Telomere length in newborns is associated with exposure to low levels of air pollution during pregnancy

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ABSTRACT

Telomere length (TL) is a biomarker of biological aging that may be affected by prenatal exposure to air pollution. The aim of this study was to assess the association between prenatal exposure to air pollution and TL in maternal blood cells (leukocytes), placenta and umbilical cord blood cells, sampled immediately after birth in 296 Danish mother-child pairs from a birth cohort. Exposure data was obtained using the high-resolution and spatial-temporal air pollution modeling system DEHM-UBM-AirGIS for PM2.5, PM10, SO2, NH3, black carbon (BC), organic carbon (OC), CO, O3, NO2, and NOx at residential and occupational addresses of the participating women for the full duration of the pregnancy. The association between prenatal exposure to air pollutants and TL was investigated using distributed lag models. There were significant and positive associations between TL in umbilical cord blood cells and prenatal exposure to BC, OC, NO2, O3, and CO during the second trimester. TL in umbilical cord blood was significantly and inversely associated with prenatal exposure to PM2.5, BC, OC, SO2, NH3, CO and NOx during the third trimester. There were similar inverse associations between TL from umbilical cord blood cells and air pollution exposure at the residential and occupational addresses. There were weaker or no associations between air pollution exposure and TL in placenta tissue and maternal blood cells. In conclusion, both the second and third trimesters of pregnancy are shown to be sensitive windows of exposure to air pollution affecting fetal TL.

1. Introduction

Maternal air pollution exposure is associated with adverse pregnancy outcomes, such as preterm birth (Porier et al., 2015; Qian et al., 2016), low birth weight (Clemens et al., 2017; Pedersen et al., 2013; Voriuflu et al., 2015), and stillbirth (Siddika et al. 2016), with chronic inflammation and oxidative stress as proposed mechanisms of action (Kannan et al. 2006). One outcome of cellular oxidative stress is oxidation of DNA, which may cause both mutations and shortening of telomeres. The telomere region in the human genome comprises of non-coding repetitive -TTAGGG- sequences in DNA forming a loop in a sheltering protein complex that protects the end of the chromosomes from degradation, recombination and end-to-end fusion (Blackburn, 2001; Blackburn et al., 2015; Møller et al., 2018). However, telomeres shorten with each DNA replication and may reach a critical limit where the cell undergoes replicative senescence or apoptotic cell death. The telomere length (TL) therefore determines the lifespan of a cell and is regarded as a biomarker of cellular aging (Benetos et al., 2001; Blackburn et al., 2015; Shammas, 2016).
2011). There is strong evidence indicating that oxidative stress and inflammation accelerate the age-dependent telomere shortening (Fyhrquist et al., 2013; Zhang et al., 2013). As air pollution exposure generates inflammation, oxidative stress and oxidatively damaged DNA in the human body (Risom et al. 2005), it is likely that the TL may become shorter in people exposed to air pollution. Indeed, earlier studies have shown inverse associations between TL in leukocytes from adults and air pollutants, such as particulate matter (PM) with aerodynamic diameter \( \leq 2.5 \mu m \) (PM\(_{2.5}\)) (Pieters et al. 2016), ambient black carbon (BC) (McCracken et al. 2010), traffic-generated air pollution (Hoxha et al. 2009), and indoor air pollution (Lin et al. 2017). However, only few studies have been published regarding associations between TL and air pollution exposure during in utero life. Two studies performed in Flanders, Belgium have reported that prenatal exposure to relatively low levels of air pollution is related to shorter TL in both placenta tissue and umbilical cord blood cells; placenta TL was found to decrease with increasing exposure to traffic related air pollution during the pregnancy (Bijnens et al. 2015), and placenta TL and umbilical cord blood cell TL were found to be inversely associated with PM2.5 exposure during midgestation (Martens et al. 2017). Another study from Wuhan city, China where the air pollution levels are much higher, reported inverse associations between TL in umbilical cord blood cells and PM\(_{2.5}\), SO\(_2\), CO, and PM with aerodynamic diameter \( \leq 10 \mu m \) (PM\(_{10}\)) (Song et al. 2019). A study from Mexico city showed inverse associations between umbilical cord blood cell TL and air pollution exposure during the first trimester and positive associations in the second and third trimester (Rosa et al. 2019). A study from Boston showed inverse associations between prenatal PM\(_{2.5}\) exposure during mid-gestation and TL in cord blood cells (Lee et al. 2020). The study also found an effect of maternal intake of antioxidants (Lee et al. 2020). There is a lack of low exposure studies with a comprehensive exposure assessment and a concomitant assessment of TL in cells from umbilical cord blood, placenta tissue and maternal blood.

We investigated whether maternal exposure to low levels of air pollution during pregnancy was related to telomere shortening in the complete in utero environment, including the maternal blood cells, placenta tissue and umbilical cord blood cells from the fetal circulation. Distributed lag models were applied to explore potential windows of sensitivity in fetal life, where exposure to particulate and gaseous air pollution components could affect the TL. Air pollution levels were modeled at both residential and occupation addresses to obtain a more complete assessment of the exposure.

2. Material and methods

This study is part of the project Maternal Stress and Placental Function that was conducted in Copenhagen, Denmark and the data collection process was previously described in detail (Dahlerup et al. 2018). In brief, the participants were pregnant women who gave birth at the Copenhagen University Hospital, Department of Obstetrics. The project aimed to investigate the general adult Danish population with an exclusion of women younger than 18 years. All women were informed about the aim of the study and gave written informed consent. The project was approved by the Regional Scientific Ethical Committee of Copenhagen (H-18058513) and the processing of personal data was approved by the Faculty of Health and Medical Sciences at University of Copenhagen on behalf of the Danish Data Protection Agency.

2.1. Population characteristics

Recruitment took place at four different locations connected to the Copenhagen University Hospital; at information meetings for all pregnant women, at information meeting for women giving birth for the first time, at information meetings specific to women giving birth by planned caesarean sections, and in waiting rooms at midwives’ offices. The participants were instructed to answer questionnaires regarding relevant personal factors such as smoking habit, pregnancy complications, and indoor air pollution exposure. The recruitment was conducted from June 2015 to May 2016. All participants that returned questionnaires and had successful sampling from placenta tissue and collection of both maternal and umbilical cord blood, directly after birth, were included in the study, resulting in 296 participants. Information on the birth and the infant were collected via hospital records.

2.2. Sample collection

Directly after birth, 20 ml of maternal blood and 20–30 ml of blood from the umbilical cord were sampled by venipuncture into 10 ml vacuum tubes containing EDTA and centrifuged for 10 min at 4000 g without brake. The plasma anduffy coat (i.e. leukocytes) were collected and stored at –80 °C.

Five full thickness samples (0.8 cm in width) were systematically collected from central or peripheral tissue in the placenta and stored at –80 °C, containing both maternal and fetal side of the placenta. One tissue sample from each placenta, taken primarily from peripheral tissue, was used for further analysis.

2.3. Analysis of the telomere length

Genomic DNA was isolated from umbilical cord blood, placenta tissue and maternal blood samples with a Quick-DNA™ Miniprep Plus Kit (Zymo Research, Irvine, CA). The umbilical cord blood and maternal blood samples (buffy coat extractions) were isolated according to manufacturer’s instructions for biological fluids, and the placenta tissue samples were isolated according to manufacturer’s instructions for solid tissues. The placenta tissue samples were homogenized before DNA isolation in homogenization buffer (see Supplementary Materials, Table S1). Small adjustments were made to the protocols to gain a higher DNA purity, including additional washing steps, longer centrifugation period for umbilical cord blood samples, and extended incubation time with double volume of Proteinase K for the homogenized placenta tissue samples. DNA concentrations were determined using a spectrophotometric analysis (Eppendorf BioPhotometer, Hamburg, Germany). A ratio of absorbance values at wavelengths at 260 nm and 280 nm (A\(_{260}/A_{280}\)) of 1.7 – 1.9 was considered acceptable. Certain samples did not have acceptable purity, thus the purity measurements were noted and divided into three categories (i.e. high (1.7 < ), medium (1.5 < 1.7), low (<1.5)), to test for differences between the purity categories. All isolated DNA were stored at –20 °C for approximately 1–2 months.

The TL was quantified by a real-time quantitative polymerase chain reaction (qPCR) method (Cawthon 2002) with some modifications; forward and reverse primers were as follows: Telomere repeat copies (T): teloF-5′-GGG TTG CCT TAC CCT TAC CCT TAC CCT TAC TGG TGG GTT GTT GTT-3′. Single copy gene (SCG): 36B4F-5′–CAT CAA GTG GGA AGG TGT AAT CC-3′ and 36B4R-5′–CCC ATT CTA TCA TCA ACG GTG ACA A-3′ (TAG Copenhagen A/S, Denmark). The cycling condition for the qPCR, was 10 min at 95 °C, followed by 30 cycles at 95 °C for 15 s, 54 °C for 60 s and 60 °C for 30 s for the measurement of TL. For the measurement of single copy gene (36B4), we used 10 min at 95 °C, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. The reaction was carried out in 384-well plates, using a 7900HDT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA), equipped to excite and read emissions from fluorescent molecules during each cycle of the PCR. The final reaction mixture (10 µL) contained primers (1 µM) and 12–18 ng genomic DNA in 1x PowerUp™ SYBR™ Green PCR Master Mix (Thermo Fisher Scientific, Vilnius, Lithuania). All samples were run in triplicates, and the inter-assay coefficient of variation (CV) was between 0.46 and 1.57% for the standard reference DNA samples, and between 0.47 and 1.37% for the negative controls. The PCR efficiencies and standard curves are presented in Figure S1. The results are reported as
the fold difference in relative TL compared to the length of a single copy gene (T/S), calculated by the following formula:

\[
\frac{T}{S} = \left[ \frac{2^{\Delta C(t)_{\text{ref}}}}{2^{\Delta C(t)_{\text{sample}}}} \right]^{-1} = 2^{-\Delta C(t)}
\]

All results were standardized as fold-difference between T/S values of the samples and the internal standard in the individual experiment. A log-transformation of the telomere lengths was used in all analyses.

2.4. Exposure assessment

Exposure data was modeled by the Danish Centre for Environment and Energy (DCE) at Aarhus University. Exposure data was modeled at residential addresses and occupational addresses with the multiscale integrated air pollution model system DEHM-UBM-AirGIS (Khan et al., 2019), which includes modeling of regional background concentration, urban background concentrations and street concentrations (Brandt et al., b, 2001a; Brandt et al., 2001c, 2003; Khan et al., 2019). The contributions from the regional background were modeled with a regional chemistry-transport model called the Danish Eulerian Hemispheric Model (DEHM) (Brandt et al. 2012) in a 5.6 km² grid resolution. The contributions from the local scale were modeled with the Urban Background Model (UBM) (Brandt et al., b, 2001a; Brandt et al., 2001c, 2003) in a 1 km² grid resolution. The street contributions were modeled with the Operational Street Pollution Model (OSPM) (Kakosimis et al., 2010; Ketzel et al., 2012), where the background concentrations from DEHM and UBM were included via the AirGIS system. The inputs generated by AirGIS (e.g. traffic and street geometry information) were based on national GIS datasets, letting the OSPM street pollution model estimate air pollution at address locations. The whole model system is validated against measurements in Hvidfeldt et al. (2018) and Khan et al. (2019).

The exposure assessment at residential addresses was modeled at street resolution with the full model system DEHM-UBM-AirGIS, and at the occupational addresses the exposure was modeled at a 1 km² grid resolution with the DEHM-UBM models only. The recruitment area was centered in the Copenhagen area in Denmark, and the distribution of the participant’s residential (n = 296) and occupational (n = 264) addresses can be seen in Supplemental Materials, Figure S2-S3. The study population was smaller for occupational addresses, due to missing data from the questionnaires.

The air pollution exposure was modeled in 7 day periods, starting from 8 weeks before the estimated start of pregnancy and one year forward. In this way, it was possible to compare the gestational weeks of the full pregnancy for the women participating.

Exposure data was modeled for PM2.5, PM10, NO2, NOx, CO, BC, O3, NH3, SO2 and OC during gestational weeks 1 to 40 for all participants, as well as 8 weeks before the estimated conception. All distributed lag models were run as single pollutant models.

The distributed lag model was set up with complete cases (no imputation). All analyses were performed with the statistical software R, version 3.6.1, dnlm package (Gasparrini 2011). We analyzed the association with TLs separately for umbilical cord blood cells, placenta tissue and maternal blood cells. Five models were run with increasing levels of adjustment for confounders chosen a priori. Model 1 was unadjusted (crude model); model 2 was adjusted for maternal age, maternal pre-pregnancy body mass index (BMI), maternal educational level; model 3 was additionally adjusted for maternal smoking status, ambient temperature, indoor exposure; model 4 was additionally adjusted for newborn gender, season of delivery, gestational age in days, pregnancy complications, parity, and mode of delivery; and model 5 was additionally adjusted for markers of morbidity in the fetus encompassing the newborns body weight, length, and circumference of head in the statistical analysis of TL in umbilical cord blood cells. Some exceptions were: model 5 was adjusted for placental symmetry (log) and placental weight (log) when the outcome was TL in placenta tissue, and for birth strain when the outcome was TL in maternal blood cells. In the text, we have reported the results from the statistical analysis of model 5 because all models essentially yielded the same association; i.e. the direction of the associations did not change in the different models as may happen by co-linearity between exposures and confounders in the studies on TL (Moller et al. 2018). The results from models 1 – 5 on the association between exposure to the modeled air pollutants at residential addresses and TL in umbilical cord blood is shown in Supplementary Materials, Table S4. The study population was smaller in model 5 compared to the crude model, due to missing data on adjusted variables from the questionnaires.

Effect estimates on TL were calculated for the three trimesters of the pregnancy (weeks 1 to 12, weeks 13 to 26, weeks 27 to 40) and for the
overall pregnancy (weeks 1 to 40), as well as for the period prior to the estimated conception (weeks –8 to 0) for an interquartile range increase in exposure by multi-variate linear regression models adjusted for maternal age, maternal BMI, maternal educational level, maternal smoking habit, ambient temperature, indoor exposure, newborn gender, season of delivery, gestational age in days, pregnancy complications, parity, mode of delivery, newborn weight, length, and circumference of head. These results are reported as percent changes (95% CI) in all three outcome variables in relation to maternal air pollution exposure at both residential and occupational addresses.

3. Results

The characteristics of the study population are presented in Table 1 (n = 296).

The distribution of maternal air pollution exposure for the full length of pregnancy is presented in Table 2.

The variation in air pollution levels at the residential addresses for full length of pregnancy is presented in Supplementary Materials, Figures S4-S13.

The correlation between the air pollutant markers are presented as a heat map in Supplementary Materials, Table S5.

The descriptive characteristics of the relative TLs in umbilical cord blood cells, placenta tissue and maternal blood cells are presented in Table 3. The mean relative TLs were 1.189 ± 0.223 for umbilical cord blood cells (n = 289), 1.006 ± 0.151 for placenta tissue (n = 291), and 1.089 ± 0.123 for maternal blood cells (n = 289).

The TL in umbilical cord blood cells and placenta tissue showed a small inverse correlation (r = -0.175, p < 0.01). The relation between TL in placenta tissue and maternal blood cells did not show statistical significance, although it was close to showing a small inverse correlation (r = -0.105, p = 0.08), whereas TL in umbilical cord blood cells and maternal blood cells did not show correlation (r = 0.019, p = 0.76).

3.1. Association between prenatal exposure to air pollution and TL

Percent changes (95% CI) in umbilical cord blood TL in relation to maternal air pollution exposure at their residential addresses are presented in Table 4. The associations between umbilical cord blood TL and air pollution exposures at occupational addresses are presented in Supplementary Materials, Table S6 and Figure S13. There were positive associations between BC, OC, NO₂, NOₓ, CO, and O₃ exposure levels at the residential addresses in the second trimester and TL at birth. For some of the exposures, positive associations were found during the first trimester (NH₃, SO₂) and prior to the conception (OC, NO₂, SO₂). There were inverse associations between PM₂.₅, BC, OC, NH₃, NO₂, CO and SO₂ exposure levels at the residential addresses in the third trimester and TL at birth. For SO₂ exposure, an inverse association was also found in the second trimester and TL at birth.

The lag-specific (weekly) distributed lag model estimates of the association between air pollution exposure at the residential address during pregnancy and umbilical cord blood TL is shown in Fig. 1. The air pollutants presented in the upper and middle panels showed similar trends, where positive associations were found around second trimester between umbilical cord blood TL and prenatal air pollution exposure to PM₂.₅ (weeks 11–17), BC (weeks 11–20), OC (weeks 11–21), NO₂ (weeks 18–22), NOₓ (weeks 20–25), CO (weeks 12–23) and O₃ (weeks 12–24). Inverse associations are shown around third trimester between umbilical cord blood TL and prenatal air pollution exposure to PM₂.₅ (weeks 29–38), BC (weeks 26–34), OC (weeks 28–35), NO₂ (weeks 32–40), and CO (weeks 29–35). In addition, there was an inverse association for early exposure to O₃ (weeks –4 to 0) and CO (weeks –1 to 5), as well as a positive association for early exposure to OC (weeks –8 to –5) and NO₂ (weeks –8 to –5). In the lower panels, it is shown that the prenatal exposure to both SO₂ and NH₃ had a positive association with TL in umbilical cord blood cells during weeks (-5)-10 and 3–15 respectively, and an inverse association with TL in umbilical cord blood cells during weeks 18–37 and 24–40 respectively. The sensitive periods were longer for SO₂ and NH₃ than for the other air pollutants, and the percentage change in umbilical cord blood TL was larger. There was no association between prenatal PM₁₀ exposure levels and TL in umbilical cord blood cells.

PM₂.₅ appears to have been the preferred exposure variable in previous studies on effects of air pollution on TL. The inverse association between PM₂.₅ and TL in the third trimester is depicted in Fig. 2, which

| Table 1 Characteristics of study population. |
|---------------------------------------------|
| Characteristics                          | Frequency (%)^a |
| Newborns                                  |                |
| Birth weight (g), mean ± SD [range] (n = 292) | 3462 ± 514     |
| [2000–5696]                               |                |
| Sex                                        |                |
| - Female                                   | 149 (50)       |
| - Male                                     | 145 (49)       |
| Gestational age (weeks), mean ± SD [range] | 39.7 ± 1.4     |
| [30–43]                                   |                |
| Season of delivery                         |                |
| - Winter (December – February)             | 65 (22.0)      |
| - Spring (March – May)                     | 98 (33.1)      |
| - Summer (June – August)                   | 44 (14.9)      |
| - Autumn (September – November)            | 89 (30.1)      |
| Apgar score 5 min after birth              |                |
| - 8                                        | 3 (1.0)        |
| - 9                                        | 10 (2.4)       |
| - 10                                       | 271 (91.6)     |
| Mothers                                    |                |
| Age (years), mean ± SD [range] (n = 289)   | 33.7 ± 4.6     |
| [23 – 49]                                  |                |
| Pre-pregnancy BMI (kg/m²), mean ± SD [range] (n = 289) | 23.0 ± 4.7 |
| Employment and educational level           |                |
| - Employed – high education                | 121 (40.9)     |
| - Employed – medium education              | 91 (30.7)      |
| - Employed – low education                 | 40 (13.5)      |
| - Not in workforce                         | 41 (13.9)      |
| Smoking during pregnancy                   |                |
| - Yes                                      | 22 (7.4)       |
| - No                                       | 274 (92.6)     |
| Indoor air pollution                       |                |
| - 0                                        | 144 (48.6)     |
| - 1                                        | 126 (42.6)     |
| - 2                                        | 24 (8.1)       |
| - 3                                        | 2 (0.7)        |
| Parity                                     |                |
| - 1                                        | 136 (45.9)     |
| - 2                                        | 117 (39.5)     |
| - ≥3                                       | 41 (13.9)      |
| Mode of delivery                           |                |
| - Cesarean delivery                        | 169 (57.1)     |
| - Vaginal delivery                         | 127 (42.9)     |
| Birth strain                               |                |
| - 0                                        | 168 (56.8)     |
| - 1–3                                      | 64 (21.6)      |
| - 4–6                                      | 49 (16.6)      |
| - ≥7                                       | 14 (4.7)       |
| Chronic diseases and/or pregnancy complications reported | |
| - Yes                                      | 126 (42.6)     |
| - No                                       | 168 (56.8)     |
| Placenta                                   |                |
| Weight (g), mean ± SD [range] (n = 287)    | 710 ± 174 [365–1475] |
| Symmetry                                   |                |
| - ≤ 1                                      | 97 (32.8)      |
| - 2                                        | 62 (20.9)      |
| - 3                                        | 30 (10.1)      |
| - 4                                        | 22 (7.4)       |
| - ≥5                                       | 38 (12.9)      |
| Weekly mean temperature (°C), mean ± SD [5th – 95th percentile] | 8.7 ± 6.4 [-1.2 – 18.9] |

Note: Parity includes the participant’s current pregnancy. Abbreviations: SD, standard deviation; BMI, body mass index.

^a Distribution percent refers to the total population (n = 296).
Table 2

Distribution of maternal air pollution exposure at both residential and occupational addresses for the full length of pregnancy.

| Air pollutant | Mean ± SD | Min | 25th | 50th | 75th | Max | IQR |
|---------------|-----------|-----|------|------|------|-----|-----|
| PM$_{2.5}$ (µg/m$^3$) | 11.5 ± 3.8 | 8.7 | 10.0 | 12.3 | 15.9 | 20.1 | 4.4 |
| PM$_{10}$ (µg/m$^3$) | 17.8 ± 5.5 | 12.2 | 15.1 | 18.4 | 21.1 | 25.4 | 7.2 |
| BC (µg/m$^3$) | 0.5 ± 0.3 | 0.2 | 0.5 | 0.7 | 0.9 | 1.1 | 0.5 |
| OC (µg/m$^3$) | 1.5 ± 0.2 | 1.0 | 1.7 | 1.9 | 2.3 | 2.5 | 0.5 |
| NH$_4$ (µg/m$^3$) | 0.6 ± 0.2 | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.3 |
| NO$_2$ (µg/m$^3$) | 16.9 ± 6.8 | 12.2 | 21.1 | 25.4 | 27.8 | 31.2 | 5.1 |
| NO$_x$ (µg/m$^3$) | 21.0 ± 12.4 | 6.5 | 12.7 | 20.5 | 27.2 | 34.0 | 9.2 |
| CO (µg/m$^3$) | 173.6 ± 24 | 64.4 | 156.1 | 182.6 | 209.1 | 251.5 | 25.5 |
| O$_3$ (µg/m$^3$) | 44.5 ± 14.1 | 11.1 | 24.4 | 32.5 | 39.7 | 44.8 | 8.5 |
| SO$_2$ (ppb) | 1.2 ± 0.9 | 0.1 | 0.7 | 0.9 | 1.4 | 1.7 | 1.0 |

Abbreviations: BC, black carbon; OC, organic carbon; Min, minimum; Max, maximum; IQR, interquartile range

Table 3

Descriptive characteristics of the relative telomere lengths in umbilical cord blood cells, placenta tissue and maternal blood cells.

| Relative telomere length | Median | Mean | SD | 95% CI | Min | Max | N |
|--------------------------|--------|------|----|--------|-----|-----|---|
| Umbilical cord blood cells | 1.153 | 1.189 | 0.223 | 1.163-1.215 | 0.721 | 2.257 | 289 |
| Placenta tissue | 1.007 | 1.006 | 0.151 | 0.989-1.023 | 0.659 | 1.367 | 291 |
| Maternal blood cells | 1.089 | 1.089 | 0.123 | 1.074-1.103 | 0.761 | 1.513 | 289 |

Abbreviations: SD, standard deviation; CI, confidence interval; Min, minimum; Max, maximum

4. Discussion

To our knowledge, this is the first study to assess the associations between air pollution exposure in the period from 8 weeks before conception until birth, and the TL in paired samples of cells in umbilical cord blood, placenta tissue, and maternal blood. This is also the first study to include air pollution exposure levels at the occupational addresses, and include such a large selection of air pollution markers. Our study has mother-newborn pairs matching umbilical cord blood, placenta tissue, and maternal blood and high-resolution exposure estimates based on both residential and occupational addresses. In comparison with more conventional approaches of averaging exposures over the entire pregnancy, the use of a distributed lag model allowed for a more comprehensive investigation of possible sensitive periods in fetal development, thus making it possible to detect the sensitive prenatal exposure windows to air pollution exposure during pregnancy.

Our study shows the second and third trimesters of the pregnancy to be susceptible windows of exposure. This was seen, as levels of PM$_{2.5}$ (weeks 11–17), BC (weeks 11–20), OC (weeks 11–21), NO$_2$ (weeks 18–22), NO$_x$ (weeks 20–25), CO (weeks 12–23) and O$_3$ (weeks 12–24) in the second trimester of pregnancy were positively associated with TL in umbilical cord blood cells. In addition, significant inverse associations were found between the TL in umbilical cord blood cells and a majority of the air pollutants; PM$_{2.5}$ (weeks 29–38), BC (weeks 26–34), OC (weeks 28–35), NO$_2$ (weeks 32–40), CO (weeks 29–35), NH$_4$ (weeks 24–40), and SO$_2$ (weeks 18–37). In recent years, four studies have assessed the effect of period-segregated prenatal air pollution exposure on TL in umbilical cord blood cells from pregnancies in Boston, US (Lee et al. 2020), Flanders, Belgium (Martens et al. 2017), Mexico city, Mexico (Rosa et al. 2019) and Wuhan city, China (Song et al. 2019).

Table 4

Associations between prenatal exposure to air pollution at residential addresses (with IQR increase) and the TL in umbilical cord blood cells: Percent change (95% CI), (n = 255).

| Air pollutants | Entire pregnancy (week 1 to 40) | Prior to conception (week –8 to 0) | 1st trimester (week 1 to 12) | 2nd trimester (week 13 to 26) | 3rd trimester (week 27 to 40) |
|----------------|-------------------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|
| PM$_{2.5}$ | 11 (–9, 36) | 4 (–8, 18) | 7 (–10, 27) | 18 (–5, 46) | –23 (–35, –9) |
| BC | 2 (–14, 21) | 4 (–8, 17) | –4 (–17, 12) | 43 (12, 84) | –22 (–35, –7) |
| OC | 23 (–4, 59) | 18 (3, 35) | –3 (–18, 15) | 67 (14, 144) | –5 (–36, 42) |
| NH$_4$ | 9 (–37, 89) | 12 (–4, 32) | –3 (–18, 15) | 67 (14, 144) | –5 (–36, 42) |
| PM$_{10}$ | 1 (–20, 28) | 7 (–10, 27) | –10 (–29, 15) | 20 (3, 39) | –20 (–31, –6) |
| NO$_2$ | 9 (–6, 27) | 11 (2, 22) | –5 (–17, 7) | 19 (3, 37) | –9 (–22, 6) |
| NO$_x$ | 9 (–5, 25) | 8 (–2, 18) | –12 (–22, 0) | 19 (3, 37) | –9 (–22, 6) |
| CO | 28 (–7, 78) | 0 (–25, 18) | –13 (–33, 13) | 70 (24, 132) | –29 (–48, –5) |
| O$_3$ | 22 (–6, 58) | 15 (–28, –1) | –17 (–35, 5) | 60 (24, 107) | –13 (–38, 22) |
| SO$_2$ | 20 (–38, 3) | 18 (4, 33) | 58 (25, 98) | –36 (–52, –25) | –33 (–47, –16) |

Notes: Model adjusted for maternal age, maternal BMI, maternal educational level, maternal smoking habit, ambient temperature, indoor exposure, newborn gender, season of delivery, gestational age in days, pregnancy complications, parity, mode of delivery, newborn weight, length, and circumference of head. Abbreviations: TL, telomere length; CI, confidence interval.
Fig. 3 summarizes the observations related to PM$_{2.5}$ exposure from these studies, since this is the only air pollution component that has been measured in all of the studies. Some discrepancy exists between the studies, as would be expected considering that they are obtained from areas with different air pollution levels and differences in adjustment for confounders, and none of the studies adjusted for differences in subpopulations of leukocytes. Inverse associations were found between umbilical cord blood cell TL and exposure to PM$_{2.5}$ during the second trimester in the studies performed in Boston (Lee et al. 2020) and Flanders (Martens et al. 2017), and inverse associations were found with exposure during the third trimester in the study performed in Wuhan city (Song et al. 2019), as in our study. A smaller period of inverse associations was found with exposure during the first trimester in the study performed in Mexico City (Rosa et al. 2019). Positive associations between umbilical cord blood TL and exposure to PM$_{2.5}$ during the second trimester were found in the study performed in Mexico City (Rosa et al. 2019), like in our study. Two very short periods of positive associations were found with exposure during the third trimester in the studies performed in Flanders (Martens et al. 2017) and Mexico City (Rosa et al. 2019).

The studies performed in Boston, Flanders and Mexico City solely investigated PM$_{2.5}$ exposure. In the Chinese study however, exposure to PM$_{10}$, NO$_2$, SO$_2$, and CO were also investigated. They found inverse associations between umbilical cord blood TL and exposure to PM$_{10}$, SO$_2$, and CO during the third trimester (Song et al. 2019). We also found inverse associations between umbilical cord blood TL and exposure to SO$_2$ and CO during the third trimester. We did not find inverse associations with exposure to PM$_{10}$, which could be attributed to the
The difference in pollution levels, as the mean concentration was 135.5 µg/m³ for PM_10 in Wuhan city (Song et al. 2019) and a much lower 17.9 µg/m³ in our study. The percentage change in TL in umbilical cord blood cells per interquartile difference in exposure to PM_2.5, BC, OC, NO₂, CO, NH₄, and SO₂ in the third trimester was between 23 and 33% shortening in our study. This range of percentage change was larger compared to the Chinese study, where the exposure in the third trimester was associated with 3–11% TL shortening per 10 µg/m³ (PM_2.5, PM_10 and SO₂) and 100 µg/m³ (CO) increase in air pollution components (Song et al. 2019). The observations from the Chinese study support the findings in our study, where inverse associations are seen between umbilical cord blood TL and exposure to several air pollutants during the third trimester. With a majority of associations found with exposures during both second and third trimester of the pregnancy among the other two studies summarized in Fig. 3, the observations from the three studies support our results that suggest the second and third trimesters of the pregnancy to be sensitive windows of exposure. Sensitive windows of exposure during fetal life are well known from studies of other outcomes such as intrauterine growth retardation (Dejmek et al. 2000).

The underlying mechanisms by which maternal air pollution exposure may lead to differences in TL in leukocytes in umbilical cord blood are not clear, although they may be similar to the mechanisms of TL shortening in circulating leukocytes in adults. Positive associations between air pollution exposures and TL in leukocytes in adults have been explained by effects of inflammatory reactions, that may alter the composition of leukocytes toward a higher number of neutrophils in the blood (Dioni et al. 2011), as it has been shown that the number of neutrophils in blood is positively associated with leukocyte TL (Mollica et al. 2009). It is also possible that low-grade systematic inflammation alters the composition of leukocytes because of clonal expansion of subpopulations with longer telomeres (T and B lymphocytes), as those will proliferate faster than the ones with shorter telomeres, resulting in an increase in average leukocyte TL (Hodes et al. 2002). Recruitment of less mature leukocytes from the bone marrow in response to the inflammation could also contribute to an increased leukocyte TL in post exposure samples (Suwa et al., 2002; Tan et al., 2000). Thus, the positive
association in the second trimester may be an effect related to differences in subpopulations of either/or granulocytes and lymphocytes in the blood, which cannot be controlled for in the statistical analysis. It is possible that a low-grade systemic inflammation in the cord blood cells arises from stimuli from the mother affected by air-pollution, such as circulating inflammatory mediators that cross the relatively thick second trimester placenta barrier more easily than environmental chemicals and PM (Benirschke 2006). The inflammation in the cord blood cells could also have arisen from stimuli from the placenta, as it previously has been shown that maternal nanoparticle exposures could cause developmental toxicity in the fetus without the direct passage of the nanoparticles (Hawkins et al. 2018). In a birth cohort study performed in the Netherlands, it was observed that long-term exposure to PM10 and NO2 was associated with elevated fetal C-reactive protein levels at birth, suggesting fetal inflammatory responses to air pollution exposures, as C-reactive protein levels frequently are used as a marker of low-grade systemic inflammation (van Den Hooven et al. 2012).

Air pollution exposure causes both a pro-inflammatory and pro-oxidation environment. The latter is strongly associated with telomere shortening. PM inhaled by the mothers during pregnancy has been found able to cross the human placenta (Bové et al., 2019; Perera et al., 2018). The third trimester of the placental development has the fastest growth period in utero, as well as the period where the placenta has the fewest cell layers between the maternal and fetal blood circulations (Syme et al., 2004; Van der Aa et al., 1998). The third trimester placenta has a relatively thin barrier for compounds to cross the placenta, including PM, pro-inflammatory cytokines and pro-oxidant species. It is possible that translocation of PM to the fetal circulation might cause oxidative stress and inflammation in the fetus. There is strong experimental evidence showing that air pollution particles cause oxidative stress, inflammation and oxidative damage to DNA in cell cultures, animal models and human beings (Moller et al. 2014). Oxidative stress mediated by PM might arise from direct production of reactive oxygen species from the surface of the particles. The oxidative stress might also arise from altered function of the mitochondria and activation of inflammation cells (Risom et al. 2005). Telomeres have a high content of guanine bases that are readily damaged by oxidants to e.g. 8-oxoguanine or strand breaks (Houben et al., 2008; Kawanishi and Oikawa, 2004). The results in our study suggest that air pollution exposure is associated with recruitment of inflammatory cells such as neutrophils, but in the third trimester the positive association related to cells with long telomeres is masked by an inverse association caused by the effect of oxidative stress. The positive association may thus be indicating the inflammation response happening from maternal air pollution exposure, and the inverse association is the combined effect of inflammation and an oxidative stress response caused by the direct transfer of air pollution particles through the placenta.

The inverse associations found in the third trimester are diluted by the positive associations in earlier trimesters, yielding an overall null association for the entire pregnancy period. This underlines the importance of assessing trimester-specific associations, or shorter periods of time during the pregnancy, like our weekly distributed lag model estimates, to be able to identify sensitive periods during the pregnancy.

Our study showed no consistent pattern in associations between air pollution exposure and TL in placenta tissue, whereas Martens et al. (2017) reported an inverse association between TL in placenta tissue and prenatal exposure to PM2.5. In a twin study performed in East Flanders, Belgium, lower telomere length was found between TL in placenta and residential proximity to major roads as an indicator of traffic-generated air pollution (Bijnens et al. 2015). The placenta tissue sampling procedure in our study differed from the procedure in Martens et al. (2017) and Bijnens et al. (2015), which may have favored subsets of placenta cell types and could be a reason for the lack of a consistent pattern in associations between air pollution exposure and TL in placenta tissue. However, as only a single exposure marker of air pollution was used in those studies, within-study consistency in associations with multiple exposure markers cannot be assessed.

Another study assessed the effect of PM2.5 and PM10 exposure on TL in maternal blood cells obtained at the 11th gestational week in a study population from Milan, Italy. Only the exposure to PM10 was associated with a shortening in maternal blood cell TL after approximately the first trimester (Iodice et al. 2018). In our study, inverse associations were found between maternal blood TL at birth and both exposure to NO2 during the first trimester, and exposure to O3 during the third trimester. Our results, together with the study by Iodice et al. (2018), suggest that it might not only be the fetus that can be affected by air pollution exposure during the pregnancy, but the mother too, strengthening the idea of pregnant women as a susceptible group. The physiological changes during the different trimesters of pregnancy poses a unique stress test for women moving disease thresholds that require significant compensation, both from immune system function and from the endocrine system. Pregnancy represents a heightened state of physiological sensitivity that can exacerbate chemical exposures effects on maternal health and should be regarded as a critical period for women’s health (Varhavsky et al. 2020).

The spectrum of gases and PM that are inversely associated with TL in umbilical cord blood cells suggests the influence of multiple sources of outdoor air pollution. The contribution from local traffic-generated exhausts is highlighted by the inverse association between the umbilical cord TL and NO2, further supported by inverse associations with other combustion-derived markers (i.e. CO, SO2, OC, BC and PM2.5). There is also an inverse association with NH4+, which is the cation of ammonium sulfate and ammonium nitrate that are generated as secondary inorganic particles from ammonia. The latter is used as fertilizer in farming. The exposure of residential addresses was modeled with data from DEHM-UBM-AirGIS, whereas the occupational exposure only was modeled with data from DEHM-UBM as the occupational addresses were often inexact. We speculate that long-range transport of pollutants is the most important contributor to the local air pollution for the participants in the present study, as similar associations were obtained from exposure models at the residential and occupational addresses.

By obtaining exposure estimates at both residential and occupational addresses for the participants, it was possible to reduce the risk of potential misclassification, as a higher contribution to the personal exposure was accounted for, although, the exact duration of time spent at each location during the pregnancy was not recorded. As the associations found between TL in umbilical cord blood cells and the exposure at each of the locations were similar, we chose to focus on the results from the residential addresses, with the knowledge at hand that those results were supported by the results from the occupational addresses. Most women in Denmark start their maternity leave around 4 – 6 weeks before their estimated delivery, resulting in minimal exposure at their occupational addresses during this time. The women probably also have spent more time at their residential addresses than at their occupational addresses during a week, underlining that the exposures at the residential addresses might be more important. Though the risk of potential misclassification is reduced in our study, as we have a well-described exposure profile of the participants, a full personal exposure profile would require exposure contributions from the participants’ daily commute routes, as well as a more detailed overall exposure including both residential and occupational exposure. Incorporation of potential residential moves and job changes during the pregnancy would also provide a more detailed overall exposure.

It should be noted that shortening of telomeres is pronounced in the first years of life, while the lifestyle during adulthood exerts a minor impact (Benetos et al., 2001; Frenck et al., 1998; Rufer et al., 1999). The relevance of the associations found between environmental factors and newborn TL can be difficult to foresee, as many more factors will influence the telomere dynamics during both childhood and later life (Zhang et al. 2013). However, the importance of early life exposures has been demonstrated in studies where increasing NO2 exposures during pregnancy was associated with a shortening of TL in blood samples from
children aged of 5 to 12 years (Clemente et al., 2019), and where telomere ranking between birth and young adulthood was inversely associated with residential traffic exposure at the birth address (Bijnens et al., 2017). No human studies concerning TL in early life and the prediction of future mortality have been performed over full lifetimes, although animal studies have shown that telomeres shorten with increasing age and greater rates of telomere shortening can predict future mortality (Barrett et al., 2013; Heidinger et al., 2012). TL shortening might therefore serve as a possible mechanism relating fetal exposures with health consequences later in life. In our study, the TL is used as a marker of early biological effect caused by exposure to air pollution throughout pregnancy.

The mean relative TL in umbilical cord blood cells was the longest, followed by the TL in maternal blood cells and placenta. Long TL in umbilical cord blood cells is in line with notion that telomeres shorten with age (Blackburn et al. 2015). The placenta and fetus develop from the same tissue (Cross, 2006; Van der Aa et al., 1998), suggesting that telomeres shorten at a faster rate in placenta than in umbilical cord blood cells. This might be caused by the limited lifespan of the placenta, along with the consequences of changes the placenta undergoes during the pregnancy (Syme et al., 2004; Van der Aa et al., 1998). The placenta has the ability to react to new situations during the pregnancy (Van Ertvelde et al., 2016; Vombotis-Dekrey et al., 2016), and the need for compensations as a reaction to an environmental challenge could therefore lead to an accelerated aging. The inverse correlation between TL in umbilical cord blood cells and placenta tissue might be explained by the placenta reacting to potential adverse changes in the maternal environment (Cross, 2006; Syme et al., 2004). By scavenging compounds the placenta may accumulate damages, while at the same time protect the fetus from hazardous effects of environmental agents, resulting in a damaged placenta with shorter TL and a fetus protected from the adverse maternal milieu. On the other hand, the lack of correlation between TL in maternal blood cells and TL in placenta and fetus may be explained by differences in cumulated exposures, where the TL in maternal blood cells reflects the life-time exposure, whereas the placenta and umbilical cord blood cells are only exposed during the pregnancy period. The lack of correlation between TL in the maternal and umbilical cord blood is in keeping with notion that the TL is elongated by increased telomerase activity in germs cells, and telomerase activity is also high in blastocytes and early embryogenic stages, whereas it decreases as the fetus matures and progenitor cells differentiate to tissue cells (Schaetzlein et al., 2004; Ulane et al., 1998; Wright et al., 1996). In addition, the heritable influence is complex as it appears that TL in children correlate with both maternal and paternal TL (Factor-Livak et al., 1996). In addition, the heritable influence is complex as it appears that TL in children correlate with both maternal and paternal TL (Factor-Livak et al., 1996). In addition, the heritable influence is complex as it appears that TL in children correlate with both maternal and paternal TL (Factor-Livak et al., 1996). In addition, the heritable influence is complex as it appears that TL in children correlate with both maternal and paternal TL (Factor-Livak et al., 1996). In addition, the heritable influence is complex as it appears that TL in children correlate with both maternal and paternal TL (Factor-Livak et al., 1996).
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