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Prenatal and Childhood Traffic-Related Air Pollution Exposure and Telomere Length in European Children: The HELIX Project

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Background: Telomere length is a molecular marker of biological aging.

Objective: Here we investigated whether early-life exposure to residential air pollution was associated with leukocyte telomere length (LTL) at 8 y of age.

Methods: In a multicenter European birth cohort study, HELIX (Human Early Life Exposome) ($n = 1{,}396$), we estimated prenatal and 1-y childhood exposure to nitrogen dioxide (NO_2), particulate matter with aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$), and proximity to major roads. Average relative LTL was measured using quantitative real-time polymerase chain reaction (qPCR). Effect estimates of the association between LTL and prenatal, 1-y childhood air pollution, and proximity to major roads were calculated using multiple linear mixed models with a random cohort effect and adjusted for relevant covariates.

Results: LTL was inversely associated with prenatal and 1-y childhood NO_2 and $PM_{2.5}$ exposures levels. Each standard deviation (SD) increase in prenatal NO_2 was associated with a −1.5% (95% CI: −2.8, −0.2) change in LTL. Prenatal $PM_{2.5}$ was nonsignificantly associated with LTL (−0.7% per SD increase; 95% CI: −2.0, 0.6). For each SD increment in 1-y childhood NO_2 and $PM_{2.5}$ exposure, LTL shortened by −1.6% (95% CI: −2.9, −0.4) and −1.4% (95% CI: −2.9, 0.1), respectively. Each doubling in residential distance to nearest major road during childhood was associated with a 1.6% (95% CI: 0.02, 3.1) lengthening in LTL.

Conclusion: Lower exposures to air pollution during pregnancy and childhood were associated with longer telomeres in European children at 8 y of age. These results suggest that reductions in traffic-related air pollution may promote molecular longevity, as exemplified by telomere length, from early life onward. https://doi.org/10.1289/EHP4148

Introduction

In the recent update of the Global Burden of Disease, Injuries, and Risk Factor study, air pollution is ranked fifth on a list of the most influential factors affecting health worldwide (Gakidou et al. 2017). Hypotheses are that oxidative stress and inflammation are important underlying mechanisms through which air pollutants could cause adverse health outcomes (Kannan et al. 2006).

Telomeres are complexes of tandem repeats of DNA (5′-TTAGGG-3′), sited at the termini of the chromosomes. Telomeres have a significant function in maintaining the integrity of chromosomes and the stability of the genome, and preventing end-to-end chromosomal fusions (Blackburn 1991). Since DNA polymerase is unable to fully replicate the 3′ end of the DNA strand, telomeres shorten with each cell division. Consequently, telomere length is considered a biomarker of biological aging, and shorter telomeres have been associated with age-related diseases such as cardiovascular disease (Chen et al. 2014; Haycock et al. 2014; Hunt et al. 2015; Zhan and Hagg 2019), type 2 diabetes (Tamura et al. 2016; Wang et al. 2016; Willeit et al. 2014), and increased mortality (Cawthon et al. 2003; Dean et al. 2017; Fitzpatrick et al. 2011; Needham et al. 2015). Furthermore, it is believed that the natural erosion of telomeres is accelerated through oxidative stress and inflammation (Kawanishi and Oikawa 2004; von Zglinicki et al. 2005).

According to the Developmental Origins of Health and Disease (DOHaD), small changes in the early-life environment shape the future probability of the development of age-related diseases (Barker 1995; Gluckman et al. 2008; Kumaran et al. 2017). The rate of telomere attrition is greatest in young children (Aubert and Lansdorp 2008), and the telomere length decline then continues at a slower rate throughout adulthood (Yamaguchi

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et al. 2005). Consequently, telomere loss in childhood is a potential important factor leading to the ultimate telomere length in adults. Environmental factors might have the greatest effect in childhood when the high telomere attrition is occurring.

Air pollution exposures may contribute to the aging phenotype, and telomere length may play a mechanistic role in linking air pollution to age-related diseases. It is thus important to study the link between early-life air pollution exposure and telomere length in childhood to gain insights into the etiology of age-related diseases. Here, we assessed, within a multicenter birth cohort study in six European countries with a wide range of exposures, the association between prenatal and 1-y childhood exposure to air pollution as exemplified by residential nitrogen dioxide (NO_2), particulate matter with aerodynamic diameter $\leq$2.5 μm ($PM_{2.5}$), residential proximity to major roads, and leukocyte telomere length (LTL) in 8-y-old children.

Materials and Methods

Study Population and Data Collection

The Human Early Life Exposome (HELIX) study is a collaborative project across six established and ongoing longitudinal population-based birth cohort studies in Europe: the Born in Bradford (BiB) study in the United Kingdom (Wright et al. 2013), the Etude de cohorte généraliste, menée en France sur les Déterminants pré et post natals précoces du développement psychomoteur et de la santé de l'Enfant (EDEN) study in France (Heude et al. 2016), the INfancia y Medio Ambiente (INMA) cohort in Spain (Guxens et al. 2012), the Kaunas cohort (KANC) in Lithuania (Grazuleviciene et al. 2009), the Norwegian Mother and Child Cohort Study (MoBa) (Magnus et al. 2016), and the RHEA Mother and Child Cohort study in Crete, Greece (Chatzi et al. 2009). The study population for the entire HELIX cohort includes 31,472 women who had singleton deliveries between 1999 and 2010, and for whom exposure to ambient air pollution during pregnancy had been estimated as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE) project (Pedersen et al. 2013). Local ethical committees approved the studies that were conducted according to the guidelines laid down in the Declaration of Helsinki. All participating women provided informed written consent. The analysis of this paper made use of the HELIX subcohort that includes mother–child pairs who were fully characterized for a broad suite of environmental exposures to be clinically examined and to have biological samples collected. A new follow-up visit was organized for these mother–child pairs. Subcohort subjects were recruited from within the entire cohorts such that there were approximately 200 mother–child pairs from each of the six cohorts. Subcohort recruitment in the EDEN cohort was restricted to the Poitiers area, and in the INMA cohort to the city of Sabadell.

Detailed information on maternal age at birth, maternal education, maternal marital status, smoking status during pregnancy, parity, and maternal ethnicity from each study participant was obtained by each cohort during pregnancy or at birth by questionnaire or medical records. The level of maternal education reported by the participant was used as the primary indicator of socioeconomic status and categorized according to the International Standard Classification of Education (ISCED) (Schneider 2013) as three levels: low (less than primary, primary, and lower secondary education, ISCED 2011 levels 0–2), middle (upper secondary and postsecondary nontertiary education, ISCED 2011 levels 3 and 4), and high (tertiary education, ISCED 2011 levels 5–8). Maternal smoking status was categorized as no active smoking during pregnancy and active smoking during pregnancy. Child ethnicity was defined for all cohorts and subdivided in seven different groups (African; Asian; white European; mixed Native American; South Asian; white, not European; or others). Perinatal parameters such as birth date and newborn sex were obtained at birth.

Blood Collection and DNA Extraction

DNA was obtained from buffy coat collected in ehylenediaminetetraacetic acid tubes. Briefly, DNA was extracted using the Chemagen DNA Blood kit (PerkinElmer) in batches of 12 samples. Samples were extracted by cohort and following their position in the original boxes. DNA concentration was determined in a NanoDrop™ 1000 UV-Vis Spectrophotometer (Thermo Scientific), and DNA integrity was tested with Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies).

Average Relative Telomere Length Measurement

Average relative telomere length was measured by a modified quantitative real-time polymerase chain reaction (qPCR) protocol as described previously (Cawthon 2009). Telomere and single-copy gene reaction mixture and PCR cycles used can be found in Martens et al. (2016). All measurements were performed in triplicate on a 7900HT Fast Real-Time PCR System (Applied Biosystems) in a 384-well format. On each run, a six-point serial dilution of pooled DNA was run to assess PCR efficiency as well as eight interrun calibrators to account for interrun variability. Relative telomere lengths were calculated using qBase+ software (Biogazelle) and were expressed as the ratio of telomere copy number to single-copy gene number (T/S) relative to the average T/S ratio of the entire sample set. We achieved coefficient of variation (CV) within triplicates of the telomere runs, single-copy gene runs, and T/S ratios of 0.84, 0.43, and 6.4%, respectively. Two batches were used to measure telomere length. Each batch contained multiple cohorts that were randomly selected.

Since blood leukocyte is a mixed cell sample and the effect of obesity may be different in different cell types, white blood cell proportions [CD4+ and CD8+ T cells, natural killer (NK) cells, monocytes, eosinophiles, neutrophils, and B cells] were estimated from raw methylation data using the Houseman algorithm (Houseman et al. 2012) implemented in the minfi R package (version R3.3.0; http://www.r-project.org) (R Development Core Team) and the Reinius reference panel (Reinius et al. 2012) in samples of 1,146 children.

Exposure Assessment

We assessed both prenatal and 1-y childhood air pollution exposure at the residential address during pregnancy and follow-up. Air pollutants used in this study included NO_2 and $PM_{2.5}$. These air pollutants were estimated using land use regression (LUR) or dispersion models, temporally adjusted to measurements made in local background monitoring stations and averaged over trimester 1, trimester 2, trimester 2, and the whole pregnancy period. For most cohorts, we used site-specific LUR models developed in the context of the ESCAPE project (Beelen et al. 2013; Eeftens et al. 2012). In EDEN, dispersion models were used to assess the NO_2 exposure (Rahmalia et al. 2012), and the ESCAPE European-wide LUR model was applied for $PM_{2.5}$, corrected for local background monitoring data (Wang et al. 2014). In BiB, $PM_{2.5}$ assessment was made based on the ESCAPE LUR model developed in the Thames Valley region of the United Kingdom and adjusted for background PM levels from monitoring stations in Bradford (Schembari et al. 2015). Additionally, we collected information on the traffic assessed as distance to nearest road (m) at mother's residence. One-year childhood air pollution included annual NO_2

and $PM_{2.5}$, assessed for the year before the telomere length measurements through site-specific ESCAPE LUR models for all cohorts except EDEN. In EDEN, a local dispersion model was used to assess the NO_2 exposure. Additionally, we assessed traffic levels as distance to nearest road (m) at the child's home residence.

The software used to make the spatial analysis were ArcGIS platform (ESRI ArcMap TM 10.0; ArcGIS Desktop 10 Service Pack 4) and SpatiaLite (version 4.11; Alessandro Furieri).

Statistical Analysis

We performed multiple imputation of exposures and confounders assuming missing at random (Royston and White 2011). The missing values were imputed using the method of chained equations (White et al. 2011), using the mice package in R (van Buuren and Groothuis-Oudshoorn 2011). We followed HELIX Statistical protocol for imputation process. The following points were taken into account: *a*) the imputation models included no more than 15 to 20 variables (van Buuren and Groothuis-Oudshoorn 2011), and quickpred function was used to reduce the number of predictors; *b*) predictive mean matching was used to impute continuous covariates, and logistic or multinomial regression was employed to impute binary or categorical exposures, respectively; *c*) variables that were functions of others [e.g., body mass index (BMI) is a function of weight of weight and height] were not imputed and were calculated after imputation; and *d*) $n = 20$ imputed datasets were created for each analyses (White et al. 2011). After imputation, we conducted diagnostics that included comparisons of the imputed and nonmissing observations using density plots and strip plots (van Buuren and Groothuis-Oudshoorn 2011). If the variables included in the imputation process were not flagged and imputations seemed plausible, we included the predictors in the imputation model (Stuart et al. 2009).

LTL showed a skewed distribution and was therefore $\log_{10}$ transformed to achieve a normal distribution. Generalized additive models were used to assess the linearity of the associations between prenatal and 1-y childhood air pollution exposure and LTL. If the *p*-value for gain was higher than 0.05, the model was considered linear. Multiple linear mixed models with a random cohort effect were applied to test the association between traffic and air pollution exposures and LTL at age 8 y (range: 5.4–12.0 y). All models were adjusted for *a priori* chosen covariates based on literature including child's age (in days), sex (male or female), qPCR batch (batch 1 or batch 2), maternal age (in years), maternal education (low, middle, high), maternal smoking status during pregnancy (yes or no), child ethnicity (listed in Table 1), child BMI (continuous), and parental smoking at the time of blood collection for the LTL measurements (neither, one, or both).

In a first step of the analysis, we studied LTL by medians of the distributions of the exposure variables considered separately. In the next step, air pollutants were treated as continuous variables and were scaled to a standard deviation (SD) difference in level for testing associations with LTL. Distance to nearest road was $\log_{10}$ transformed to assure normality. Multiple-pollutant models simultaneously considering $PM_{2.5}$ and NO_2 levels in each exposure window were assessed. Finally, we used models that mutually adjusted for prenatal and 1-y childhood exposure to assess which period had the largest effect on LTL.

To test whether the results were robust, we ran different sensitivity analyses in which we tested the sex interaction between air pollution exposure and sex on LTL in children by including its interaction term in the full models. Additionally, we ran another sensitivity analysis in which we stratified our exposure analyses by group of children who lived at the same address at both time points (pregnancy and 1-y childhood) vs. those who moved between those two time points. Because blood leukocyte is a mixed cell sample, we included an additional analyses in which we included the proportion of white blood cells (CD4+ and CD8+ T cells, NK cells, monocytes, eosinophiles, neutrophils, and B cells) as covariates in our model. The sensitivity of the findings was also examined by removing one cohort at the time from the analysis and recalculating the estimates.

Analyses were performed using the SAS statistical software (version 9.3; SAS Institute Inc.). Results were indicated as statistically significant when the *p*-value was lower than 0.05.

Table 1. General characteristics of the complete case study population ($n = 1,396$).

	Mean ± SD or *n* (%)
Children	
Sex	
Girls	643 (46.1)
Boys	753 (53.9)
Ethnicity	
African	12 (0.9)
Asian	21 (1.5)
White European	1,223 (87.4)
Mixed Native American	13 (0.9)
Other	22 (1.6)
South Asian	79 (5.7)
White, not European	26 (1.9)
Cohort	
INMA	428 (30.6)
MoBa	213 (15.3)
BiB	205 (14.7)
RHEA	202 (14.5)
KANC	199 (14.3)
EDEN	149 (10.6)
Moved	
Yes	191 (13.7)
No	1,205 (86.3)
Age at telomere length assessment, years	8.0 ± 1.5
Relative telomere length	1.0 (0.9–1.1)
BMI	0.48 ± 1.2
Mothers	
Age at delivery, years	30.5 ± 4.9
Missing	15 (1.1)
Education	
Low	219 (15.8)
Middle	480 (34.5)
High	643 (46.2)
Missing	54 (3.5)
Active smoking during pregnancy	
Yes	1,121 (83.3)
No	229 (13.4)
Missing	46 (3.30)
Parity	
1	635 (45.5)
2	498 (35.7)
≥3	228 (16.3)
Missing	35 (2.5)
Parental smoking at 8 y	
Neither	827 (59.3)
One	394 (28.2)
Both	156 (11.2)
Missing	19 (1.3)

Note: Continuous covariates expressed by mean and standard deviation (SD) or geometric mean and 25th–75th percentiles; categorical covariates described by number and frequencies (percent). Data are complete for all observations unless otherwise indicated. BiB, Born in Bradford study; BMI, body mass index; EDEN, Etude de cohorte généraliste, menée en France sur les Déterminants pré et post natals précoces du développement psychomoteur et de la santé de l'Enfant; INMA, INfancia y Medio Ambiente cohort; KANC, Kaunas cohort; MoBA, Norwegian Mother and Child Cohort Study; RHEA, Mother and Child Cohort study.

Table 2. Exposure characteristics of the complete case study population ($\mu g/m^3$).

			Percentiles					Correlation[a]	
	n	Mean ± SD	5th	25th	50th	75th	95th	Prenatal	1-y childhood
NO_2									
Prenatal	1,237	25.0 ± 13.9	9.6	14.8	20.4	32.9	51.4	1	—
1-y childhood	1,366	23.1 ± 12.2	7.3	11.9	23.3	32.2	42.2	0.74[b]	1
$PM_{2.5}$									
Prenatal	1,307	15.1 ± 2.6	10.7	13.5	15.0	16.9	19.6	1	—
1-y childhood	1,366	13.2 ± 3.3	7.3	11.0	13.3	15.0	19.1	0.48[b]	1

Note: Continuous variables expressed by mean ± standard deviation (SD). —, no data; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter ≤2.5 μm.
[a]Spearman correlation coefficient between prenatal and 1-year childhood exposure.
[b]$p < 0.0001$.

Results

Characteristics of the Study Population

Table 1 describes the general characteristics of the study population ($n = 1{,}396$). The children were mainly white European (87.4%) and had a mean (SD) age of 8 (1.5) y. Forty-six percent of the children were girls. The mean (SD) maternal age at delivery was 30.5 (4.9) y. Overall, 643 (46.1%) of the mothers were highly educated, 635 (45.5%) of the mothers were primiparous, and 229 (13.4%) of the mothers actively smoked during pregnancy. There were few missing covariate data. We reported missing data for maternal education (3.5%), active smoking during pregnancy (3.3%), parity (2.5%), and parental smoking (1.3%) (Table 1). The characteristics for the individual cohorts are presented in Table S1.

Table 2 displays the average outdoor prenatal and 1-y childhood air pollution exposures. Average (25th–75th percentile) mean prenatal exposure was 25.0 (14.8–32.9) and 15.1 (13.5–16.9) $\mu g/m^3$ for NO_2, and $PM_{2.5}$, respectively. Average (25th–75th percentile) mean 1-y childhood exposure was 23.1 (11.9–32.2) and 13.2 (11.0–15.0) $\mu g/m^3$ for NO_2, and $PM_{2.5}$, respectively. Prenatal and 1-y childhood NO_2 were highly correlated, whereas a similar analysis for $PM_{2.5}$ showed a moderate correlation (Table 2). The exposure characteristics for the individual cohorts are presented in the Table S2. The distributions of the NO_2 and $PM_{2.5}$ exposure levels across the different HELIX cohorts are presented in Figures S1 and S2.

Association between Leukocyte Telomere Length and Maternal and Child Characteristics

We observed shorter LTL in boys compared with girls (0.98 vs. 1.02; $p < 0.0001$), while LTL was not significantly correlated with child´s age within our narrow age range ($r = -0.038$; $p = 0.15$). Shorter LTL in children was associated with higher child BMI ($r = 0.073$; $p = 0.007$). Telomere lengths in children were positively associated with maternal age ($r = 0.09$; $p = 0.0006$).

Association between Prenatal and Childhood Air Pollution and Leukocyte Telomere Length at Age of Eight Years

Associations between prenatal and 1-y childhood air pollution exposures and LTL did not deviate from linearity, as p-value of gain was higher than 0.05 (Figure S3).

In analyses treating exposure to air pollution as continuous variables, increasing NO_2 exposure during pregnancy was associated with a shortening in LTL (−1.5% per SD increment; 95% CI: −2.8, −0.2). Each SD increment in NO_2 during trimester 1, trimester 2, and trimester 3 exposure was associated with shorter telomeres of −1.6% (95% CI: −2.8, −0.4), −1.3% (95% CI: −2.6, −0.04), and −1.6% (95% CI: −2.9, −0.4), respectively (Table 3). Prenatal $PM_{2.5}$ exposure was not significantly associated with telomere length (entire pregnancy: −0.7%; 95% CI: −2.0, 0.6) (Table 3). One-year childhood NO_2 was associated with statistically significant shorter LTL (−1.6% per SD increment; 95% CI: −2.9, −0.4) at age 8 y. Furthermore, 1-y childhood $PM_{2.5}$ was inversely (−1.4%; 95% CI: −2.9, 0.1) associated with telomere length at age 8 y, although this was only borderline statistically significant. Doubling of the residential distance to nearest road during pregnancy was not significantly associated with 1-y childhood telomere length (0.2%; 95% CI: −1.3, 1.6), whereas a doubling in residential distance to nearest road in 1-y childhood was associated with significant longer childhood telomere length (1.6%; 95% CI: 0.02, 3.1) (Table 3). Table S3 presents the results in the original metric without conversion to percent difference in telomere length.

Ambient air pollutants (NO_2 and $PM_{2.5}$) were weakly correlated with each other (($r = 0.20$, $p < 0.0001$; $r = 0.15$, $p < 0.0001$) for prenatal and 1-y childhood exposure, respectively). Therefore, multipollutant models that included both NO_2, and $PM_{2.5}$ did not alter interpretation of the results (Table 4). In the prenatal period, the estimates for NO_2 exposure were a bit greater when adjusted for $PM_{2.5}$ exposure, whereas the estimates for $PM_{2.5}$ attenuated slightly. In childhood, there was a very small attenuation of the estimates for both NO_2 exposure and $PM_{2.5}$ exposure. Prenatal and 1-y childhood NO_2 were highly correlated, whereas a similar analysis for $PM_{2.5}$ showed a moderate correlation (Table 2); therefore, it is difficult to distinguish the effects of prenatal and

Table 3. Association between leukocyte telomere length (LTL) and traffic-related air pollution exposure and distance to nearest road.

	Percent difference (95% CI)	p-Value
NO_2		
Trimester 1	−1.6 (−2.8, −0.4)	0.01
Trimester 2	−1.3 (−2.6, −0.04)	0.04
Trimester 3	−1.6 (−2.9, −0.4)	0.01
Entire pregnancy	−1.5 (−2.8, −0.2)	0.02
1-y childhood	−1.6 (−2.9, −0.4)	0.01
$PM_{2.5}$		
Trimester 1	−0.8 (−2.3, 0.7)	0.5
Trimester 2	−0.1 (−1.3, 1.1)	0.9
Trimester 3	−0.3 (−1.5, 0.8)	0.6
Entire pregnancy	−0.7 (−2.0, 0.6)	0.3
1-y childhood	−1.4 (−2.9, 0.1)	0.08
Distance to nearest road		
Pregnancy	0.2 (−1.3, 1.6)	0.8
1-y childhood	1.6 (0.02, 3.1)	0.04

Note: Effect size was estimated as a percent difference in LTL for each standard deviation (SD) increment in ambient air pollution exposure. Models included a variable for only one pollutant (NO_2 or $PM_{2.5}$) during one time period [during pregnancy (trimester 1, 2, 3, or entire pregnancy)] or the year before LTL measurement. Models were adjusted for child's age, sex, quantitative real-time polymerase chain reaction (qPCR) batch, maternal age, maternal education, maternal smoking status during pregnancy, child ethnicity, child body mass index (BMI), and parental smoking at 8 y. CI, confidence interval; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter ≤2.5 μm.

Table 4. Percentage difference in leukocyte telomere length (LTL) at approximately 8 y of age in association with standard deviation (SD) increases in average NO_2 and $PM_{2.5}$ throughout pregnancy (prenatal exposure) and childhood (average annual concentration 1 y before the LTL measurement).

Model	Prenatal exposure	1-y childhood exposure
NO_2		
One pollutant and time period[a]	−1.5 (−2.8, −0.2)	−1.6 (−2.9, −0.4)
Adjusted for $PM_{2.5}$ during the same time period[b]	−1.9 (−3.3, −0.6)	−1.5 (−2.7, −0.4)
One pollutant during both time periods[c]	−0.7 (−2.7, 1.3)	−1.2 (−3.0, 0.7)
Both pollutants during both time periods[d]	−0.5 (−2.2, 1.2)	−1.1 (−2.8, 0.7)
$PM_{2.5}$		
One pollutant and time period[a]	−0.7 (−2.0, 0.6)	−1.4 (−2.9, 0.1)
Adjusted for NO_2 during the same time period[b]	−0.6 (−2.7, −0.4)	−1.5 (−3.2, 0.2)
One pollutant during both time periods[c]	0.3 (−1.4, 2.0)	−1.6 (−3.6, 0.4)
Both pollutants during both time periods[d]	0.4 (−1.1, 1.9)	−1.2 (−3.0, 0.5)

Note: NO_2 SD: prenatal = 13.9 $\mu g/m^3$, 1-y childhood = 12.2 $\mu g/m^3$; $PM_{2.5}$ SD: prenatal = 2.6 $\mu g/m^3$, 1-y childhood = 3.3 $\mu g/m^3$. All models included random effects for study cohort and were adjusted for quantitative real-time polymerase chain reaction (qPCR) batch; maternal age, education, and active smoking during pregnancy; child's ethnicity and gender; child's age; child's body mass index (BMI) z-score; and parental smoking at the time of blood collection for the LTL measurement. NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter ≤2.5 μm.

[a]Models included a variable for only one pollutant (NO_2 or $PM_{2.5}$) during one time period (during pregnancy or the year before LTL measurement).

[b]Models included variables for one pollutant (NO_2 or $PM_{2.5}$) during two time periods (during pregnancy and during the year before LTL measurement).

[c]Models included variables for both pollutants (NO_2 and $PM_{2.5}$) during the same time period (during pregnancy or the year before LTL measurement).

[d]Models included variables for both pollutants (NO_2 and $PM_{2.5}$) during both time periods (during pregnancy and the year before LTL measurement).

childhood air pollutants. However, results from models that mutually adjusted for prenatal and 1-y childhood exposure suggest that the effects of air pollutants on telomere length could be stronger in childhood rather than in the prenatal period (Table 4). For NO_2, the estimates for both prenatal and 1-y childhood exposure were attenuated, although the attenuation was greater for prenatal exposure than for childhood exposure. Furthermore, the association with 1-y childhood $PM_{2.5}$ exposure became slightly stronger when adjusted for prenatal $PM_{2.5}$. In a model including variables for both pollutants (NO_2 and $PM_{2.5}$) during both time periods (prenatal and 1-y childhood), the estimates for prenatal and 1-y childhood NO_2 and $PM_{2.5}$ were attenuated.

Sensitivity Analyses

Including an interaction term between air pollution exposure and sex in the full models showed that the sex interaction was not significant for the different models ($p = 0.89$ and $p = 0.60$ for prenatal NO_2 and $PM_{2.5}$ exposure, respectively, and $p = 0.91$ and $p = 0.53$ for 1-y childhood NO_2 and $PM_{2.5}$ exposure, respectively). Including white blood cell proportions as covariates in our model as an additional analysis did not substantially change our reported results (Table S4). The sensitivity of the significant findings was further examined by removing one cohort at the time from the analyses and recalculating the percent difference in telomere length (Table S4). The percent difference in telomere length for each increment in prenatal NO_2 exposure decreased to −3.2% when INMA was excluded and to −1.7% when MoBa was excluded, while excluding RHEA lead to an increase in telomere length to −1.1%. Excluding BiB, KANC, or EDEN did not change our reported percentage changes in telomere length. Additionally, the percent change in telomere length for each increment in 1-y childhood NO_2 exposure decreased to −2.4% when INMA was excluded and to −1.7% and −2.0% when MoBa and KANC were excluded, respectively, while excluding BiB and RHEA led to an increase in telomere length of −1.4% and −1.1%, respectively. Excluding EDEN did not alter our reported percentage changes in telomere length. Finally, association for 1-y childhood exposure was much stronger when the analysis was limited to 191 HELIX participants who moved (e.g., for average 1-y childhood NO_2: −10.0%; 95% CI: −17.9, −1.3 compared with −1.2%; 95% CI: −2.7, 0.4) (Table S4).

Discussion

To our knowledge, the present study, including six populations across Europe, is so far the largest study of air pollution exposure and LTL in children. Here we showed that prenatal (entire pregnancy) and childhood (1 y prior blood collection) traffic-related air pollution were associated with shorter leukocyte telomeres in children. For each SD increment in prenatal NO_2 (13.9 $\mu g/m^3$), LTLs were −1.5% shorter in children. Additionally, for each SD increment (12.2 $\mu g/m^3$, 3.3 $\mu g/m^3$) in 1-y childhood NO_2 and $PM_{2.5}$ exposure, LTL was −1.6% and −1.4% shorter, respectively.

Extensive epidemiological studies showed associations between ambient air pollution and adverse health outcomes, including premature mortality and cardiovascular and respiratory disease, both with short-term (Levy et al. 2001; Nawrot et al. 2011) and chronic exposure (Beelen et al. 2014a, 2014b; Hamra et al. 2014; Laden et al. 2006). The biological mechanisms by which air pollutants may cause adverse health outcomes are not completely understood, but oxidative stress and inflammation are thought to be of importance. The ability of oxidative stress to damage nucleic acids provides a potential mechanism by which oxidative stress could interfere with telomere DNA (Epel et al. 2006). It is assumable that telomeres are a sensitive target for reactive oxygen species (ROS)–induced damage, as telomeres contain a high amount of ROS-sensitive guanine bases (Grahame and Schlesinger 2012). It is believed that ROS can induce DNA breakage, and single-strand breaks in telomeric DNA are ineffectively repaired, which could lead to increased telomere shortening (Kawanishi and Oikawa 2004).

We found a significant inverse association between prenatal and 1-y childhood air pollution exposure and telomere length at 8 y of age. Our findings of prenatal exposure and LTL in children are in line with studies in newborns (Bijnens et al. 2015; Martens et al. 2017). In the East Flanders Prospective Twin Survey ($n = 221$), maternal residential proximity to a major road was associated with placental telomere length; a doubling in the distance to the nearest major road was associated with 5.32% longer placental telomere length at birth (Bijnens et al. 2015). In 641 newborns of the ENVIRonmental influence ON early AGEing (ENVIRONAGE) birth cohort, cord blood and placental telomere length were significantly inversely associated with $PM_{2.5}$ exposure during midgestation, with approximately 8.8% and 13.2% shorter cord blood and placental telomere length at birth for each 5 $\mu g/m^3$ increase in residential $PM_{2.5}$ exposure, respectively (Martens et al. 2017). The mean $PM_{2.5}$ exposure was comparable (13.4 $\mu g/m^3$) with the mean exposure of our study. We found that the association between telomere length and exposure to air pollution is persistent into childhood. However, it is difficult to distinguish between potential effects on telomere length during the individual time periods (prenatal vs. 1-y childhood), given that the exposures are well correlated. In contrast to our current study and previous studies (Hoxha et al. 2009; Lee et al. 2017; McCracken et al. 2010; Pieters et al. 2016), a study of 333 school children (8–9 y) in London reported that annual air pollution exposure was associated with longer telomeres in saliva, DNA

coming from a mixture of different cell types (Walton et al. 2016). The authors suggested that these increases in telomere length may be due to the effect on telomere-associated proteins, telomerase activation, or clonal expansion of less mature leukocytes (Hodes et al. 2002; Weng et al. 1997). It could also be due to difficulties in measuring telomeres in the saliva matrix. Furthermore, the mean NO_2 exposure in this study (43.6 $\mu g/m^3$) was much higher than the mean NO_2 exposure of our study. The mean $PM_{2.5}$ exposure was comparable (13.7 $\mu g/m^3$) with the mean exposure of our study.

How do our results in childhood compare to the evidence in adults? Among 165 never-smoking men, the Normative Aging Study found an inverse association between long-term exposure to ambient black carbon (BC) and telomere length in adulthood (−7.6% for each 0.25 $\mu g/m^3$ increment in BC; 95% CI: −12.8, −2.1) (McCracken et al. 2010). A cross-sectional study on 77 traffic officers and 57 indoor office workers found that traffic officers (LTL = 1.02; 95% CI: 0.96, 1.09) had shorter leukocyte telomeres than did office workers (LTL = 1.22; 95% CI: 1.13, 1.31), suggesting that long-term exposure to traffic-related air pollution may shorten telomere length (Hoxha et al. 2009). Furthermore, a study in the Cooperative Health Research in the Augsburg Region (KORA) F4 cohort ($n = 1{,}777$) found that telomere length was inversely associated with BC exposure in men ($\beta = -0.28$; 95% CI: −0.47, −0.1) (Ward-Caviness et al. 2016). Among 166 nonsmoking elderly participants, a 5-$\mu g/m^3$ increment in annual $PM_{2.5}$ concentration (range: 15–23 $\mu g/m^3$) was associated with a relative decrease of 16.8% in telomere length (Pieters et al. 2016). In this elderly study, they found also that short-term (last month) exposure to $PM_{2.5}$ was associated with increased telomere length. Short-term personal exposure to PM was associated with 5.2% longer telomeres in a study with highly exposed steelworkers (Hou et al. 2012). The discrepancies in the results of the studies associating telomere length with air pollution exposure are mainly driven by differences in exposure windows. Long- and short-term exposure to air pollution can change the telomere length in different directions (Miri et al. 2019). As seen above, long-term exposure to PM has been associated with shorter telomere length, whereas short-term exposure to PM has been associated with longer LTL. Whether this acute increase in telomere length is due to the effects on telomere-associated proteins, telomerase activation, or to the proliferative capacity and clonal expansion of less mature leukocytes needs to be evaluated (Hodes et al. 2002; Weng et al. 1997).

Telomere length synchrony across somatic tissues has been observed (Daniali et al. 2013; Friedrich et al. 2000). However, limited evidence is available of the correlation between respiratory-specific cells and blood telomere length. In this regard, Saferali et al. (2014) found a significant correlation between blood telomere length and lung tissue telomere length ($n = 51$; $r = 0.348$; $p = 0.012$). Furthermore, Snetselaar et al. (2017) found a very strong association between blood telomere length and alveolar type 2 cells ($n = 9$; $r = 0.82$; $p = 0.007$), whereas whole-lung biopsy telomeres did not correlate with blood telomeres. It has been shown that air pollutants can cross the respiratory tract and enter the blood circulation (Nemmar et al. 2002; Saenen et al. 2017). Consequently, the exposure may directly affect blood cells. In this regard, it has been shown that air pollution exposure leads to increased lung and systemic inflammation and oxidative stress (Chuang et al. 2007; Colicino et al. 2017), which both are processes involved in telomere shortening. However, whether our results found in blood are exactly representative for respiratory cell telomeres is unclear.

Traffic-related air pollution in the early-life environment, as exemplified by residential ambient NO_2 exposure, both prenatal and during childhood, may increase the risk for chronic diseases in adulthood. Indeed, although telomeres of children are long compared with adults, shortening due to early-life exposure to air pollution may decrease the buffer capacity to cope with inflammation and oxidative stress later in life, and therefore, it is reasonable to assume that it might lead to faster shortening of critical telomere length at older age.

Our study needs to be interpreted within the context of its potential limitations. Firstly, the traditional method to determine telomere length is telomere restriction fragment (TRF) analysis. In this study, we used a real-time PCR method, which has, in general, a higher assay variability compared with the TRF method (Aviv et al. 2011; Kimura et al. 2010). However, an interlaboratory comparison of our method showed that the coefficient of variation was less than 7%. Secondly, the assessment of telomere length at 8 y of age represents only a snapshot in childhood. We were not able to evaluate telomere dynamics throughout the entire pregnancy and the childhood period. Thirdly, our results are based on exposure at the residential address, and potential misclassification may be present because we could not take into account other exposure sources that contribute to personal exposure, such as exposure during commute and elsewhere. However, proxies of exposure, such as residential proximity to major roads, have been shown recently to be associated with internal exposure to nanosized particles, reflecting exposure to BC (Saenen et al. 2017). Fourthly, we only looked at two exposure periods during the child's life, *in utero* exposure during pregnancy and childhood exposure during the year before telomere length was measured. However, the exposures in these periods were highly correlated and therefore difficult to distinguish. Furthermore, blood leukocyte is a mixed cell sample that would include granulocytes (neutrophils and eosinophils) as well as mononuclear cells. The effects of increased exposure to air pollution may be different in different cell types. It is known that average telomere length is shorter in neutrophils than lymphocytes, and the half-life of neutrophils is much shorter than that of lymphocytes. However, we did a sensitivity analyses where we adjusted for the proportion of the different white blood cells: NK cells, B cells, CD4T, CD8T, eosinophils, mononuclear cells, and neutrophils. The results of these analyses did not differ from those without these additional adjustments. Finally, our results are based on exposure at the home address, and potential misclassification may be present because we could not account for other exposure sources that contribute to personal exposure, such as exposure during commute, at work or school, and elsewhere.

In conclusion, air pollution exposure in early life and childhood is an important contributing factor for later telomere length. Its potential importance for the susceptibility to later-life diseases underscores the relevance of identifying early-life and childhood determinants of telomere length. In a large multicenter European cohort, we showed that traffic-related air pollution exposure in early life and childhood was associated with childhood telomere length, indicating a potential higher susceptibility for the development of telomere length–associated diseases later in life when exposed to higher concentrations of air pollution. Reduction of traffic-related air pollution levels may promote molecular longevity, as exemplified by telomere length, from early life onward, leading to improved health in later life.

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