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Prenatal lead exposure, telomere length in cord blood, and DNA methylation age in the PROGRESS prenatal cohort

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ABSTRACT

Background: Lead is a ubiquitous pollutant with deleterious effects on human health and remains a major current public health concern in developing countries. This heavy metal may interfere with nucleic acids via oxidative stress or epigenetic changes that affect biological markers of aging, e.g., telomere length and DNA methylation (DNAm). Telomere shortening associates with biological age in newborns, and DNA methylation at specific CpG sites can be used to calculate "epigenetic clocks".

Objective: The aim of this study was to examine the associations of prenatal lead exposures with telomere length and DNA-methylation-based predictors of age in cord blood.

Design: The study included 507 mother–child pairs from the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) study, a birth cohort in Mexico City. Maternal blood (second trimester, third trimester and at delivery) and bone lead levels (one month postpartum) were measured using inductively coupled plasma-mass spectrometry and X-ray fluorescence, respectively. Cord blood leukocyte telomere length was measured using quantitative PCR and apparent age by DNA methylation biomarkers, i.e., Horvath's DNA methylation age and the Knight's predictor of gestational age.

Results: Average maternal age was 28.5 ± 5.5 years, and 51.5% reported low socioeconomic status. Children's mean telomere length was 1.2 ± 1.3 relative units, and mean DNA methylation ages using the Horvath's and Knight's clocks were −2.6 ± 0.1 years and 37.9 ± 1.4 weeks (mean ± SD), respectively. No significant associations were found between maternal blood and bone lead concentrations with telomere length and DNAm age in newborns.

Conclusion: We found no associations of prenatal lead exposure with telomere length and DNA methylation age biomarkers.

1. Introduction

Low and middle-income countries are disproportionately affected by environmental exposure to toxic substances and compounds (Mamtani et al., 2011; Trasande et al., 2011). There are specific stages of increased vulnerability to such exposures that lead to alterations in human health. Specifically, the prenatal stage is highly susceptible and a critical stage where exposure to contaminants causes various biological alterations (Grandjean, 2013; Rosa et al., 2019).

Lead (Pb), a common neurotoxic heavy metal and natural constituent of the Earth's crust, enters the human environment through anthropogenic activities (Chin-Chan et al., 2019). The main sources of Pb exposure include: drinking water, soil, air, food, cigarettes, industrial processes, and household resources, as well as gasoline, home paints,

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batteries, pipes, pewter utensils, glazed ceramics, cosmetics, toys, traditional medicines, dust, mineral fertilizers, pesticides, and others (Fralick et al., 2016; Engwa et al., 2019; Obeng-Gyasi, 2019). Pb is a ubiquitous pollutant in the environment with deleterious effects on human health (Lermen et al., 2021). While Pb exposure levels have declined globally in recent decades (CDC. Centers for Disease Control and Prevention. QuickStats, 2016; Mielke et al., 2019; ATSDR, 2020), Pb remains a major current public health concern, especially in developing countries such as Mexico (Liu et al., 2019).

Intrauterine Pb exposure has been associated with an alteration in prenatal programming with adverse outcomes during pregnancy (Nye et al., 2016; Rygiel et al., 2021). It has even been shown that Pb exposure can have an impact on the maternal-fetal interface, and it is also known that this heavy metal can cross the placental barrier (Hu et al., 2006; Taylor et al., 2016). Pb may interfere with nucleic acids via oxidative stress or epigenetic changes that affect gene silencing in DNA regions that impact pathophysiological changes leading to premature aging (Senut et al., 2012; Khalid and Abdollahi, 2019). Pb exposure also affects coding of DNA repair, cell cycle, and metabolic enzymes as well as the organization of chromatin, DNA methylation profile, and telomere shortening (Banfalvi et al., 2012; Pawlas et al., 2015; Montrose et al., 2020).

Increased production of oxidative stress may be one biological pathway linking prenatal Pb exposure with the shortening of telomeres in newborns; because telomeres have a high guanine content, they are more susceptible to attack by free radicals (Zota et al., 2015; Wai et al., 2020). Studies have shown that reactive oxygen species can cause single-strand breaks in the telomere regions; which have a deficient repair system for this type of damage; additionally, oxidative stress has been associated with a decrease in the activity of telomerase reverse-transcriptase, which is highly expressed during gestation and is responsible for adding repeats of telomeric DNA to the chromosome ends (Houben et al., 2008; Coluzzi et al., 2014; Rosen et al., 2020). Therefore, Pb exposure, via these alterations in biological processes, may accelerate aging at both the cellular and molecular levels. Given that early life is a critical window for telomere dynamics and epigenetic programming, it is essential to understand how environmental factors influence the initial setting of these biomarkers (Flora et al., 2008; Vaiserman et al., 2018; Cowell et al., 2020).

Telomeres are composed of repetitive sequences rich in guanine located at the ends of chromosomes. They play an important role in maintaining genomic integrity and cell proliferation (Epel et al., 2004). Critical shortening of telomeres can cause genomic instability, a primary factor in the development of diseases related to aging, and can increase the risk of developing cancer (Lindqvist et al., 2015; Yeh and Wang, 2016; Møller et al., 2018). Telomeres are susceptible to oxidative stress because of their high guanine content and their inefficient single-strand break repair system (Wang et al., 2010). Telomere length (TL) is longest at birth and shortens with progressive aging. TL is considered a biomarker of chronological aging and age-related morbidity as affected by cell division (von Zglinicki and Martin-Ruiz, 2005; Rizvi et al., 2014; Vaiserman and Krasnienkov, 2021). Shortened telomeres in embryonic and fetal tissues may indicate early aging of newborns (Menon et al., 2012, 2016; Rosa et al., 2019), and select toxic metals may affect TL (Herlin et al., 2019).

Epigenetic modifications are associated with aging and age-related diseases (Bohlin et al., 2016; Javed et al., 2016; Vyas et al., 2019). Methylation levels from specific CpG sites have been used to construct "epigenetic clocks" reflecting biological age. The difference between epigenetic and chronological age can predict all-cause mortality and multiple age-associated diseases (Ashapkin et al., 2019). The most widely used method for estimating epigenetic age is the Horvath clock. This epigenetic clock, based on measurement of biological age, can be used in various cell types. More recently, Knight et al. (2016) built a DNA methylation (DNAm)-based predictor of gestational age from cord blood samples. This calculator of epigenetic gestational age uses 148 CpG sites that strongly correlate with gestational age at birth.

Epigenetic clocks may reflect biological development of the organism (Breitling et al., 2016; Horvath and Raj, 2018; Everson et al., 2019). DNAm age acceleration has been linked to exposures to environmental contaminants during early life, to high levels of maternal stress, and to pregnancy complications (Simpkin et al., 2016; Bell et al., 2019; Palma-Gudiel et al., 2019). Girchenko et al. (2017) observed that DNAm age acceleration correlates with adverse effects on newborns, e.g., lower birth weights, heights at birth, head circumferences at birth, placental weights, and 1-min Apgar scores. Regarding prenatal Pb exposure, Javed et al. (2016) found no significant differences between quartiles of prenatal Pb levels and DNAm age in newborns using the Horvath clock; however, Pb exposure during pregnancy has been associated with alterations in prenatal programming, with adverse effects occurring at the cellular and molecular levels in infants. Despite this, information about the prenatal influence of Pb on aging biomarkers is still scarce (Zhang et al., 2012; Cowell et al., 2020), and few studies, to our knowledge, have evaluated associations between prenatal Pb exposure and TL or DNAm age in newborns. Therefore, we examined whether exposure to Pb during pregnancy associates with cord blood leukocyte telomeres and DNAm age at birth.

2. Methods

2.1. Study population

This study included mother–child pairs from the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) study (Burris et al., 2013; Rodosthenous et al., 2017), a prospective birth cohort that recruited participants between December 2007 and July 2011. The eligibility criteria were: women from Mexico City with conceptuses at gestational age <20 weeks, ≥ 18 years old, expectation to live in Mexico City for the next 3 years, completed primary education, without medical history of heart or kidney diseases, and did not consume alcohol daily nor use any steroid or anti-epilepsy medications. Of the 1054 participants, the current analyses included 507 mother–child pairs, those with available maternal blood and bone Pb levels and cord blood DNA samples for TL quantification and DNA methylation assays.

At enrollment and follow-up visits (second trimester, third trimester, and delivery), participant information was collected via questionnaire, including maternal age, smoking status, environmental smoking status, alcohol consumption, educational level, marital status, parity, and socioeconomic status (SES). Women enrolled in this cohort voluntarily provided written informed consent. This study was approved by institutional review boards at the Harvard School of Public Health, Icahn School of Medicine at Mount Sinai, and the Mexican National Institute of Public Health.

2.2. Covariates

The selection of covariates for multivariate linear regression analyses was performed based on previous studies that have reported their influence on the outcomes (Rosa et al., 2017; Dolcini et al., 2020); additionally, the variables that showed a correlation with the TL and DNAm age were taken into account. The information of the covariates was obtained from the questionnaires applied during pregnancy. The mother's age and child's sex were collected at delivery. The calculation of gestational age was performed on self-reported last menstrual period and the Capurro method. The socioeconomic status classification was carried out based on the guidelines established by the Asociación Mexicana de Agencias de Investigación de Mercados y Opinión Pública (AMAI) (Carrasco, 2002); that were collapsed into a three-level index of low, medium and high. The body mass index was calculated by dividing the weight by the height squared (kg/m^2) for the second trimester, third trimester and at delivery; meanwhile, the information about the

secondhand smoking (binary variable) was obtained during the second and third trimester during pregnancy. The blood cell types were measured through peripheral blood collected by trained research staff using the Coulter ACT-5 DIFF Hematology Analyzer (Beckman Coulter Inc.).

2.3. Maternal blood lead measurements

Maternal blood samples were collected during the second trimester, third trimester, and delivery in trace metal-free tubes and stored at −20 °C. Samples were thawed on ice, and 1 mL digested with 1 mL of nitric acid (HNO_3) for 48 h and diluted in 10 mL of deionized water after addition of 0.5 mL of hydrogen peroxide (30%). Digested samples were analyzed in an Agilent 8800 ICP Triple Quad (ICP-QQQ) instrument (Agilent Technologies, Inc., Delaware, USA) in MS/MS mode with Lutetium as the internal standard. This procedure was carried out with an external calibration and seven calibration points; additionally, quality controls and quality assurances were taken into account in the experiments. The recovery rates for quality control standards and spiked samples were between 85% and 115%, and the precision parameter (given as % relative standard deviation) was <10% for samples with concentrations above the limit of quantitation. The limit of detection for Pb by this procedure was 0.065 μg/dL and limit of quantitation was 0.22 μg/dL.

2.4. Maternal bone lead measurements

To quantify chronic Pb exposure, maternal tibia (cortical bone) and patella (trabecular bone) measurements were determined one month postpartum with a K-shell X-ray fluorescence instrument (Hu et al., 1991; Renzetti et al., 2017; Heiss et al., 2020). In each leg, measurement of Pb concentration was evaluated for 30 min, and the final estimate was calculated as the average of both measurements weighted by the inverse of the measurement error. Tibia Pb measurements are estimated to reflect periods of exposure >10 years, while patella measurements reflect exposures between 1 and 5 years (Hu et al., 1998). Because the values of true bone Pb concentrations were sometimes nearly 0, negative values were obtained during measurements, as the instrument produces a continuous unbiased point estimate that fluctuates around the true value of bone Pb (Kim et al., 1995). However, if negative values are set to zero, the relative position of each participant within the study population would be lost; therefore, negative values were retained to maintain the true shape of distribution of the measures in the study population (Téllez-Rojo et al., 2004; Renzetti et al., 2017).

2.5. Leukocyte telomere length assay

Total blood samples from newborns were obtained from cord bloods at the time of delivery and stored in PAXgene Blood DNA Tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland). DNA extraction and quantification was performed as described previously (Rosa et al., 2017; Heiss et al., 2020). Briefly, for an initial batch of samples, DNA extraction was performed using the QIAamp DNA Blood Kit (QIAGEN), and the DNA stored at −80 °C. For remaining samples, the conventional phenol–chloroform method of extraction was used after red blood cell lysis by a second laboratory, and the DNA stored at 4 °C. After normalization and quantification, 503 samples had enough DNA to measure leukocyte telomere length (LTL) using the qPCR method described previously (Cawthon, 2002; Pavanello et al., 2011).

Quantification of relative TL was performed by duplex quantitative polymerase chain reaction (qPCR) through the ratio of telomere repeat copy number to single gene (albumin) copy number (T/S ratio). This test used iQ SYBR Green Supermix, which contains an antibody-mediated hot-start iTaq DNA polymerase and a passive reference dye, fluorescein. Primer sequences and amplification conditions for qPCR were as described previously (Rosa et al., 2019).

Each sample was run in triplicate, and a pooled quality control (QC) sample normalized to 2 ng/μL was run on each plate. The QC sample was made with an equal mass of total DNA from all samples. The standard curve was made from the same study-specific pooled sample starting at 30 ng/μL. The T/S ratio was calculated from Bio-Rad software using the study-specific standard curve (Cq = slope * Log 10 (Sq) + intercept). To determine variation in the measurements, the coefficient of variation (CV) was calculated from each sample triplicate with a threshold of 16.3, which was established by the inter-quartile range. We found no significant difference in TL measurements based on the extraction method (Levene test $p > 0.3$).

2.6. DNAm measurement and methylation clocks

DNA methylation was measured from DNA extracted from whole umbilical cord blood collected from newborns. Quantification of DNAm was carried out on the Infinium MethylationEPIC Beadchip (EPIC, Illumina, Inc., San Diego, CA). Samples and a subset of technical replicates were randomized with respect to bisulfite conversion plate, chip, and position within a chip. The experiments took into account several quality controls evaluated using the *ewastools* R Package as described previously (Heiss and Just, 2018). A total of 420 cord blood samples and 16 technical replicates passed quality control. Unreliable measurements due to low fluorescence were filtered following the method described by Heiss and Just (2019), with a detection *p*-value threshold of 0.01, which is stringent to a cut-off around $1e\text{-}^{40}$ when using *p*-values provided by the Illumina GenomeStudio Software. The method of REgression on Logarithm of Internal Control probes (RELIC) was used to correct the dye bias of fluorescence intensity (Xu et al., 2017) and converted into methylation levels (β-scale). DNAm age was calculated using Horvath's publicly available online calculator (https://dnamage.genetics.ucla.edu/home) as well as the approach for estimating gestational age based on DNAm data published by Knight et al. (Horvath, 2013; Knight et al., 2016).

Regarding the determination of the epigenetic clock of Knight et al. (2016), the data for 6 CpG sites of the 148 CpG sites used by Knight et al. for the calculation of their epigenetic clock were lost; therefore, the calculation of this epigenetic clock was performed with the 142 CpG sites that passed quality control.

2.7. Statistical analysis

We examined the relationship of maternal blood and bone Pb concentrations with LTL and DNAm age through multivariable linear regression. These analyses included mothers who had at least one Pb measurement at any time during pregnancy. For blood Pb, we averaged Pb levels across pregnancy. In multivariable linear regression models, we included the mother's age, child's sex, gestational age at birth, SES, second-hand smoking, maternal body mass index (BMI), and blood cell type composition (percentages of monocytes, basophils, lymphocytes, eosinophils, and neutrophils) as covariates. We did not adjust for maternal active smoking, as only a few women (n = 3) reported smoking.

We used Multiple Imputations Chained Equations (MICE) with to impute missing covariate data with 5 iterations using all available variables in each model as predictors (van Buuren and Groothuis-Oudshoorn, 2011). For LTL multivariable models, there were some cases missing gestational age at birth, sex of the child, mother's age, and maternal BMI. For the DNAm age models, percentage of monocytes, eosinophils, neutrophils, basophils, maternal BMI, and second-hand smoking had missing values (Table 1S). R version 4.1.0 was used for all statistical analyses, and statistical significance was defined as *p*-values <0.05.

Table 1
General characteristics of the study population.

Variable	Mean ± SD or n (%)
Maternal characteristics	
Age	28.5 ± 5.5
BMI 2nd trimester (kg/m^2)	26.9 ± 4.3
BMI 3rd trimester (kg/m^2)	29.5 ± 4.3
Educational level (years)	
<High school	200 (39.5)
High school	187 (37.0)
>High school	120 (23.7)
Socioeconomic status	
Low	261 (51.5)
Medium	187 (36.9)
High	59 (11.6)
Exposure to second-hand smoking 2nd trimester	150 (29.7)
Exposure to second-hand smoking 3rd trimester	91 (20.3)
Newborn characteristics	
Sex	
Male	279 (55.1)
Female	227 (44.9)
Gestational age at birth (weeks)	38.4 ± 1.9
Preterm birth (<37 weeks)	52 (10.3)
≥37 weeks	454 (89.7)
Birth weight (kg)	3.1 ± 0.5
Telomere length (relative units)	1.2 ± 1.3
DNA methylation age-Horvath clock (years)	−2.6 ± 0.1
DNA methylation gestational age-Knight clock (weeks)	37.9 ± 1.4

Body mass index (BMI) according to the World Health Organization (WHO). SD: standard deviation. BMI—we added 2 kg to traditional cut-off points to account for normal weight gain in the first trimester of pregnancy, as previously reported (Rasmussen and Yaktine, 2009; Burris et al., 2015).

3. Results

The study population comprised 507 mother–child pairs. Table 1 shows the population's general characteristics. Mean maternal age was 28.5 ± 5.5 years, mean third-trimester maternal BMI was in the overweight range, but that for the second trimester was within the normal range (Rasmussen and Yaktine, 2009; Burris et al., 2015). Overall, 39.5% of the study population had an education level of less than high school, and the majority of participants reported a low SES (51.5%) calculated according to the index created by AMAI (Carrasco, 2002; Rosa et al., 2017). Few (n = 3) women reported active smoking, but 29.7% of mothers reported exposure to second-hand smoke in the second trimester of pregnancy, and 20.3% did so for the third trimester. About half (55.1%) of newborns were male. The average gestational age at birth was 38.4 weeks, and only 10.3% of newborns were premature, defined as delivery before 37 completed weeks of pregnancy. The average birthweight was 3.1 kg. Mean LTL was 1.2 ± 1.3 relative units, and the means for DNAm age using the Horvath and Knight clocks were −2.6 ± 0.1 years and 37.9 ± 1.4 weeks (mean ± SD), respectively.

Table 2 shows the distribution of maternal blood and bone Pb concentrations. The geometric mean for blood Pb levels was 3.08 μg/dL (95% CI: 2.92, 3.24 μg/dL) in the second trimester, 3.12 μg/dL (95% CI: 2.94, 3.31 μg/dL) in the third trimester, and 3.33 μg/dL (95% CI: 3.13, 3.55 μg/dL) at delivery. The geometric mean of Pb levels was 4.96 μg/g (95% CI: 4.54, 5.40 μg/g) for tibia and 5.41 μg/g (95% CI: 4.92, 5.95 μg/g) for patella. Blood Pb levels at the different timepoints were highly correlated ($p < 0.01$, Table 2S).

Table 3 presents unadjusted and covariate-adjusted regression analysis of the relationship between maternal blood and bone Pb concentrations and cord blood LTL in newborns. Pb levels in maternal blood and bone were not associate ($p > 0.05$) with cord blood LTL in multivariable regression models. Similar results were obtained in LTL multivariable analyses performed without multiple imputations (Table 3S). Table 4 shows unadjusted and covariate-adjusted regression analysis of maternal blood and bone Pb concentrations with DNAm age calculated by the Horvath clock. Similar to LTL, there were no statistically significant associations. Further, these results were consistent with the data obtained in DNAm age multivariate models carried out without multiple imputations (Table 4S).

Results for the Knight's epigenetic clock are shown in Tables 5 and 5S. In the unadjusted models, maternal concentration of Pb in blood at delivery showed a marginal positive association with DNAm age in newborns (β = 0.05; 95% CI: 0.01, 0.10; $p = 0.09$); however, this

Table 3
Association of lead levels in maternal blood at pregnancy and of lead levels in bone one month postpartum with cord blood telomere length.

Lead exposure	N	β	95% confidence interval	*p-value
Unadjusted				
Maternal blood				
2nd trimester levels	475	−4.43e-03	−0.02, 0.01	0.58
3rd trimester levels	424	4.03e-03	−0.01, 0.02	0.60
At delivery levels	471	6.01e-03	−7.50e-03, 0.02	0.38
Overall average	500	1.30e-03	−0.02, 0.02	0.88
Maternal bone, one month postpartum				
Tibia levels	364	1.54e-03	−3.74e-03, 6.82e-03	0.57
Patella levels	361	−2.23e-03	−7.99e-03, 3.54e-03	0.45
Adjusted				
Maternal blood				
2nd trimester levels	475	−6.53e-03	−0.02, 0.01	0.42
3rd trimester levels	424	2.78e-03	−0.01, 0.02	0.72
At delivery levels	471	5.64e-03	−0.01, 0.02	0.42
Overall average	500	1.59e-05	−0.02, 0.02	0.99
Maternal bone, one month postpartum				
Tibia levels	364	7.30e-04	−4.69e-03, 6.15e-03	0.79
Patella levels	361	−4.15e-03	−0.01, 1.89e-03	0.18

*p-values were obtained from linear regression models. $p<0.05$ was considered statistically significant. The models were adjusted for the mother's age, child's sex, gestational age at birth, socioeconomic status, percentage of monocytes, and maternal body mass index.

Table 2
Sample size and distribution of lead concentrations in maternal blood at pregnancy and in bone at one month postpartum.

Lead levels	n	GM	95% CI	Minimum	Percentile			Maximum
					25th	50th	75th	
Maternal blood								
2nd trimester (μg/dL)	481	3.08	2.92, 3.24	0.75	2.00	2.88	4.46	17.81
3rd trimester (μg/dL)	429	3.12	2.94, 3.31	0.02	2.03	3.02	4.85	28.25
At delivery (μg/dL)	476	3.33	3.13, 3.55	0.00	2.17	3.27	5.20	22.74
Maternal bone at one month postpartum								
Tibia (μg/g)	369	4.96	4.54, 5.40	−32.60	−1.55	3.22	7.34	29.70
Patella (μg/g)	366	5.41	4.92, 5.95	−15.90	−0.78	3.74	8.89	40.22

GM: geometric mean; 95% CI: 95% confidence interval.

Table 4
Association of lead levels in maternal blood at pregnancy and of lead levels in bone one month postpartum with Horvath's DNA methylation age.

Lead exposure	N	β	95% confidence interval	*p value
Unadjusted				
Maternal blood				
2nd trimester levels	342	1.81e-03	−0.01, 4.00e-03	0.53
3rd trimester levels	313	−5.11e-04	−0.01, 0.01	0.86
At delivery levels	347	2.64e-03	−3.00e-03, 0.01	0.35
Overall average	363	−1.20e-04	−6.30e-03, 6.06e-03	0.97
Maternal bone, one month postpartum				
Tibia levels	279	−1.21e-04	−1.91e-03, 1.67e-03	0.89
Patella levels	277	1.11e-03	−8.08e-04, 3.02e-03	0.26
Adjusted				
Maternal blood				
2nd trimester levels	342	−1.50e-03	−0.01, 4.08e-03	0.60
3rd trimester levels	313	−4.32e-04	−0.01, 0.01	0.88
At delivery levels	347	1.39e-03	−4.28e-03, 0.01	0.68
Overall average	363	−1.17e-03	−7.20e-03, 4.86e-03	0.70
Maternal bone, one month postpartum				
Tibia levels	279	−8.52e-04	−2,81e-03, 1.11e-03	0.40
Patella levels	277	5.31e-04	−1.59e-03, 2.65e-03	0.62

*p-values were obtained from linear regression models. $p < 0.05$ was considered statistically significant. The models were adjusted for the mother's age, child's sex, gestational age at birth, socioeconomic status, smoke exposure, maternal body mass index, and cell type percentages (of monocytes, basophils, eosinophils and neutrophils) in peripheral blood.

Table 5
Association of lead levels in maternal blood at pregnancy and of lead levels in bone one month postpartum with the Knight's methylation-based predictor of gestational age.

Lead exposure	N	β	95% confidence interval	*p value
Unadjusted				
Maternal blood				
2nd trimester levels	342	7.06e-03	−0.05, 0.06	0.81
3rd trimester levels	313	0.01	−0.04, 0.07	0.67
At delivery levels	347	0.05	−7.56e-03, 0.10	0.09
Overall average	363	0.03	−0.04, 0.09	0.41
Maternal bone, one month postpartum				
Tibia (μg/g)	279	0.01	−4.34e-03, 0.03	0.15
Patella (μg/g)	277	8.46e-03	−8.65e-03, 0.03	0.33
Adjusted				
Maternal blood				
2nd trimester levels	342	0.02	−0.03, 0.06	0.47
3rd trimester levels	313	5.90e-03	−0.04, 0.05	0.79
At delivery levels	347	0.01	−0.03, 0.05	0.50
Overall average	363	0.01	−0.03, 0.06	0.58
Maternal bone, one month postpartum				
Tibia levels	279	0.01	−2.63 e−03, 0.02	0.12
Patella levels	277	6.45e-03	−8.10e-03, 0.02	0.39

*p-values were obtained from linear regression models. $p < 0.05$ was considered statistically significant. The models were adjusted for mother's age, child's sex, gestational age at birth, socioeconomic status, smoke exposure, maternal body mass index, and cell type percentages (of monocytes, basophils, eosinophils and neutrophils) in peripheral blood.

association was not observed in adjusted models (β = 0.01; 95% CI: 0.03, 0.05; $p = 0.50$).

4. Discussion

Pb is a neurotoxic pro-oxidant metal that can cross the placental barrier and reach the fetus. The adverse effects of Pb on child health and development are well documented (Wani et al., 2015; Dórea, 2019; Montrose et al., 2020). However, the mechanisms underlying these effects remain unclear. In particular, it is not known if Pb exposure causes early acceleration of aging. The results of this study showed no association between prenatal exposure to Pb and well-characterized biomarkers of aging in cord blood.

There is currently no consensus on the association of Pb and LTL in newborns. Wai et al. (2018) evaluated the association of maternal urine concentrations of multiple metals in the third trimester and LTL in Myanmar and found no association between prenatal Pb levels and LTL. Conversely, Cowell et al. (2020) reported an association between urinary prenatal Pb levels (mean ± SD: 0.45 ± 0.44 ng/mL) and relative LTL in newborns. Similarly, studies of Polish children and adults found that LTLs decrease as blood Pb concentrations increase (Pawlas et al., 2015, 2016). In contrast, Wai et al. (2018) reported for a Myanmar population a urine Pb concentration of 1.8 μg/g creatinine (interquartile range: 1.0–3.2 μg/g creatinine); they did not obtain an association between Pb levels and TL in newborns. An association between prenatal Pb exposure and LTL in newborns is expected as several studies have reported that Pb promotes production of reactive oxygen species with an increase in cellular oxidative stress, one of the main factors influencing TL variation and a factor that alters proteins involved in telomere maintenance (Patra et al., 2011; Pottier et al., 2013; Zota et al., 2015). Our findings may differ from previous studies, possibly due to variations in the degree of exposure to Pb, as well as the frequency and duration of exposure in the differing study populations. Unfortunately, a direct comparison of maternal Pb levels is difficult because previous studies of the maternal Pb–newborn LTL relationship used urinary Pb measures (Wai et al., 2018; Cowell et al., 2020), with variations in reported values depending on the analytical method used for measurement; as well as the final units used in each study. In this regard, it has been previously reported that the measurement of Pb in total blood is the most reliable biomarker with the best capacity to show the level of acute exposure among individuals; while urine determinations tend to be more imprecise in exposures to low concentrations of Pb (Barbosa et al., 2005; Sommar et al., 2014).

In relation to the quantification of Pb in blood, Herlin et al. (2019) reported a concentration of maternal Pb in blood of 21 μg/L (range: 6.9–99 μg/L); also determined the Pb concentration in the infant through the quantification in placenta and cord blood, with the following results: 5.8 μg/kg (range: 1.2–38 μg/kg) and 14 μg/L (range: 6.3–60 μg/L); respectively. They found a negative association between the cord blood Pb concentration and the TL in the male newborns measured in this same matrix; however, no results were reported between maternal Pb concentration and TL variation in the newborns. This is consistent with studies suggesting that male children may be more susceptible to neurotoxic Pb exposure during pregnancy (Llop et al., 2013; Joo et al., 2018). In the present study, the sex of the child did not influence the relationship between maternal Pb levels and TL in newborns (data not shown).

To our knowledge, this study is one of only two studies to evaluate the possible toxic effects of prenatal exposure to Pb on DNAm age in newborns. In the present study, there were no significant associations between DNAm age in cord blood and maternal Pb concentrations in blood or bone. Our findings are consistent with those of Javed et al. (2016), who did not find significant differences in Horvath's DNAm age across quartiles of Pb levels. Although current information on the variation in DNAm age by exposure to environmental pollutants is scarce, epidemiological studies have shown that exposure to adverse environmental events in the prenatal period predicts an increased risk of age-related diseases (Langie et al., 2012; D'Onofrio et al., 2013; Girchenko et al., 2017).

Additionally, there are studies that have evaluated the epigenome-wide association (EWAS) in newborns with prenatal lead exposure, and have identified several biologically relevant CpG sites associated with prenatal exposure to this heavy metal (Wu et al., 2017; Zeng et al., 2019; Park et al., 2021). In addition, it is important to note that in a previous epigenome-wide association study in this same birth cohort

from Mexico City, not found associations between Pb prenatal exposure and cord blood DNA methylation (Heiss et al., 2020). However, none of these studies have focused on determining the possible associations of prenatal Pb exposure to CpG sites specifically related to cellular aging in newborns; therefore, we consider it is important to strengthen this point in future studies; as well as to determine if these results of analysis in EWAS would relate to the information obtained by DNAm age.

Moreover, in comparison between blood and bone Pb levels found in this study with a previous studies carried out in the Mexican population (Hernández-Serrato et al., 2006, Caravanos et al., 2014; Jansen et al., 2018; Liu et al., 2019), it could be shown that the concentrations found in this study could be considered relatively low, which might suggest that the level of Pb exposure in this study population was not sufficient to evidence an effect on TL and DNAm age in the newborns. Nevertheless, it is important to note that, due to the limited number of studies that have evaluated the effect of prenatal Pb exposure on these outcomes, it is difficult to establish at which concentrations of this heavy metal these adverse effects could be evidenced for this type of population.

This study has some strengths and weakness. It is, to our knowledge, the first to evaluate two ageing biomarkers, i.e., TL and DNAm age for an effect of prenatal Pb exposure. We also used two different DNAm clocks and leveraged a relatively large population study with data on socio-demographic and anthropometric characteristics, allowing us to control potentially relevant confounding factors. However, generalization of these results could be limited due to composition of the cohort, which consisted of Mexican women belonging mainly to a low SES; because previous studies in the Mexican population have shown that the exposure sources, as well as the type of exposure to this metal differ according to the socioeconomic characteristics of the study population (Farias et al., 1996; Tellez-Rojo et al., 2020).

In summary, we analyzed the influence of prenatal exposure to Pb on biomarkers of aging in newborns, i.e., TL and DNAm age, and found no association between maternal Pb exposure at any stage of pregnancy with these markers. Considering the importance of the prenatal period for establishing telomere length and epigenetic marks that may influence aging (Flora et al., 2008; Vaiserman et al., 2018; Cowell et al., 2020), and due to the limitation of studies, we consider that it is necessary to develop more studies to understand the impact of different environmental pollutants on LTL and DNAm age in newborns.

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Human subjects

This study was approved by institutional review boards (IRBs) at the Harvard School of Public Health, Icahn School of Medicine at Mount Sinai (IRB protocol number: 12–00751), and Mexican National Institute of Public Health (IRB protocol number: 560).

Declaration of competing interest

The authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112577.

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