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MATERNAL PRO-INFLAMMATORY STATE DURING PREGNANCY AND NEWBORN LEUKOCYTE TELOMERE LENGTH: A PROSPECTIVE INVESTIGATION

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1 Introduction

Telomere biology plays a crucial role in genome integrity and chromosomal stability (Blackburn et al., 2006). Telomeres consist of a deoxyribonucleic acid (DNA)-protein-complex that forms the protective caps at the ends of the eukaryotic chromosomes. Telomeres shorten with every cell division cycle, constituting a well-established indicator for cellular aging processes (Blackburn et al., 2015; Blackburn and Gall, 1978; Blackburn, 2000, 2001; Blackburn, 2005). More recent evidence suggests that the integrity of telomeres affects not only the replicative capacity of the cell, but also underlies other changes that

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engender a self-perpetuating pathway of global epigenetic changes to affect the integrity of overall chromatin structure (DNA folding) that protects against senescence and cellular aging (Karlseder, 2009; O'Sullivan et al., 2010). A substantial body of research has linked shortened telomeres to age-related physiological risk factors, physical and mental disorders, and mortality (e.g. Haycock et al., 2014; Mons et al., 2017; Rehkopf et al., 2016; Rode et al., 2015; Tamura et al., 2016; Tellechea and Pirola, 2017).

Studies in animals and humans converge to provide a strong rationale for the importance of newborn and infant telomere biology in long-term health and disease risk (Entringer et al., 2018; Okuda, 2002). Telomere length (TL), at any given age during life, is a joint function of *a*) the initial (newborn) TL, and *b*) telomere attrition rate over time (Aviv, 2008). Thus, the shorter the telomere length at birth, the earlier the telomeres may reach a critical short length, sending the cell in replicative senescence and leading to the impairment of tissue functionality. Animal models of telomere dynamics *over the life span* and *across generations* suggest that *initial TL* and *TL attrition rate in early life* is *a*) a better predictor of realized life span than TL and TL attrition rate in later life (Asghar et al., 2015; Bateson et al., 2015; Heidinger et al., 2012), *b*) the effects of early life TL persist over and beyond those of risk exposures in later life (Asghar et al., 2015; Bateson et al., 2015; Heidinger et al., 2012), and *c)* TL changes are more dramatic in early life than later in life (Baerlocher et al., 2007; Fairlie et al., 2016; Frenck RW Jr et al., 1998; Rufer et al., 1999). In humans, a longitudinal multi-cohort study investigating the stability of LTL in adulthood showed that the overwhelming majority of individuals maintained their LTL ranking over a 12 year-long follow-up period (Benetos et al., 2013), suggesting that most of the inter-individual variation in adult LTL originates early in life. A similar findings has been reported in a life course study of TL dynamics in sheep (Fairlie et al., 2016).

The determinants of variation in newborn telomere length are not yet fully understood. Although the heritability of TL is high, studies to date have reported that known genetic variants associated with TL collectively account for only a small proportion of the observed variation in TL (Codd et al., 2013; Prescott et al., 2011). The mother-offspring correlation in TL is substantially larger than the father-offspring correlation (Broer et al., 2013). Shared environmental influences between the maternal and fetal compartments during pregnancy might account for this stronger maternal-offspring TL correlation, in contrast to the weaker paternal-offspring TL correlations (De Meyer and Eisenberg, 2015). Taken together, this suggests a major role for maternal effects in the initial setting of the TL system.

In support of this premise that prenatal conditions may ‘program’ the initial setting of the telomere system, several experimental and observational studies in animals and humans have, indeed, established associations between exposure to adverse intrauterine conditions (such as stress, suboptimal diet, obesity, and obstetric complications) and shorter offspring TL at birth and in adult life (Blaze et al., 2017; Entringer et al., 2012; Entringer et al., 2018; Entringer et al., 2011; Entringer et al., 2013; Haussmann et al., 2011; Martens et al., 2016; Send et al., 2017; Shalev et al., 2013; Tarry-Adkins et al., 2016). How, specifically, might the effects of such conditions be transmitted to the fetus to program offspring telomere length? The predominant proximate pathway by which maternal states and conditions influence fetal development is ultimately biological in nature, and it implicates maternal–

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placental–fetal gestational physiology. Several previously-published studies have shown that inflammation during pregnancy is related to most of the adverse conditions (listed above) that have been associated with reduced offspring telomere length (Challier et al., 2008; Coussons-Read et al., 2005; Entringer et al., 2010; Ratnayake et al., 2013; Segovia et al., 2017; Wilder, 1998). Thus, in terms of fetal programming of the telomere system, maternal-placental-fetal immune activation, characterized by an increased expression of pro-inflammatory cytokines in response to various adverse conditions during pregnancy, may have the potential to impact fetal TL (Howerton and Bale, 2012; Lin et al., 2012). In adults, a high pro-inflammatory load, as demonstrated by tumor necrosis factor (TNF)-α and interleukin (IL)-6, has been linked to shorter leukocyte telomere length (LTL, O'Donovan et al., 2011). In a recent study, *in vitro* pro-inflammatory gene expression profiles were associated with shorter TL in peripheral blood mononuclear cells (Lin et al., 2018). Inflammation can impact TL attrition through an increase in oxidative stress, and activation of transcription factors, such as *Nuclear factor-kappa B* (NF-κB, Zhang et al., 2016). Although several studies exist on the association between inflammation and TL in adults, there is a lack of research investigating the potential link between prenatal maternal inflammatory load and newborn TL.

Initial evidence supporting a pivotal role of the maternal pro-inflammatory milieu for programming of offspring TL was derived from a murine model pointing towards a specific importance of TNF-α during pregnancy in this context. In detail, the intraperitoneal administration of the pro-inflammatory cytokine TNF-α to pregnant mice induced telomere shortening in various tissues including blood cells in the offspring (Liu et al., 2016). A recent study in mice replicated these findings and supports a causal role of TNF-α-induced expression of activation transcription factor 7 (ATF7) in telomere shortening (Maekawa et al., 2018).

In the current study, we operationalized maternal pro-inflammatory state as the ratio of maternal concentrations of TNF-α and IL-10 based on the following considerations. In vivo, cytokines function as part of an elaborate and complex network of molecules that mediate the innate immune response. Recognizing this, we have previously considered cytokines in functional clusters and created a measure of pro- and anti-inflammatory cytokine balance (Simhan and Krohn, 2009). TNF-α is a potent multifunctional cytokine that plays an important role in autocrine and paracrine processes during reproduction, including placental differentiation, embryogenesis and parturition (Alijotas-Reig et al., 2017; Hunt, 1993). Elevated levels of TNF-α are related to major pregnancy complications (Kupferminc et al., 1994; Sadowsky et al., 2006; Saito et al., 2010). During pregnancy, higher perceived stress is prospectively associated with increased TNF-α concentrations in peripheral blood (Lindsay et al., 2018). Women genetically predisposed to increased TNF-α production are more likely to develop clinical infection during labor, highlighting its relevance as a risk biomarker during pregnancy (Simhan et al., 2003). TNF-α is directly secreted primarily by macrophages but also other immune cells such as natural killer cells, and antigen-stimulated T-cells, as well as by placental cells (Alijotas-Reig et al., 2017; Hunt, 1993; Olszewski et al., 2007; Vitoratos et al., 2006). In the case of inflammation, TNF-α (along with interleukin-1) has a central role in the pro-inflammatory cytokine cascade: TNF-α increases the expression of other cytokines, such as interleukin-6 and interleukin-8, and the secretion of

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adrenocorticotropic hormone in the central nervous system, induces pro-coagulant activity on endothelial cells and the production of free radicals (Cavaillon and Adib-Conquy, 2002). The overexpression of TNF-α is implicated in a number of pathological conditions related to chronic inflammation and aging (Rangel-Zuniga et al., 2016).

IL-10, a potent anti-inflammatory cytokine, is known to inhibit TNF-α production, which represents an interplay between pro-inflammatory and anti-inflammatory responses (Choy and Panayi, 2001; Moore et al., 1993). IL-10 is secreted by immune cells such as monocytes and t*h*2 T helper cells as well as by decidual cells (Ouyang et al., 2011; Trautman et al., 1997). In the context of pregnancy, IL-10 is considered the pregnancy promoting cytokine with respect to its anti-inflammatory function and the mediation of placenta and decidua signal exchange in normal pregnancy (Denney et al., 2011; Murphy et al., 2005; Thaxton and Sharma, 2010). An increase in the ratio of TNF-α and IL-10, representing the balance between pro- and anti-inflammatory cytokines, promotes complications during pregnancy such as implantation failure, fetal loss, hypertensive syndromes, and gestational diabetes (Majetschak et al., 2002; Quenby et al., 2002; Wedekind and Belkacemi, 2016; Wilder, 1998). Based on the interplay of pro-inflammatory and anti-inflammatory signaling, it is crucial to consider the relationship between TNF-α and IL-10 in the context of pro-inflammatory state during pregnancy and its effects on telomere biology of the newborn.

Thus, the aim of the present study was to examine the prospective relationship between maternal pro-inflammatory state (TNF-α/IL-10 ratio) during pregnancy and newborn LTL. We conducted a prospective, longitudinal study in a sample of healthy pregnant women from early gestation through birth until the early newborn period of life. We hypothesized that higher levels of the TNF-α/IL-10 ratio during pregnancy is associated with shorter newborn LTL after adjustment for relevant and previously-established determinants of LTL in the newborn, including maternal age (Muezzinler et al., 2013), pre-pregnancy body mass index (BMI; Kim et al., 2009; Martens et al., 2016; Muezzinler et al., 2016; Muezzinler et al., 2014), sex of the child (Factor-Litvak et al., 2016; Gardner et al., 2014; Lapham et al., 2015), birth weight, and gestational age at birth (Lee et al., 2017).

2 Materials and Methods

2.1 Participants

A prospective, longitudinal study was conducted at the University of California, Irvine's Development, Health and Disease Research Program in a population of mother-child dyads. Healthy women with a singleton pregnancy were recruited in early gestation. The study protocol included three maternal study visits during early (T1: M=13.1, SD=1.8 weeks of gestation, N=106), mid (T2: M=20.5, SD=1.4 weeks of gestation, N=112), and late pregnancy (T3: M=30.6, SD=1.4 weeks of gestation, N= 112), and a child visit for newborn blood sampling within two to four weeks after birth (M=3.4, SD=1.5 weeks postnatal age, n=98). Each study visit during pregnancy entailed the collection of a fasting maternal antecubital venous blood sample for the measurement of cytokine concentrations and a structured socio-demographic interview. Exclusion criteria included twin pregnancies, uterine, placental or cord anomalies, and fetal congenital malformations. Study visits were

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not conducted if the participant presented with an acute infection like common cold, upper respiratory infection.

The study was in accordance with the Declaration of Helsinki and approved by the UC Irvine Institutional Review Board, and all participants provided written, informed consent. The maternal sociodemographic and clinical newborn characteristics are provided in Table 1.

2.2 Measures

Sociodemographic information.—A structured sociodemographic interview was conducted by trained study personnel to obtain information about maternal age, years of education, level of education, household income, and race/ethnicity.

Obstetric risk factors and birth outcomes.—Presence of obstetric risk factors (e.g., preeclampsia, diabetes, infections, etc.) and information about birth outcomes were abstracted after delivery from medical records. The prevalence of obstetric risk factors in the current sample was low (see Table 1). Preterm birth, defined by the World Health Organization as birth at less than 37 weeks completed gestation, occurred in 5.6% of the women (n= 6). Mean gestational age at birth was 39.2 weeks (SD = 1.4, n= 108).

TNF-α and IL-10.—In order to quantify maternal serum cytokine concentrations during pregnancy, maternal antecubital venous fasting blood samples were collected in serum tubes (BD Vacutainer) at all three pregnancy visits. By using fasting blood samples we controlled for potential confounding effects of dietary intake on cytokine measurements (Zhou et al., 2010). Serum samples were allowed to clot for 30 minutes at room temperature before they were centrifuged at 4 °C at 1800 x g. Serum was then separated and stored at −80 °C. Maternal serum concentrations of TNF-α and IL-10 were quantified using a commercially-available Multiplex Bead-Based Kit, the V-PLEX Proinflammatory Panel 1 (10-Plex, Milliplex MAP Human Cytokine/Chemokine Kit; Millipore, Billerica, MA, USA) in accordance with the kit-specific protocols provided by the manufacturer. Assay sensitivity was 3.2 - 10000 pg/mL. The assay yielded reliable values for the assessed cytokines: coefficient of variation (CV)<2.97, lower limit of detection (LLD)=0.04 pg/mL for IL-10 and CV<5.54, LLD=0.06 pg/mL for TNF-α. Plates were read on a Luminex FLEXMAP 3D System and analyzed using xPONENT® software (Luminex). Cytokine distributions were inspected for outliers separately for each plate. Values for cytokine measurements below the detection limit (none for TNF-α, 1.62% for IL-10) were set to the plate-specific lowest standard concentration. Overall, TNF-α and IL-10 concentrations did not correlate significantly (rs: −.094-.101, ps>.324; for detailed information see supplement figure 1 and table 1). The Friedman test for a nonparametric repeated ANOVA indicated no significant change within the repeated cytokine measurements across pregnancy and the measures were highly correlated (rs: .999-.240, ps: <.001-.012; for detailed information see supplement figure 1 and table 1). The average cytokine concentrations across gestation were used to compute the TNF-α/IL-10 ratio across pregnancy. Table 2 in the Results section provides an overview of the cytokine concentrations for each assessment time point as well as the averages.

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Newborn Telomere Length.—Newborn Telomere Length. Neonatal DNA was isolated from whole blood obtained by heel stick and collected from the newborns within the first 4 weeks (n=98) after delivery using EDTA microtainer tubes (BD, Franklin Lakes, NJ). After plasma separation the buffy coat was removed and blood cell pellets were stored at −80°C until DNA extraction procedure. Genomic DNA was extracted from the blood cell pellets using Gentra Puregene Blood Kit (Qiagen Inc., Valencia, CA) according to the manufacturer recommended protocol. The DNA concentration was determined by optical density at 260nm and DNA purity was assessed using OD260/OD280 ratio. Measurement of relative LTL (telomere repeat copy number to single gene copy number [T/S ratio]) using quantitative polymerase chain reaction (qPCR) was performed as previously described (Cawthon, 2002; Lin et al., 2010). The qPCR LTL inter-assay coefficient of variation (CV) was 4.1%. The mean LTL at birth from whole blood as measured by the T/S-Ratio was 1.485 and ranged from .735 to 2.385. All samples were measured in one batch.

Statistical Analysis.—All statistical analyses were performed in R version 3.5.1. To examine the effect of the pro-inflammatory ratio (TNF-α/IL-10) across pregnancy on the T/S-ratios at birth we used bivariate linear regression analyses. In a second step we adjusted this association for the effects of other potential determinants of newborn telomere length (maternal age, maternal pre-pregnancy BMI, sex of the child, and birth weight percentile of the child) using multiple linear regression analysis. Because birth weight and gestational age at birth have previously been associated with shortened telomeres, we summarized these correlated measures using birth weight percentiles adjusted for gestational age (Oken et al., 2003). Birth weight percentiles capture is the birth weight adjusted for length of gestation, thereby considering collinearity rather than using birth weight and length of gestation as two highly correlated predictors in the statistical models. We selected all covariates based on previous empirical work to increase precision of the model. Scatterplots and regression diagnostics were performed to examine the potential presence of outlier-driven associations.

3 Results

Descriptive statistics of the concentrations of TNF-α, IL-10, and the pro-inflammatory ratio TNF-α/IL-10 are displayed in Table 2. For each cytokine, the concentrations were serially correlated across the three study visits (TNF-α: all $r > .206$, all $p < .001$; IL-10: all $r > .526$, all $p < .001$; pro-inflammatory Ratio TNF-α/IL-10 all $r > .462$, all $p < .001$; for detailed information see Supplement Table 1).

The bivariate linear regression analysis revealed a significant effect of the mean TNF-α/IL-10 ratio across pregnancy on newborn telomere length (β = −0.222, $p = 0.018$; R^2=.05, $F(1, 110)=5.72$, p=.018, Table 3). The results of the unadjusted and adjusted models are provided in Table 3, and a graphical representation is given in Figures 1 A and Figure 1 B. The telomere length of newborns of women in the upper quartile of the TNF-α/IL-10 ratio across pregnancy was on average 9.963% shorter compared to newborns of women in the lowest quartile of the TNF-α/IL-10 ratio (see Figure 1 B). After adjustment for maternal age, maternal pre-pregnancy BMI, child’s sex, and birth weight percentile, the effect remained significant, and unchanged in its direction and magnitude (β= - .205, $p = .030$, R^2=.10, $F(5, 106)=2.27$, p=.053, Table 3). Additionally, pre-pregnancy BMI was

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significantly and negatively associated with LTL at birth (β = - .219, p = .023, Table 3). Other covariates were not significantly associated with LTL at birth in the current study sample (all $p > .05$; Table 3).

Based on graphical inspection of the scatter plot for the associations described above, potential outlier driven effects were examined to ensure model fit and robustness of the results (see supplemental material). We conducted several measures of regression diagnostics, including the variance inflation factors/tolerances (Supplement, Table 2), a q-q-plot (Supplement, Figure 2) and an influence plot (Supplement, Figure 3). Based on our regression diagnostics, there was no indication of a concerning amount of multi-collinearity based on the variance inflation factor (VIF) in the present set of predictors. The four data points located on the right of Figure 1A were not leverage points. In summary, regression diagnostics did not provide evidence for an outlier-driven association between the pro-inflammatory ratio TNF-α/IL-10 across pregnancy and LTL at birth[1].

In a sensitivity analysis, the women with severe infection during pregnancy (N=4) were excluded to ensure that the reported association was not confounded by increased inflammation due to acute infection. The association between LTL at birth (T/S-Ratio) and pro-inflammatory ratio TNF-α/IL-10 across pregnancy remained significant and unchanged in its magnitude and direction for both the unadjusted and the adjusted (β=−0.208, p=0.030). In this sensitivity analysis, the adjusted overall model no longer reached statistical significance after exclusion of four participants (R^2=.10, F(5, 102)=2.23, p=.057; for summary of the sensitivity analysis see supplement information Table 4).

4 Discussion

To the best of our knowledge these findings represent the first report in humans linking a shift towards a pro-inflammatory state immunological state during pregnancy with shorter offspring LTL. This effect persists after adjusting for a number of potential confounders, including maternal age, pre-pregnancy body mass index, sex of the child, birth weight, and gestational age at birth. Each unit increase in the maternal mean pro-inflammatory ratio during pregnancy was linearly associated with a decrease in newborn LTL by −.205 units. The magnitude of the observed effect can be quantified as a difference of approximately ten percent in telomere length at birth between newborns of mothers in the lower and upper quartiles of the pro-inflammatory ratio during pregnancy. Thus, the study supports the growing recognition that intrauterine conditions during gestation may have "programming" effects on the development and initial setting of the telomere system.

As of our knowledge, there are no studies investigating the balance between pro-inflammatory and anti-inflammatory cytokines and LTL to this date. Importantly, we focused on the ratio rather than on a single cytokine because the period of pregnancy after implantation and before induction of labor is characterized by an anti-inflammatory immune profile (reviewed in Mor et al., 2017). Therefore, it is crucial to take potential inhibitors of

[1]Robust regressions by iterated re-weighted least squares (R package 'rlm' weighing function: Huber weights) yielded similar results (for details see supplement Table 3).

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inflammatory signaling (e.g., IL-10) into account when investigating the association between maternal pro-inflammatory state and telomere biology of the newborn. Our results are consistent with previous studies on the relationship between telomere biology and immune states. These findings are consistent with those of an animal model in which mice received an *in utero* TNF-α treatment. The offspring exposed to a prolonged pro-inflammatory state *in utero* exhibited shorter telomeres in adulthood than their unexposed counterparts (Liu et al., 2016). Our findings are also consistent with findings in human adults of cross-sectional associations between higher concentrations of pro-inflammatory cytokines, such as TNF-α, as well as C-reactive protein and shorter TL (Aviv et al., 2006; Lin et al., 2018; O'Donovan et al., 2011; Wolkowitz et al., 2011).

It is important to highlight four previously-described key molecular mechanisms that may account for the observed associations between pro-inflammatory state and shortened LTL. Firstly, inflammation and the accompanying increased proliferative activity of cells results in increased loss of telomeric repeats due to increased cell division rates. Secondly, increased TNF-α concentrations have been associated with replicative senescence, inhibition of telomerase activity, telomere shortening, and decreased hTERT expression in hematopoietic cells (Beyne-Rauzy et al., 2004). TNF-α binds to two cell surface receptors, TNFa-R1 and TNFa-R2 (Chen and Goeddel, 2002). Binding of TNF-α to TNF-R1 is known to trigger the intracellular activation of transcription factors, particularly activation transcription factor 7 (ATF7) and nuclear factor κB (NF-κB). The administration of TNF-α leads to the phosphorylation of ATF7. ATF7 protects the telomeric sequence by supporting the formation of heterochromatin. The release of ATF7 from the telomeres through TNF-α is accompanied by the release of telomerase, resulting in TNF-α-induced and ATF7-dependent telomere shortening (Liu et al., 2016; Maekawa et al., 2018). Thirdly, NF-κB activation has been associated with prolonged growth arrest and decreased telomerase activity (Akiyama et al., 2003; Akiyama et al., 2004), thereby contributing to telomeric disruptions (shortening, losses and fusions) (Beyne-Rauzy et al., 2004). A mouse model in which systematic chronic inflammation was induced by knocking out one repressive subunit of the NF-κB pathway (Jurk et al., 2014) showed accumulation of telomere dysfunctional senescent cells. Lastly, another potential molecular mechanism implicates increased oxidative stress previously linked to shortened telomeres (Aubert and Lansdorp, 2008). Chronic inflammation, through increased NF-kB activation, is known to cause excess production of free radicals and depletion of antioxidants, thus leading to heightened oxidative stress (Federico et al., 2007). (Haddad, 2002; Weisberg et al., 2003). An increase in oxidative stress is known to directly produce DNA damage in the telomere sequence via the toxic effects of free radicals (Houben et al., 2008).

How might a pro-inflammatory state be transmitted from the maternal to the fetal compartment? Studies in rodents suggest that the placenta is highly sensitive to pro-inflammatory signaling in early pregnancy, in that TNF-α acts on the placenta by binding to placental TNFa1 receptors (Carpentier et al., 2011), and the placenta produces TNF-α after being stimulated by maternal TNF-α (Racicot et al., 2014). Further, TNF-α is produced by placental tissue after lipopolysaccharide stimulation in rodents (Boles et al., 2012). In humans, maternal pro-inflammatory cytokine concentrations are correlated with cord blood pro-inflammatory cytokine concentrations, providing evidence for transmission from the

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maternal to the fetal compartment (Rakers et al., 2017; Ross et al., 2016). Elevated blood concentrations of maternal TNF-α during pregnancy have been shown to alter the inflammatory secretion profile of the placenta, which, in turn, is associated with an increase in pro-inflammatory cytokine concentrations in the fetal compartment (Siwetz et al., 2016). Furthermore, if the mother is treated with TNF-α during pregnancy, TNF-α antagonists deployed to counteract an increase in maternal TNF-α can be measured in the newborn, suggesting a possible passage through the placenta (Mahadevan et al., 2013). However, a perfusion study in human placentas examining the placental passage of TNF-α suggests only minimal direct maternal-fetal and fetal-maternal transfer of cytokines (Zaretsky et al., 2004). A second study found no evidence trans-placental TNF-α passage (Aaltonen et al., 2005). Taken together, these findings in humans and animals suggest that maternal immune signals might be transmitted indirectly through stimulation of the placental immune milieu, and may thereby have the potential to induce pro-inflammatory cytokine release in the fetal compartment. In addition, it is possible that the maternal pro-inflammatory state is a response to gestational / fetal conditions (e.g, trophoblast ischemia, decidual inflammation) that can contribute to a maternal inflammatory state supporting a bidirectional relationship between the maternal and fetal compartment.

Our study findings also suggest that the presence of a maternal clinical condition/disorder associated with inflammation (such as infection) is *not* a prerequisite for the link between maternal pro-inflammatory state and newborn telomere length described here. Moreover, previous studies in humans have demonstrated programming effects of a diverse range of prenatal conditions on offspring telomere biology, including psychosocial stress, obesity, gestational diabetes, tobacco exposure, and air pollution during pregnancy (Hjort et al., 2018; Martens et al., 2017; Oerther and Lorenz, 2018; Salihu et al., 2016; Salihu et al., 2015; Whiteman et al., 2017). We note that each of these conditions has the potential to trigger a pro-inflammatory state in the mother and suggest that inflammation may represent an underlying mediating pathway.

We suggest several features of our study represent strengths. First, we employed a prospective design. Second, by using the pro-inflammatory ratio TNF-α/IL-10 we adopted a systemic approach, because the two cytokines represent the relevant and functionally intertwined axes of the immune system – the Th_1 and the Th_2 system. The pro-inflammatory ratio TNF-α/IL-10, derived from three measurements across early, mid and late gestation, thereby provides a robust indicator of the overall pro-inflammatory state in pregnancy. Third, we measured newborn telomere length in immune cells soon after birth. Thus, these measures are unlikely to have been confounded by additional postnatal conditions or the postnatal environment.

The study also has some limitations. First, LTL was not adjusted for immune cell type. This was not feasible because the heel stick procedure does not yield enough sample volume to analyze cell population distribution. However, there is considerable synchrony in newborns in telomere length within immune cells of the hematopoietic cell lineage, as well as across tissues (Kimura et al., 2010; Okuda, 2002; Youngren et al., 1998). There is also a sufficient degree of inter-correlation between LTL of different immune cell types within a person in contrast to inter-individual variation (Lin et al., 2016). Given that hematopoietic stem cells

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ensure the life-long production of blood cells through self-renewal and differentiation into all blood lineages (including peripheral leukocytes), LTL dynamics across the course of life mimic telomere dynamics in the hematopoietic stem cells (Kimura et al., 2010; Sidorov et al., 2009), and newborn LTL may therefore represent a good indicator of general immune cell telomere dynamics. Second, our findings are based on a predominantly healthy, low-risk normative population, with a relatively low pro-inflammatory ratio. Further, future studies should examine the relationship between newborn LTL and immune profiles in high-risk obstetric populations, such as mothers with hypertension, type 2 diabetes, or preeclampsia, to compare the magnitude of effects between our population and these high-risk populations. Third, our study did not incorporate a child follow up period. Future studies would benefit from a follow-up through infancy, childhood, and beyond. Fourth, paternal age at conception is another determinant of offspring telomere length at birth (Broer et al., 2013). Although this information was not assessed in our study, we note that maternal age is typically highly correlated with paternal age (Eisenberg and Kuzawa, 2018). Fifth, we were not sufficiently powered to test a potential mediating effect of inflammatory pathways in the context of maternal stress (and other adverse prenatal exposures/conditions) and programming of offspring telomere biology which we suggest should be addressed in future studies.

To conclude, our study demonstrates a significant prospective association of maternal pro-inflammatory state during pregnancy with the initial setting of her child’s telomere system. The effect size of being ten percent shorter, for babies in the highest quartile of maternal inflammation, if maintained over time, is presumed to have clinical significance later in life. Thus, our findings add further evidence to the growing recognition that pre-disease pathways for common, complex age-related disorders may have their foundations very early in life and implicate the immune pathways as a key mediator in this context.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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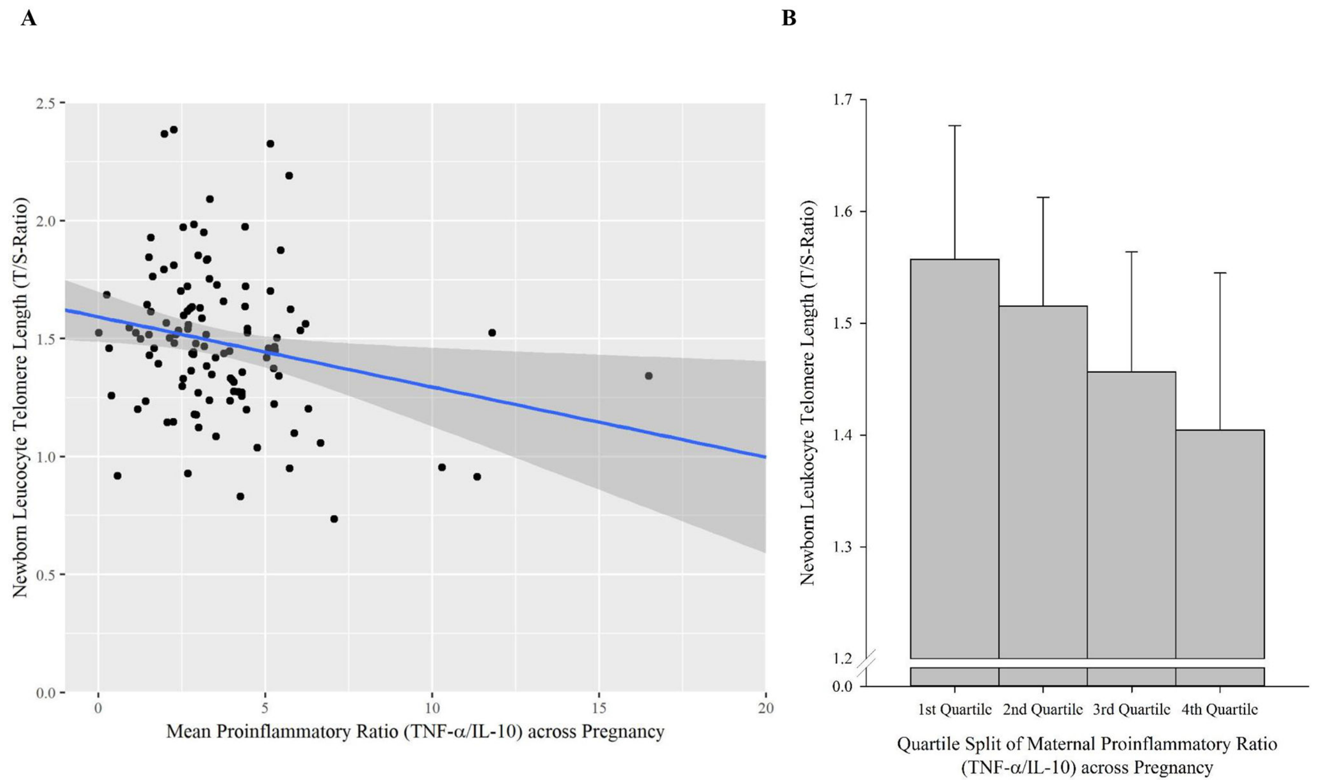

Figure 1.

A Scatterplot of the unadjusted association between mean pro-inflammatory ratio (TNF-α/IL-10) across pregnancy and newborn LTL (T/S-Ratio). Blue line is the fitted linear regression line. Shaded area around the regression line is the +/− 95% confidence interval.
B Bar chart of newborn LTL (T/S-ratio) for groups based on quartile split of maternal pro-inflammatory ratio (TNF-a/IL-10) across pregnancy. Error bars display ± 2 standard errors.

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Table 1.

Sample characteristics: maternal sociodemographic variables, obstetric risk factors, and infant outcomes.

Maternal characteristics	***N*=112**
Mean pro-inflammatory ratio TNF-α/IL-10 across pregnancy (*M* ± *SD)*	3.619 ± 2.321
Maternal age (years, *M* ± *SD*)	28.0 ± 5.4
Education (*n*, %)	
Some high school or less	23 (21.5%)
Technical or Vocational School or Certificate	12 (11.2%)
Some college or associates degree	40 (37.4%)
Bachelor’s degree or higher	32 (29.9%)
Income (USD, *n*, %)	
<15k	11 (10.2%)
15-50k	43 (39.8%)
50-100k	41 (38.0%)
>100k	13 (12.0%)
Race/ethnicity (*n*, %)	
Non-Hispanic White	42 (39.6%)
Hispanic White	41 (38.7%)
Other	23 (21.7%)
Pre-pregnancy BMI (*M* ± *SD*)	27.5 ± 6.6
Normal weight (< 25)	51 (45.6%)
Overweight (25-30)	32 (28.6%)
Obese (> 35)	29 (25.9%)
Table continues on the next page.	
Obstetric risk characteristics	
Obstetric risk factors (*n*, %)	14 (13.0%)
Severe Infections	4 (3.7%)
Preeclampsia	3 (2.7%)
Diabetes	7 (6.3%)
Parity (*n*, %)	
0	42 (39.3%)
1	29 (27.1%)
2	21 (19.6%)
>2	15 (14.0%)
Infant characteristics	*N*=112
Newborn LTL (T/S-ratio, *M* ± *SD*)	1.485 ± .311
Child’s sex (*n*, %)	
Female	51 (45.5)
GA at birth (weeks, *M* ± *SD*)	39.3 ± 1.4
Birthweight percentile (Oken et al., 2003, *M* ± *SD*)	47.24 ± 27.97
Birth length (cm, *M* ± *SD*)	49.25 ± 4.93
Birth weight (grams, *M* ± *SD*)	3354.7 ± 509.6

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Maternal characteristics	***N*=112**
Infant age at blood draw (weeks, *M* ± *SD*)	3.4 ± 1.5

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Table 2.

Median, means, interquartile range, standard deviations and range of cytokines TNF-α, IL-10 and the pro-inflammatory ratio TNF-α/IL-10 for each of the three visits (T1, T2, T3) and across pregnancy.

	M	Mdn	SD	IQR	Minimum	Maximum
TNF-α (pg/ml)						
T1	8.338	7.890	3.518	4.308	1.140	19.360
T2	8.535	7.823	3.816	5.044	1.160	23.309
T3	8.833	8.000	3.755	4.465	1.354	19.732
Mean	8.462	8.246	2.797	3.548	1.150	14.800
IL-10 (pg/ml)						
T1	14.457	2.425	106.406	1.476	.470	1028.222
T2	6.753	2.470	38.224	1.388	.750	393.451
T3	7.324	2.361	40.416	1.495	.670	405.615
Mean	8.677	2.361	57.400	1.447	.630	609.096
TNF-α/IL-10						
T1	3.788	3.285	2.936	2.750	.011	22.085
T2	3.561	2.996	2.206	2.351	.024	14.24
T3	3.790	3.405	2.404	2.781	.013	15.075
Mean	3.619	3.160	2.323	2.112	.014	16.487

Note. Sample size at each visit and across pregnancy was at T1: n=97, at T2: n=109, at T3: n=103, across pregnancy N=112.

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Table 3.

Summary of the unadjusted and adjusted models derived from linear regression analysis (*N*=112).

LTL at birth [T/S-ratio]	***B***	***SE B***	β	CI_L	CI_U	***t***	***p***
Unadjusted Model							
Intercept	1.592	.053		1.486	1.698	29.832	<.001
Mean TNF-α/IL-10	−.030	.012	−.222	−.054	−.005	−2.393	.018
Adjusted Model							
Intercept	1.799	.214		1.373	2.225	8.375	<.001
Mean TNF-α/IL-10	−.027	.012	−.205	−0.052	−0.003	−2.203	.030
Maternal age	.002	.006	.043	−0.009	0.014	.433	.666
Pre-pregnancy BMI	−.010	.004	−.219	−0.019	−0.001	−2.309	.023
Sex of the child	.000	.058	.001	−0.115	0.116	.007	.994
Birthweight percentile	−.000	.001	−.004	−.002	.002	−.035	.972

Notes. B= unstandardized coefficient, *SE B*= standard error of the unstandardized coefficient, β=standardized coefficient, CI_L 95% Confidence Interval of *B* lower bound, CI_U 95% Confidence Interval of *B* upper bound.