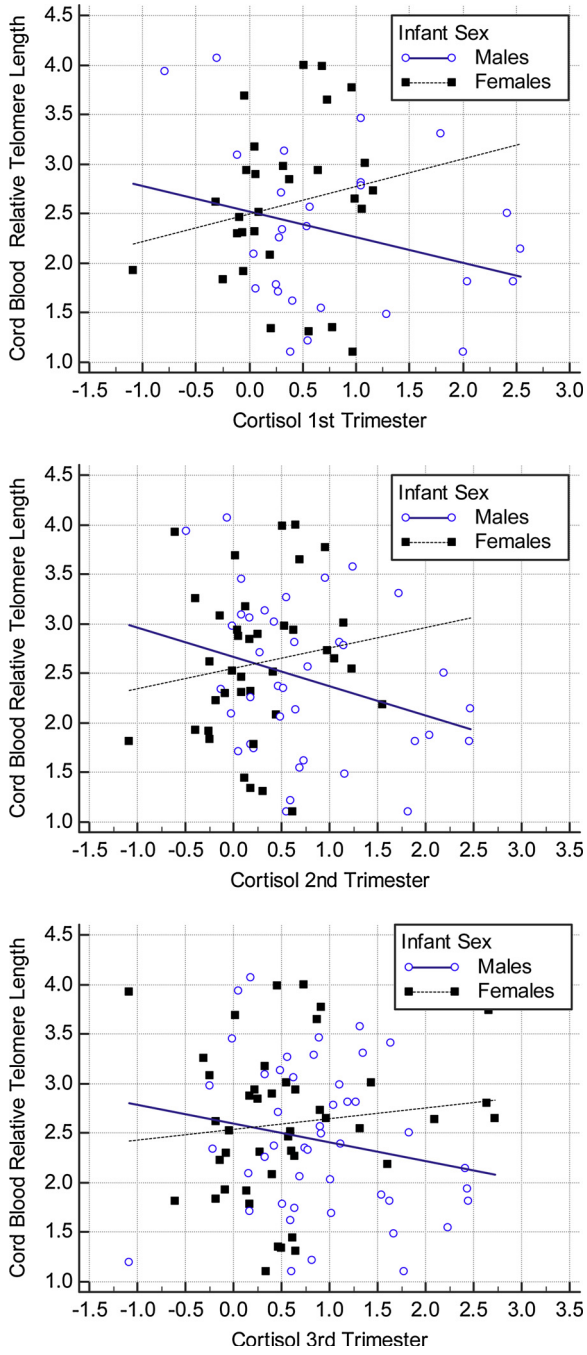

Fig. 2. Associations between maternal hair cortisol level (log transformed) for each trimester and leukocyte cord blood relative telomere length for male and female infants. Lines represent fitted regression lines for male (solid lines) and female (dotted lines) infants.

zero, indicating that the difference correlation coefficient for male versus female infants was significant.

4. Discussion

The overall goals of this study were to examine whether maternal cortisol output during pregnancy predicts newborn telomere length and whether the association between maternal cortisol in pregnancy and newborn telomere length differs by infant sex. An additional goal was to examine whether maternal cortisol levels across pregnancy differs by fetal sex, as suggested by others (DiPietro et al., 2011). When examined in the sample as a whole, maternal HPA axis activity during pregnancy, assessed via hair cortisol levels across trimesters, was not associated with newborn telomere length. However, sex-specific findings emerged, with females demonstrating a significant positive association between maternal cortisol output during pregnancy and newborn telomere length and males failing to show a significant association. Moreover, tests of the difference correlation coefficient were significant, indicating that the nature of the association of maternal hair cortisol in pregnancy with newborn telomere length differed between males and females. In addition, we found that mothers of male infants evidenced greater cortisol output across pregnancy compared to mothers of female infants.

One prior study examined associations between multiple hormones in cord blood and newborn leukocyte telomere length (Liu et al., 2017) and found that shorter telomere length was associated with higher levels of reactive oxygen species (ROS) and lower levels of dehydroepiandrosterone (DHEAS) but was not associated with cortisol or estradiol levels. There were important differences between this study and the current study. First, Liu and colleagues' study relied on cortisol levels measured from cord blood, which the authors acknowledged are relatively unstable, potentially substantially changing within tens of minutes, thus increasing random measurement error. Moreover, such measures represent relatively short-term assessments of HPA axis functioning and thus may not provide an accurate depiction of the fetus's overall cortisol exposure. The current study utilized hair cortisol, which is hypothesized to characterize longer-term HPA axis activity during pregnancy (D'Anna-Hernandez et al., 2011; Kalra et al., 2007). Additionally, Liu and colleagues' study did not examine associations between cord blood hormone levels and telomere length by infant sex, which may have obscured findings. In the current study, associations between prenatal cortisol exposure and newborn telomere length did not emerge in the sample as a whole but only when the sample was stratified by sex. Thus, the findings across the studies suggest that associations between prenatal cortisol exposure and newborn telomere length may only emerge when long-term measures of maternal HPA axis are utilized and infant sex is considered.

The current findings are consistent with a growing body of evidence demonstrating sex-specific effects of prenatal exposures on a range of developmental outcomes and extend this literature to include newborn telomere biology. The implications of these findings are as yet unknown. Although shorter telomere length generally has been considered a marker of health vulnerability, both shorter and longer telomere length at birth have been described as indicating increased risk (Drury et al., 2015; Factor-Litvak et al., 2016). In adults, shorter telomere length has been associated fairly consistently to a number of risk factors associated with poor health (e.g., elevated BMI; smoking) as well as to increased morbidity and mortality, although longer telomere length has been associated with major cancers (Baragetti et al., 2015; Factor-Litvak et al., 2016; Hochstrasser et al., 2012; Mundstock et al., 2015; Rode et al., 2015). Telomere length at birth establishes an individual's initial telomere length setting, thus influencing lifetime telomere biology (Factor-Litvak et al., 2016; Martens et al., 2016). Therefore, shortened telomere length at birth may be expected to predict poorer health. However, some have suggested that longer telomere length at birth predicts more rapid attrition across the life course and thus may be an indicator of vulnerability (Drury et al., 2015). Our findings of a significant association between maternal cortisol levels in pregnancy and newborn telomere length among female but not male infants may indicate that female fetuses more consistently adopted a strategy to respond to the in utero environment than male fetuses. Notably, these findings fit with theories that female fetuses show greater adaptation than male fetuses to in utero adversity but at a potential cost to health in later development (Doyle et al., 2015). Research is needed to determine the health implications of newborn telomere length to establish whether shorter and/or longer telomere length at birth is associated with various health outcomes and whether such effects differ by sex.

The current findings also suggest sex differences in fetal cortisol exposure over the course of pregnancy and are somewhat consistent with previous findings. DiPietro et al. (2011) also reported increases in cortisol output among mothers of males and females at the end of pregnancy, with differences in the pattern of maternal cortisol production by fetal sex. However, DiPietro et al. found that mothers of male fetuses demonstrated relatively higher levels of cortisol in mid-pregnancy (24–30 weeks) and mothers of female fetuses demonstrated relatively higher levels in later pregnancy (after 30 weeks). Notably, that study had important methodological differences from the current study, including relying on weekly salivary cortisol levels rather than trimester hair cortisol levels and only measuring cortisol from 24 to 38 weeks. Further research is needed to establish more firmly the nature of these differences. Together, both studies suggest that fetal sex may influence maternal cortisol production during pregnancy; thus, characteristics of the fetus may contribute to newborn telomere biology by influencing maternal HPA axis functioning.

Several mechanisms have been proposed to underly associations between glucocorticoid exposure and telomere biology. Some have hypothesized that glucocorticoids may affect the oxidative balance via genomic or non-genomic mechanisms and that exposure to increased oxidative stress is a primary cause of telomere shortening (Angelier et al., 2018; Nelson et al., 2018). Glucocorticoids may also influence telomere length by modulating telomerase activity (Angelier et al., 2018; Choi et al., 2008). In addition, the HPA axis interacts with multiple other physiological systems involved in oxidative stress and/or telomere biology, including immune activation and metabolic processes that result in increased production of reactive oxygen species (e.g., glucose and lipid mobilization) (Angelier et al., 2018). Specifically in pregnancy, maternal cortisol production may stimulate changes to placental physiology and maternal-fetal HPA axis functioning, impacting the initial setting and on-going regulation of newborn telomere length via epigenetic and other gene-regulating processes across cells (Entringer et al., 2011; Entringer et al., 2013). Thus, disruptions to maternal HPA axis activity in pregnancy may influence newborn telomere biology via various routes. Future studies should consider assessing the multiple physiological mechanisms that may contribute to newborn telomere biology and explore whether mechanistic pathways differ by infant sex.

The literature theorizing about the impact of cortisol on telomere biology has largely speculated that increased cortisol exposure is associated with greater telomere attrition (Choi et al., 2008; Epel et al., 2006; Tomiyama et al., 2012). Notably, in adults, indicators of both hyper- and hypoactivity of the HPA axis have been associated with shorter telomere length (Tomiyama et al., 2012; Wikgren et al., 2012). During pregnancy, regulation of the maternal HPA axis changes dramatically to support fetal development, with particularly large increases in cortisol production at the end of pregnancy considered normative (Davis and Sandman, 2010). Together, these data suggest that fetal exposure to reduced, as well as to elevated, maternal cortisol may be considered maladaptive with unknown effects on offspring telomere biology. In the current study, mothers of female fetuses demonstrated lower levels of hair cortisol than male fetuses, and only females showed an association between maternal cortisol levels and newborn telomere length, with lower cortisol levels associated with shorter telomere length. This raises the possibility that the sex differences found in the association between maternal cortisol levels and newborn telomere length may have been attributable to sex differences in levels of in utero cortisol exposure. Notably, studies suggest that, depending on the intensity and chronicity of exposure, glucocorticoids may increase both oxidation and antioxidant protection as well as down-regulate and up-regulate telomerase activity (Angelier et al., 2018). Research is needed to determine whether the effects found here reflect sex differences in fetal adaptation to maternal cortisol levels or whether the effects suggest potential "optimal" moderate levels of cortisol exposure in relation to telomere biology that are similar for males and females.

This study offers a number of strengths. It is the first study to provide evidence that maternal HPA axis functioning in pregnancy is associated with newborn telomere length, specifically when infant sex is considered. It responds to a recent call to "use hair cortisol or other measures of chronic activation of the HPA system to index maternal stress exposure across pregnancy," given documented associations between prenatal stress and telomere length at birth and in adulthood (Entringer et al., 2011, 2013; Nelson et al., 2018). The use of hair cortisol to quantify fetal cortisol exposure is relatively novel and offers some benefits over more conventional measures of maternal HPA axis functioning in pregnancy that have relied on saliva or serum. Such measures represent relatively short-term assessments of HPA axis functioning and thus may not provide an accurate depiction of the fetus's overall cortisol exposure. Given that the sample was drawn from the community, the findings may be more generalizable than studies using samples recruited by clinical status or other pre-determined risk factors. However, the sample was skewed toward higher socioeconomic status than the general population, and within sex analyses were limited by the sample size. Studies with larger sample sizes are needed to replicate the findings and determine if the pattern of associations apply to populations with more extreme characteristics (e.g., high stress/trauma exposure; low socioeconomic status).

Future research with larger samples should also explore whether associations between maternal HPA axis functioning and newborn telomere length are influenced by race/ethnicity. Documented race/ethnicity differences in telomere length have varied widely in nature and degree across studies of infants as well as children and adults (Drury et al., 2015; Factor-Litvak et al., 2016; Martens et al., 2016; Needham et al., 2012; Okuda et al., 2002). Research suggests that race/ethnicity may moderate physiological responses to stress exposures, including HPA axis functioning in pregnancy (Suglia et al., 2010). Additionally, one study suggests potential interactive effects between sex and race in predicting newborn telomere length, with Black female infants showing longer telomere length than Black males or White infants of either sex and White infants showing no sex differences in telomere length (Drury et al., 2015). In the current sample, the pattern of results was upheld when controlling for maternal race/ethnicity. However, maternal race and infant sex were associated with hair cortisol, with Black mothers of males evidencing higher cortisol each trimester than White mothers of males; there were no differences in hair cortisol level by maternal race for mothers of females (analyses not shown). Race/ethnicity was not associated with newborn telomere length in the current sample but has been in others, with evidence that race/ethnicity differences in newborn telomere length may depend on infant sex and maternal social context (e.g., socioeconomic status) (Drury et al., 2015; Needham et al., 2017). Further analysis of race/ethnicity effects was beyond the scope of the current study and limited by the sample size but should be explored in futures studies.

Limitations of the current study may include the use of peripheral blood mononuclear cells (PBMC) to estimate newborn telomere length. PBMC telomere length is an average across different cell subpopulations (T cells, B cells, NK cells, monocytes); telomere attrition may not be uniform across cell types, and relative ratios of cell types may vary across individuals. However, PBMC telomere length is the metric most commonly associated with morbidity and mortality (Geronimus et al., 2015). Moreover, telomere length from different tissues and cell types from the same individual are highly correlated within individuals from the fetal period through adulthood, suggesting PBMC telomere length is a surrogate parameter for relative telomere length in other tissues (Friedrich et al., 2000; Okuda et al., 2002; Price et al., 2013). The use of cord blood to measure newborn telomere length has been validated by others (De Carli et al., 2017; Okuda et al., 2002) and is common in the field (e.g., Entringer et al., 2015; Marchetto et al., 2016; Send et al., 2017). However, concerns have been raised that cord blood may be contaminated by maternal blood during labor or sample collection, with data suggesting that maternal DNA is present in 1–17% of

umbilical cord samples (Scaradavou et al., 1996). Thus, cord blood telomere length measures may reflect, in part, maternal telomere length. Future studies in this area may consider use of newborn blood samples (e.g., blood spots) or other sampling procedures that do not risk or can control for contamination by maternal factors. There are also limitations to using hair cortisol as a measure of HPA axis activity, as prior research has suggested a number of potential confounders of hair cortisol concentration measures (e.g., hair treatment, season and mode of delivery, health behaviors) (Braig et al., 2015; Hoffman et al., 2014; Slominski et al., 2015). We tested many of these variables and found no relations or inconsistent relations between such variables and hair cortisol concentrations in the current sample. There may be unmeasured variables that influenced the validity of the hair cortisol measure (e.g., activity level, method of hair preparation) (Gerber et al., 2013; Slominski et al., 2015). Relatedly, hair growth profiles may have varied across participants, influencing the accuracy of the timing of the cortisol assessment, particularly between individuals from different racial/ethnic backgrounds (Loussouarn, 2001; Loussouarn et al., 2005). Creating a latent variable for hair cortisol using measures across trimesters and controlling for race/ethnicity in the analyses may have mitigated any such effects. Although the current analyses accounted for cortisol levels across trimesters when data were available, analyses did not test for timing effects. Data were not available for all participants across all trimesters, and there is some evidence that hair segments dating back more than 6 months may contain lower cortisol levels due to washout effects (Dettenborn et al., 2010). Notably, cortisol levels were highly correlated across trimesters, and the measures from each trimester loaded highly on the cortisol factor in the SEM models, suggesting little variability in relative standing between individuals across trimesters. However, future studies with larger samples should test the influence of the interaction among cortisol levels, timing of exposure, and sex on newborn telomere length given evidence that male and female fetuses experience different patterns of cortisol exposure across pregnancy and that prenatal stress effects on other health outcomes vary by both timing of exposure and fetal sex (DiPietro et al., 2011; Van den Bergh et al., 2017). Hair cortisol measures were used as a proxy of fetal cortisol exposure. However, individual differences in 11β-HSD activity likely influenced the amount of maternal cortisol that crossed the placenta to the fetus (Monk et al., 2016; O'Donnell et al., 2012; Stirrat et al., 2018). Importantly, research suggests sex differences in 11β-HSD activity in response to maternal environmental exposures (Appleton et al., 2013). This study focused on hair cortisol as the assessment of maternal HPA axis functioning in pregnancy given its purported utility as a measure of long-term fetal exposure to cortisol. To date, most animal and human studies examining associations between glucocorticoid exposure and telomere length have focused on baseline circulating levels, with some calling for research on HPA axis stress reactivity and telomere dynamics (Angelier et al., 2018). Support for this position comes from studies demonstrating that telomere length is more strongly associated with cortisol stress reactivity and other dynamic aspects of cortisol production (e.g., waking response) than to basal levels or static measures of cortisol (Gotlib et al., 2015; Nelson et al., 2018). Thus, other maternal cortisol measures during pregnancy (e.g., stress reactivity, diurnal rhythms) may contribute additional information to our understanding of maternal HPA axis functioning on newborn telomere biology and should be considered in future research. Finally, prior research indicates that telomere length heritability is relatively high (Broer et al., 2013); the current study did not take into account heritability factors, including paternal factors, which may have contributed to the findings.

4.1. Conclusions

The current findings are consistent with the hypothesis that exposure to cortisol influences telomere length and are the first to demonstrate this association among newborn infants. The findings further suggest that there are sex-specific effects of maternal HPA axis functioning in pregnancy and of in utero cortisol exposure on fetal telomere biology. These findings may contribute to our understanding of mechanisms underlying sex-specific effects of maternal pregnancy risk factors on offspring health outcomes. These results may also help explain sex differences in associations between prenatal risk factors and newborn telomere length emerging in the literature (Bosquet Enlow et al., 2018). This study provides support for future research to test whether maternal HPA axis functioning in pregnancy is mechanistically responsible for previously identified associations between maternal risk factors (e.g., maternal pregnancy health, stress, psychopathology) and newborn telomere biology and highlights the necessity of considering sex effects in such studies. This research builds on our understanding of maternal-fetal processes that have long-term implications for offspring health and points to mechanisms that may contribute to documented sex differences in vulnerability to prenatal experiences for poor health and developmental outcomes.

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Declarations of interest

None.

Competing interests

None.

Contributors/Authorship

All authors participated in the design of the study and/or acquisition of data and/or analysis and interpretation of data and drafting the manuscript or revising it critically for important intellectual content. All authors provided final approval of the version submitted.

Data statement

Data will be made available upon request.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.12.222.

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