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Life-course exposure to air pollution and biological ageing in the Lothian Birth Cohort 1936

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ABSTRACT

Background: Exposure to air pollution is associated with a range of diseases. Biomarkers derived from DNA methylation (DNAm) offer potential mechanistic insights into human health differences, connecting disease pathogenesis and biological ageing. However, little is known about sensitive periods during the life course where air pollution might have a stronger impact on DNAm, or whether effects accumulate over time. We examined associations between air pollution exposure across the life course and DNAm-based markers of biological ageing.
Methods: Data were derived from the Scotland-based Lothian Birth Cohort 1936. Participants' residential history was linked to annual levels of fine particle ($PM_{2.5}$), sulphur dioxide (SO_2), nitrogen dioxide (NO_2), and ozone (O_3) around 1935, 1950, 1970, 1980, 1990, and 2001; pollutant concentrations were estimated using the EMEP4UK atmospheric chemistry transport model. Blood samples were obtained between ages of 70 and 80 years, and Horvath DNAmAge, Hannum DNAmAge, DNAmPhenoAge, DNAmGrimAge, and DNAm telomere length (DNAmTL) were computed. We applied the structured life-course modelling approach: least angle regression identified best-fit life-course models for a composite measure of air pollution (air quality index [AQI]), and mixed-effects regression estimated selected models for AQI and single pollutants.
Results: We included 525 individuals with 1782 observations. In the total sample, increased air pollution around 1970 was associated with higher epigenetic age (AQI: b = 0.322 year, 95 %CI: 0.088, 0.555) measured with Horvath DNAmAge in late adulthood. We found shorter DNAmTL among males with higher air pollution around 1980 (AQI: b = −0.015 kilobase, 95 %CI: −0.027, −0.004) and among females with higher exposure around 1935 (AQI: b = −0.017 kilobase, 95 %CI: −0.028, −0.006). Findings were more consistent for the pollutants $PM_{2.5}$, SO_2 and NO_2.
Discussion: We tested the life-course relationship between air pollution and DNAm-based biomarkers. Air pollution around birth and in young-to-middle adulthood is linked to accelerated epigenetic ageing and telomere-associated ageing in later life.

1. Introduction

Ambient air pollution is one of the greatest environmental threats to human health, with serious consequences for morbidity and mortality (World Health Organization, 2021), and is responsible for an estimated $3.5 trillion welfare and $144 billion labour income losses annually

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worldwide (World Bank, 2016). Based on the Global Burden of Diseases Study 2015, 4.2 million annual deaths and 103 million disability-adjusted life years can be attributed to exposure to fine particulate matter with an aerodynamic diameter of ≤ 2.5 μm ($PM_{2.5}$) (Cohen et al., 2017). Gaseous pollutants, such as nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and ozone (O_3) have been also associated with hazardous health effects and increased mortality (World Health Organization, 2021). Research has established the impact of long-term exposure to air pollution on cardiovascular and respiratory diseases, and cancer (Cohen et al., 2017; Manisalidis et al., 2020), and there is growing evidence for its effect on the risk of neurodegenerative (Russ et al., 2021) and mental disorders (Braithwaite et al., 2019).

Prolonged air pollution exposure can lead to leukocyte telomere length attrition (i.e. attrition of nucleoprotein complexes located at the ends of the chromosome) (Miri et al., 2019) and to epigenetic alterations, key hallmarks of ageing (Micheu et al., 2020; Dhingra et al., 2018; López-Otín et al., 2013). One epigenetic mechanism that contributes to the regulation of gene expression is DNA methylation (DNAm) at 5-methylcytosine: the process of adding a methyl group to the fifth carbon of cytosine nucleotides in the genome, usually found at cytosine-phosphate-guanine (CpG) dinucleotides (Mathews and Janusek, 2011; Moore et al., 2013). Ageing cells undergo substantial changes in genome-wide DNAm levels; given this association with chronological age (i.e. calendar time since birth) (Horvath et al., 2012), epigenetic age estimators (or epigenetic clocks) have been developed as markers of biological ageing representing the workings of epigenetic maintenance systems (Horvath, 2013; Hannum et al., 2013; Horvath and Raj, 2018). Epigenetic clocks are derived from human tissues and calculated from the methylation level of sets of CpGs sites: whereas earlier clocks selected CpGs sites as best predicting chronological age, a surrogate of biological ageing, second generation clocks focus more on providing a prediction of healthy lifespan using other surrogate measures (Horvath and Raj, 2018). Accelerated ageing is a predictor of all-cause mortality (Marioni et al., 2015) and worse health outcomes (Horvath and Raj, 2018), and it is associated with lower physical and cognitive fitness (Marioni et al., 2015).

Recent evidence indicates accelerated biological ageing, including telomere shortening and epigenetic alterations, among individuals exposed to long-term ambient air pollution (measured usually as annual average concentration prior to outcome assessment) (Miri et al., 2019; Nwanaji-Enwerem et al., 20162016; Isaevska et al., 2021); however, evidence is lacking on the relationship covering longer periods across the life course. It remains largely unknown whether there are sensitive or critical periods where exposure to air pollution has a prominent and/or long-lasting effect on biological ageing or – alternatively – whether the impact of air pollution gradually accumulates over time (Rider and Carlsten, 2019). These are profound questions as individuals' ranking in leukocyte telomere length is relatively stable from birth to adulthood (Martens et al., 2021). Therefore, it is plausible that associations identified in later life (Nwanaji-Enwerem et al., 2016; White et al., 2019; Ward-Caviness et al., 2016) are the consequence of exposures earlier in the life course combined with limited geographical mobility or moving to areas with similar air pollution levels (Pearce et al., 2018). Prolonged air pollution exposure can often have a stronger impact on DNAm patterns than short-term exposure (a few days) (Rider and Carlsten, 2019), which raises the question as to whether air pollution effects may accumulate over the life course.

The current study addresses this research gap by applying the life-course approach (studying the long-term effects of physical and social exposures operating across an individual's life course) (Kuh, 2003) and exploring associations between air pollution from birth onwards and DNAm-based biomarkers in late adulthood (i.e. epigenetic clocks, DNAm proxy for telomere length). Using a cohort of older, 1936-born Scottish adults with life-course residential addresses linked to historical air pollution concentrations and DNAm data in their 70s, we investigated whether local levels of pollution at different points across the life course (or their accumulation) are associated with biological ageing. Analyses focussed on four air pollutants ($PM_{2.5}$, SO_2, NO_2, O_3) with previously established impacts on health and wellbeing (World Health Organization, 2021).

2. Material and methods

2.1. Study participants

We used data from the Lothian Birth Cohort 1936 (LBC1936) (Taylor et al., 2018). Participants were born in 1936 and took part in the Scottish Mental Survey 1947, a nationwide school-based cognitive test at the age of 11 (Taylor et al., 2018). Between 2004 and 2007, 1091 surviving men and women of the Scottish Mental Survey 1947 living in the City of Edinburgh and in the Lothian area of Scotland were retraced and recruited for participating in the LBC1936. The average age was 70 in the first wave. Follow-up waves took place at age ~ 73 (2007–2010; n = 866), ~76 (2011–2013; n = 697), and ~ 79 (2014–2017; n = 550) (Taylor et al., 2018). In 2014, LBC1936 participants were asked to complete a lifegrid questionnaire (Berney and Blane, 2003) supported by 'flashbulb' memory prompts (e.g. 9/11 attacks in New York) aiming to capture residential histories from birth to the date of completing the lifegrid (Taylor et al., 2018). Out of 704 individuals remaining in the study at that point, 593 participants provided 7423 addresses, which were georeferenced using automatic geocoders and historical building databases (Pearce et al., 2018). The LBC1936 study was conducted according to the Declaration of Helsinki guidelines with ethical permission obtained from the Multi-Centre Research Ethics Committee for Scotland (MREC/01/0/56), Lothian Research Ethics Committee (wave 1, LREC/2003/2/29), and the Scotland A Research Ethics Committee (waves 2–4, 07/MRE00/58). Written consent was obtained from all participants.

2.2. Historical air pollution exposure

Annual concentrations of $PM_{2.5}$, SO_2, NO_2, and O_3 were estimated using the EMEP4UK (Vieno et al., 2010; Vieno et al., 2014; Vieno et al., 2016) atmospheric chemistry transport model for the model years of 1935, 1950, 1970, 1980, 1990 and 2001 (Fig. 1) (details on data generation and the feasibility of using historical air pollution estimates in epidemiological research has been published previously) (Russ et al., 2021). The model has a horizontal resolution of $0.5^\circ \times 0.5^\circ$ used to provide the boundary condition for a nested UK domain with a horizontal resolution of $0.055^\circ \times 0.055^\circ$ (~5×6 km^2). The EMEP4UK model has been extensively evaluated for the UK and globally (Lin et al., 2017; Ge et al., 2021).

Individual exposure was derived based on latitudes and longitudes of geocoded residential addresses. We extracted annual concentrations of pollutants for each UK-based address using time bands around EMEP4UK model output years (i.e. 1935 output linked to pre-1943 addresses, 1950 to 1943–1959, 1970 to 1960–1975, 1980 to 1976–1985, 1990 to 1986–1995, and 2001 to 1996–2006 addresses). Since participants could reside at multiple locations within a given time band, we calculated the unweighted mean level of exposure per time point, resulting in no more than six estimates per pollutant for each participant. (Russ et al., 2021).

Within each measurement period, exposure to pollutants was highly correlated: we found strong positive associations between $PM_{2.5}$, SO_2 and NO_2 (Pearson correlation coefficients ranged between 0.58 and 1.00), and they were negatively correlated with O_3 (ranged between −0.63 and −0.97) (Fig. S1). Correlation coefficients were particularly high for the modelling years of 1935 and 1950. To reflect this high degree of shared variance, we constructed a composite air quality index (AQI), an additive cumulative measure of multi-pollutants exposure. (Giang and Castellani, 2020) We first scaled and centred $PM_{2.5}$, SO_2, NO_2 and O_3 values, and then summed them for each measurement periods (i.e. AQI in 1935, 1950, 1970, 1980, 1990, and 2001).

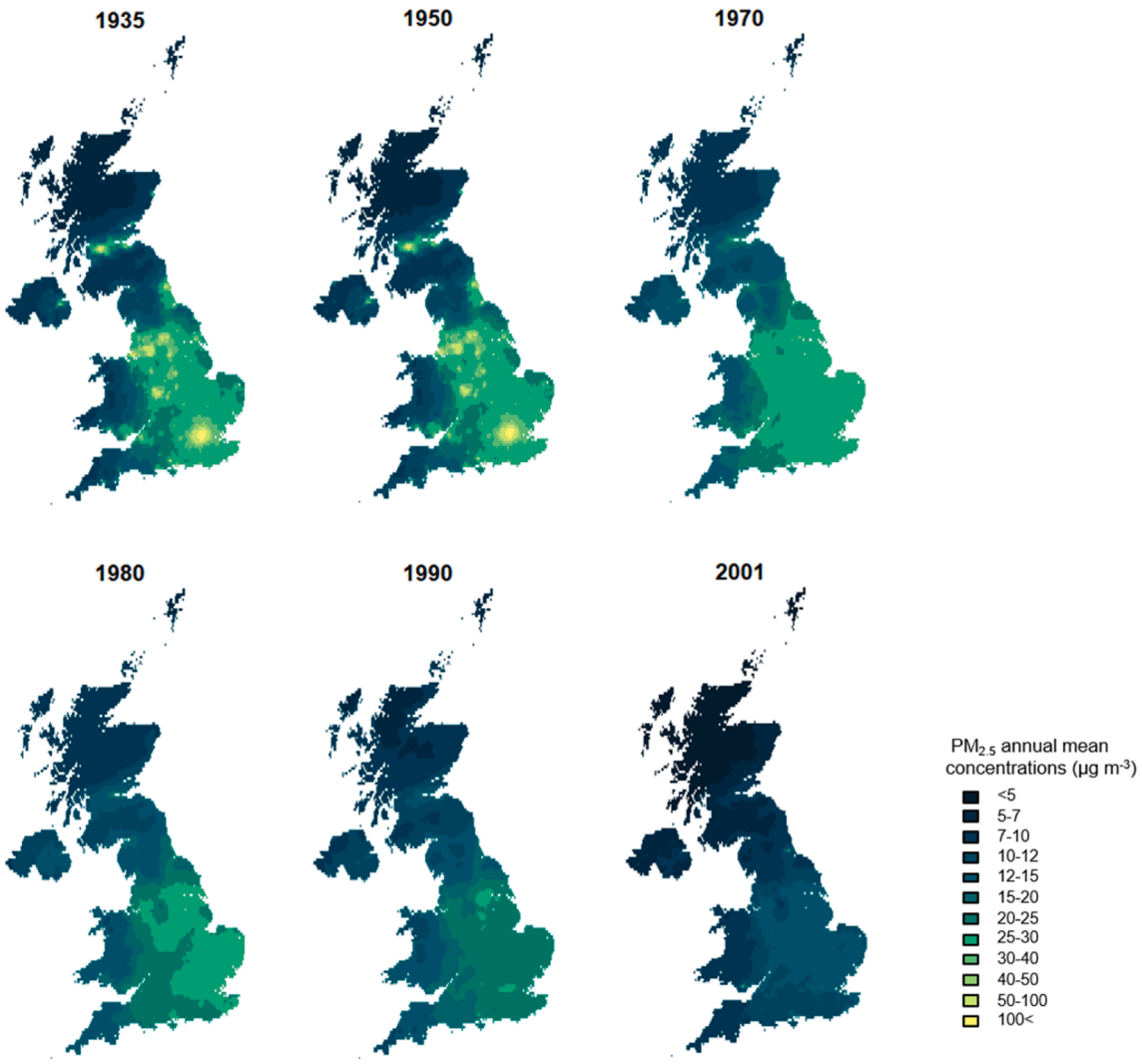

Fig. 1. $PM_{2.5}$ annual mean concentrations for the UK in 1935, 1950, 1970, 1980, 1990, and 2001 estimated with the EMEP4UK atmospheric chemistry transport model.

2.3. DNAm-based biomarkers

DNA methylation was derived from blood samples collected from LBC1936 participants in waves 1–4 (mean ages ~ 70, 73, 76, 79 years). Methylation was measured at 485,512 CpG sites using the Illumina HumanMethylation450BeadChips array; details are published elsewhere (Shah et al., 2014). Extensive quality control was carried out by removing (a) probes with low detection rate (<95%); (b) low-quality samples (e.g. inadequate hybridization); (c) samples with a low call rate (i.e. below 450,000 probes); and (d) samples where sex based on XY probes (predicted based on the median intensity of the X and Y chromosomes) did not match reported sex (Marioni et al., 2015; Shah et al., 2014). After quality control, there remained 895 samples in wave 1, 792 in wave 2, 611 in wave 3 and 499 in wave 4.

Five biomarkers were computed from the available DNA methylation data using an online calculator (https://dnamage.genetics.ucla.edu/) (Horvath, 2013). First generation clocks derived from chronological age included (1) Horvath's multi-tissue epigenetic clock based on 353 CpGs (Horvath DNAmAge) (Horvath et al., 2012), and (2) Hannum's epigenetic clock based on 71 CpGs (Hannum DNAmAge) (Hannum et al., 2013). We included also two second generation clocks: (3) DNAm PhenoAge, where CpGs were identified based on a composite measure of phenotypic age (Levine et al., 2018), and (4) DNAm GrimAge, a predictor of mortality trained on time-to-death, which is derived from a linear combination of age, sex, and DNAm surrogates for seven plasma proteins and smoking pack-years (Lu et al., 2019). Finally, (5) a DNA methylation-based proxy for telomere length (DNAmTL) was derived based on 140 CpGs selected by regressing leukocyte telomere length on methylation data (Lu et al., 2019).

2.4. Covariates

Covariates are presented in a directed acyclic graph (DAG) taking into consideration the years of air pollution exposure and the timing of covariates during the life course (Fig. 2). We considered age at the time of outcome assessment, sex (male, female) and parental occupational social class (OSC) (professional-managerial [I/II] versus skilled, partly skilled and unskilled [III/IV/V]) (Office of Population Censuses and Surveys, 1980) as common confounders for all life-course models. Childhood smoking (initiating $\leq$ 16 years; yes, no) was considered as a confounder from adolescence, years spent in full-time education from young adulthood, adult smoking (initiating $>$ 16 years; yes, no) and adult OSC (I/II versus III/IV/V) (Office of Population Censuses and Surveys, 1980) from middle adulthood onwards, whereas BMI at age 70 was considered as a confounder during late adulthood.

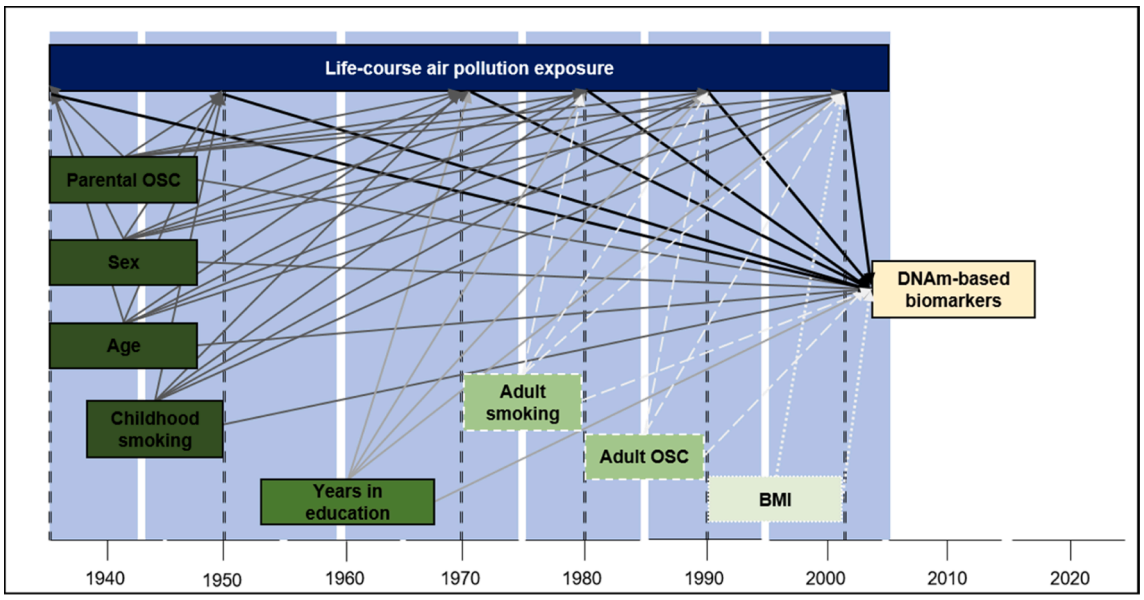

Fig. 2. Directed acyclic graph depicting the associations between air pollution exposure across the life course, markers of biological ageing, and their life-course confounders. Double dashed lines are years with modelled air pollution concentrations (i.e. EMEP4UK atmospheric chemistry transport models), light blue bars show time bands where addresses were linked to a respective air pollution modelling year. Differently shaded covariates and arrows indicate time-specific confounding: dark green covariates are confounders from childhood, medium green covariates from young adulthood, dashed light green covariates from middle adulthood, and dotted light green covariates from late adulthood onwards. Arrows present associations, black solid arrows are the associations of interest. Links between covariates are not shown for simplicity. BMI = body mass index; OSC = occupational social class.

2.5. Statistical analysis

To explore the associations between exposure to air pollution across the life course and DNAm-based biomarkers in late adulthood, we applied the two-stage structured life-course modelling approach originally developed by Mishra et al (Mishra et al., 2009) and modified by Smith et al (Smith et al., 2016) for continuous exposures. As different life-course models might be appropriate for biological ageing among males and females (Ward-Caviness et al., 2016; Marini et al., 2020), sex-stratified models are also presented. A flowchart outlining analyses can be found in Fig. S2.

In the first stage, the life-course model(s) most strongly supported by the observed data were selected from multiple simultaneously competing ones by applying the least angle regression (LARS) (Smith et al., 2016). LARS is a variable selection algorithm which implements the least absolute shrinkage and selection operator (lasso), and indicates the best lasso fit for each number of selected variables (Efron et al., 2004; Smith et al., 2015). This approach always identifies the variable with the strongest association to the outcome in the observed data, and then it selects further variables based on their strength of association, applying an absolute value penalty; the process continues until all variables have been selected (Smith et al., 2015). In order to identify the most appropriate variable(s) supported by LARS, we used the covariance test for the lasso indicating whether additional variables significantly ($p < 0.05$) improve explained outcome variance (Lockhart et al., 2014). Five biomarkers were tested in our study; to minimise type 1 errors arising from multiple comparisons, we provided false discovery rate (FDR) adjusted p-values (p_{FDR}) for the covariance test across outcomes. As LARS cannot accommodate multilevel data structure with individuals having repeated outcome measurements, we conducted model selection for one wave of DNAm-based biomarkers by choosing the wave with the largest sample size. This approach is based on a causal assumption concerning the exposure, outcome and confounders and requires having the same measurement error for all life-course models (Smith et al., 2016).

We investigated seven life-course models, which were inputted as variables into LARS (Smith et al., 2016). Six sensitive periods (SP_i) captured average exposure to air pollution at the six measurement periods outlined in section 2.2. ($SP_{1935} = \overline{X}_{1936-1942}$; $SP_{1950} = \overline{X}_{1943-1959}$; $SP_{1970} = \overline{X}_{1960-1975}$; $SP_{1980} = \overline{X}_{1976-1985}$; $SP_{1990} = \overline{X}_{1986-1995}$; $SP_{2001} = \overline{X}_{1996-2006}$). Accumulation of air pollution exposure across the life course was conveyed as the sum of sensitive periods, weighted with the number of years (t_i) spent in the respective period ($A = \sum_i t_i SP_i; i = 1935, 1950, 1970, 1980, 1990, 2001$). We found stronger correlations between sensitive periods closer to each other in time, and between sensitive periods and accumulation (Fig. S4). To adjust for confounding before model selection, we produced model residuals after regressing life-course models on their specific confounders picked individually using the DAG (Table S1).

In the second stage, we estimated effect sizes for selected life-course models utilising linear mixed-effects regression with random intercepts making use of all available epigenetic data across waves 1 and 4. Random slopes for chronological age were not considered as trajectories because age acceleration did not change significantly during shorter follow-ups (Marioni et al., 2015). All presented models in this stage were adjusted for white blood cell proportions as fixed effects (i.e. CD8T, CD4T, NK, Bcell, Mono, Gran), extracted from the same blood sample as for DNAm using Houseman's algorithm (Houseman et al., 2012). Methylation data were generated across three separate set of laboratory experiments (Table S2); position on array, plate and set of experiment were included in the models as random effects (other technical variables were dropped due to high collinearity). Moreover, we adjusted for the same life-course specific confounders in the regression as used in the model selection stage. In addition to primary findings for the AQI (coefficients expressed as 1-unit increase), we also reported results separately for $PM_{2.5}$, SO_2, NO_2, and O_3 (expressed as *1-$\mu g\ m^{-3}$*). To aid the comparison of the magnitude of associations across all findings, we presented standardized coefficients as βs. Potential sex-differences identified in the male and female samples were formally investigated in the non-stratified sample using the interaction term of AQI × sex.

Five sets of analyses were carried out to assess the sensitivity and robustness of our findings. First, we reran model selection using the second largest wave to verify life-course models identified in the main analysis. Second, instead of accounting for relevant life-course

confounders in the model selection stage, we regressed life-course models on all confounders (i.e. age, [sex], parental OSC, childhood smoking, years spent in education, adult smoking, adult OSC, BMI) to reduce the likelihood of unmeasured confounding (e.g. there is high correlation between BMI assessments from adolescence onwards (Simmonds et al., 2016), but LBC1936 only captures BMI data from late adulthood). After selecting the best-fit life-course models, we also presented main models with adjustments for all confounders. Third, we ran two-pollutant models to explore co-pollutant confounding, whereby each pollutant was added as a covariate in the models for every other pollutant and calculated variance inflation factors to report collinearity between them. Fourth, in a *post-hoc* analysis we estimated all life-course models for the outcomes identified as being associated with air pollution, to explore missed life-course models and help the interpretation of findings (p_{FDR} are also provided). Last, air pollution exposure around 1935 was originally derived from addresses between 1936 and 1942; in further *post-hoc* analysis we presented findings for relevant models using 1936 addresses only, providing a stronger case for exposure around birth and excluding exposures overlapping with World War II.

All analyses were conducted in R 4.1.0. (R Core Team. R, 2021).

3. Results

3.1. Study sample

The sample included 525 individuals; 437 participated in wave 1, 489 in wave 2, 455 in wave 3 and 401 in wave 4. The majority of excluded LBC1936 participants dropped out before the lifegrid questionnaire was distributed in 2014 ($n = 387$) or did not provide address history ($n = 111$). A comparatively small number of individuals had missing information on air pollution exposure in at least one time period, due to living outside of the UK ($n = 22$), or missing covariate or outcome data ($n = 46$) (Fig. 3). Excluded individuals were more likely to have smoked in childhood, had higher BMI at age 70 and more likely belonged to the 'skilled, partly skilled and unskilled' OSC in adulthood (Table S3). Descriptive statistics for the total sample and stratified by sex are presented in Table 1. Between waves 1 and 4, participants aged on average 10 years; the biological ageing process during these years materialised in increasing epigenetic clock estimates and shortening DNAmTL. Correlation coefficients between markers of biological ageing were moderate-to-strong – for DNAmTL the direction was negative (Fig. S4). There was also high correlation between the same biomarkers measured across waves ($r > 0.49$) (Fig. S5). Table S4 shows white blood cell proportions across waves. Participants' residential exposure to air pollution changed markedly during their life course, with $PM_{2.5}$ and SO_2 levels monotonically dropping from the 1950s, whereas exposure to NO_2 and O_3 increased across the life course (Fig. 4; Table S5).

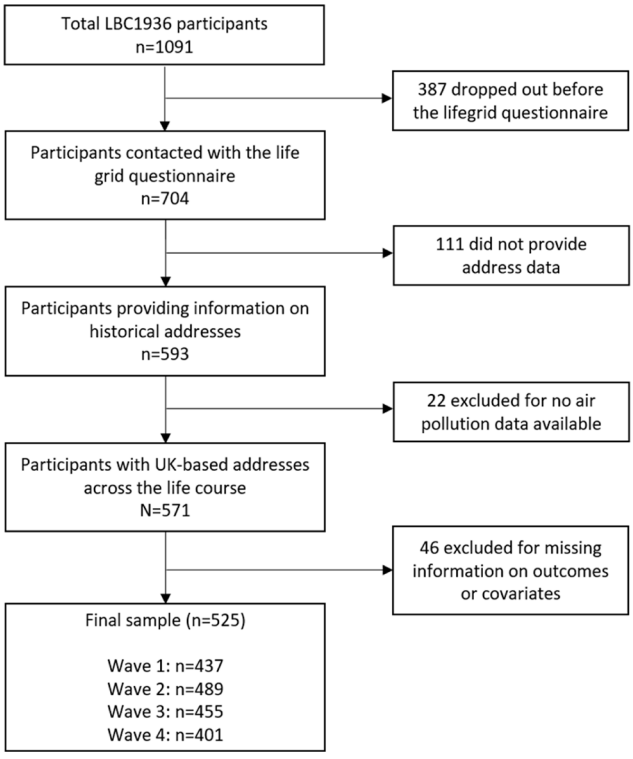

Fig. 3. Flowchart indicating sample selection. We utilised wave 2 data for model selection, the complete sample with repeated measurements for model estimation. LBC1936 = Lothian Birth Cohort 1936.

3.2. Stage 1: Identifying best-fit life-course models

For the model selection stage, we utilised outcome data from wave 2 as it provided the largest sample size ($n = 489$), thus allowing the best approximation of the total sample. The covariance test for the lasso indicated that air pollution exposure around 1970 reduced outcome variance for Horvath DNAmAge ($R^2 = 0.009$), accounting for 0.9% of the residual variance after adjusting for life-course confounders selected based on the DAG (i.e. age, sex, parental OSC, childhood smoking, years spent in education, adult smoking). In the non-stratified sample, air pollution was not associated with any other biomarkers (Table 2). Among males ($n = 265$), we found that a sensitive period around 1980 was the most appropriate life-course model for DNAmTL, accounting for 3.1% of the residual variance ($R^2 = 0.031$). In the female subsample ($n = 224$), air pollution exposure around 1935 explained 2.6% of the residual variance in DNAmTL ($R^2 = 0.026$) (Table 2).

3.3. Stage 2: Estimating best-fit life-course models

Selected life-course models (i. sensitive period around 1970 for Horvath DNAmAge; ii. sensitive period around 1980 for DNAmTL among male; and iii. sensitive period around 1935 for DNAmTL among female) were estimated in mixed-effects regressions using all available epigenetic data ($n = 525$; $obs = 1782$) and adjusting for white blood cell proportions, technical variables and life-course specific confounders.

We found that 1-unit increase in AIQ around 1970 was associated with 0.322 years (95% CI: 0.088, 0.555) higher epigenetic age measured in Horvath DNAmAge (Table 3); no sex differences were identified ($b = 0.072$, 95% CI: −0.399, 0.542; Fig. S6). Estimating the associations for single air pollutants showed that 1 μg m^{-3} increase in $PM_{2.5}$, SO_2 and NO_2 levels around 1970 was associated with an epigenetic age increase of 0.299 (95% CI: 0.046, 0.552), 0.078 (95% CI: 0.005, 0.151) and 0.115 (95% CI: 0.010, 0.220) years, respectively (Table 3).

Among males ($n = 277$; $obs = 950$), 1-unit increase in AQI around 1980 was associated with 0.015 kilobase (95% CI −0.027, −0.004) reduction in DNAmTL. A 1 μg m^{-3} increase in $PM_{2.5}$ and NO_2 levels around 1980 was associated with −0.018 kilobase reduction (95% CI: −0.034, −0.003) and −0.007 kilobase reduction (95% CI: −0.012, −0.002) respectively, while 1 μg m^{-3} increase in O_3 was associated with 0.012 kilobase increase (95% CI: 0.001, 0.023) in estimated telomere length (Table 3). In the female subsample ($n = 248$; $obs = 832$), we found that 1-unit increase in AQI around 1935 was associated with an estimated telomere attrition of −0.017 kilobase (95% CI −0.028, −0.006); higher $PM_{2.5}$ (−0.002, 95% CI: −0.004, −0.001), SO_2 (−0.001, 95% CI: −0.002, −0.000) and NO_2 (−0.006, 95% CI: −0.010, −0.002) exposure with shorter, and higher O_3 (0.015, 95% CI: 0.004, 0.027) with longer estimated telomeres (Table 3) (note that correlation coefficients between pollutants were > 0.9 in 1935). Testing for sex differences found DNAmTL attrition only present among males when exposed to higher air pollution around 1980 (AQI: $b = −0.018$, 95% CI: −0.035, −0.001); but we could not confirm a clear sex-difference in the 1935 air pollution and DNAmTL relationship ($b = 0.011$, 95% CI: −0.005, 0.026) (Fig. S6).

Table 1
Descriptive statistics for the analytical sample, Lothian Birth Cohort 1936.

Characteristics	Total (n = 525)	Male (n = 277)	Female (n = 248)	p
Parental occupational social class, n (%)				
I and II	141 (26.86%)	77 (27.80%)	64 (25.81%)	
III, IV and V	384 (73.14%)	200 (72.20%)	184 (74.19%)	0.678
Childhood smoking (≤16 years), n (%)				
Yes	437 (83.24%)	68 (24.55%)	20 (8.06%)	
No	88 (16.76%)	209 (75.45%)	228 (91.94%)	<0.001
Years spent in education (mean ± SD)	10.77 ± 1.10	10.78 ± 1.12	10.77 ± 1.08	0.924
Adult smoking (>16 years), n (%)				
Yes	174 (33.14%)	88 (31.77%)	86 (34.68%)	
No	351 (66.86%)	189 (68.23%)	162 (65.32%)	0.539
Adult occupational social class, n (%)				
I and II	321 (61.14%)	160 (57.76%)	161 (64.92%)	
III, IV and V	204 (38.86%)	117 (42.24%)	87 (35.08%)	0.112
BMI at wave 1 (mean ± SD)	27.39 ± 4.04	27.68 ± 3.72	27.06 ± 4.35	0.082
Chronological age (mean ± SD)				
Wave 1[a]	69.50 ± 0.84	69.50 ± 0.83	69.50 ± 0.85	0.973
Wave 2[b]	72.47 ± 0.71	72.47 ± 0.70	72.49 ± 0.71	0.641
Wave 3[c]	76.24 ± 0.69	76.27 ± 0.68	76.21 ± 0.69	0.376
Wave 4[d]	79.27 ± 0.62	79.27 ± 0.60	79.26 ± 0.64	0.861
Horvath DNAmAge (mean ± SD)				
Wave 1[a]	64.78 ± 7.21	65.22 ± 7.26	64.27 ± 7.14	0.172
Wave 2[b]	68.10 ± 6.69	68.54 ± 6.29	67.58 ± 7.11	0.116
Wave 3[c]	72.13 ± 6.51	72.89 ± 6.75	71.27 ± 6.13	0.007
Wave 4[d]	74.78 ± 5.95	75.58 ± 6.46	73.87 ± 5.19	0.003
Hannum DNAmAge (mean ± SD)				
Wave 1[a]	71.43 ± 5.67	72.65 ± 5.58	70.03 ± 5.45	<0.001
Wave 2[b]	73.01 ± 5.77	74.40 ± 5.62	71.38 ± 5.51	<0.001
Wave 3[c]	77.64 ± 5.74	79.09 ± 5.84	75.99 ± 5.17	<0.001
Wave 4[d]	82.39 ± 5.56	84.43 ± 5.93	80.10 ± 4.04	<0.001
DNAm GrimAge (mean ± SD)				
Wave 1[a]	66.64 ± 4.70	68.54 ± 4.34	64.48 ± 4.15	<0.001
Wave 2[b]	69.57 ± 4.64	71.46 ± 4.34	67.34 ± 3.95	<0.001
Wave 3[c]	72.60 ± 4.91	74.59 ± 4.66	70.35 ± 4.17	<0.001
Wave 4[d]	75.37 ± 4.56	77.34 ± 4.26	73.20 ± 3.85	<0.001
DNAm PhenoAge (mean ± SD)				
Wave 1[a]	66.31 ± 7.17	67.07 ± 7.02	65.44 ± 7.26	0.018
Wave 2[b]	66.85 ± 7.09	67.38 ± 6.26	66.22 ± 7.92	0.078
Wave 3[c]	70.43 ± 7.78	71.09 ± 7.69	69.68 ± 7.83	0.054
Wave 4[d]	73.38 ± 6.62	74.65 ± 7.05	71.94 ± 5.81	<0.001
DNAmTL (mean ± SD)				
Wave 1[a]	6.75 ± 0.22	6.68 ± 0.20	6.83 ± 0.21	<0.001
Wave 2[b]	6.71 ± 0.21	6.65 ± 0.21	6.78 ± 0.20	<0.001
Wave 3[c]	6.62 ± 0.22	6.56 ± 0.22	6.69 ± 0.21	<0.001
Wave 4[d]	6.55 ± 0.22	6.48 ± 0.22	6.63 ± 0.20	<0.001

P-values are based on two-sample t-tests for mean difference, and chi-squared tests for differences in distribution. DNAmTL = DNAm based telomere length; SD = standard deviation.
[a] Sample size n = 437.
[b] Sample size n = 489.
[c] Sample size n = 455.
[d] Sample size n = 401.

Finally, fully standardized coefficients are presented in Fig. 5. This indicates that all associations were of small effect size (Sullivan and Feinn, 2012). Associations with DNAmTL were generally numerically stronger than for Horvath DNAmAge, and coefficients for O_3 were weaker compared to $PM_{2.5}$, SO_2 and NO_2. (However, these were not formally tested, and we point out that the 95% CIs overlap in all cases.).

3.4. Sensitivity and robustness analyses

LARS identified the exact same life-course models when the second largest wave (i.e. wave 3) was used for model selection (Table S6). Similarly, adjusting life-course models with all confounders independently of their timing during the life course (i.e. sex, age, parental OSC, childhood smoking, years spent in full-time education, adult smoking, adult OSC, and BMI) resulted in a similar selection of life-course models as in the main analysis (Table S7); estimating models after these adjustments did not alter the results (Table S8). In two-pollutant models, we did not find evidence for one pollutant having an independent effect above any other (Table S9). Rather the single pollutant models with comparable effect sizes and the two-pollutant models with high collinearity indicated that the generalised effect of air pollution cannot be attributed to any single component of poor air quality in the current sample. Still, we observed that around 1935 and 1970 $PM_{2.5}$ and SO_2, and around 1980 NO_2 and $PM_{2.5}$, had stronger associations in comparison to their co-pollutants, and O_3 was always more weakly associated with biological ageing markers.

We explored *post-hoc* whether there were other life-course models associated with DNAmAge and DNAmTL not identified through LARS model selection (see Fig. S7 for standardized effect sizes). We found that in addition to exposure around 1970 ($\beta = 0.078$, 95% CI: 0.021, 0.135; $p = 0.007$; $p_{FDR} = 0.049$), air pollution around 1980 was associated with Horvath DNAmAge ($\beta = 0.060$, 95% CI: 0.003, 0.118; $p = 0.041$; $p_{FDR} = 0.126$) suggesting a longer sensitive period stretching from young-to-middle adulthood, especially among males. DNAm estimation of telomere shortening was not only associated with 1980 exposure among males ($\beta = -0.131$, 95% CI: −0.229, −0.033; $p = 0.010$; $p_{FDR} = 0.059$)

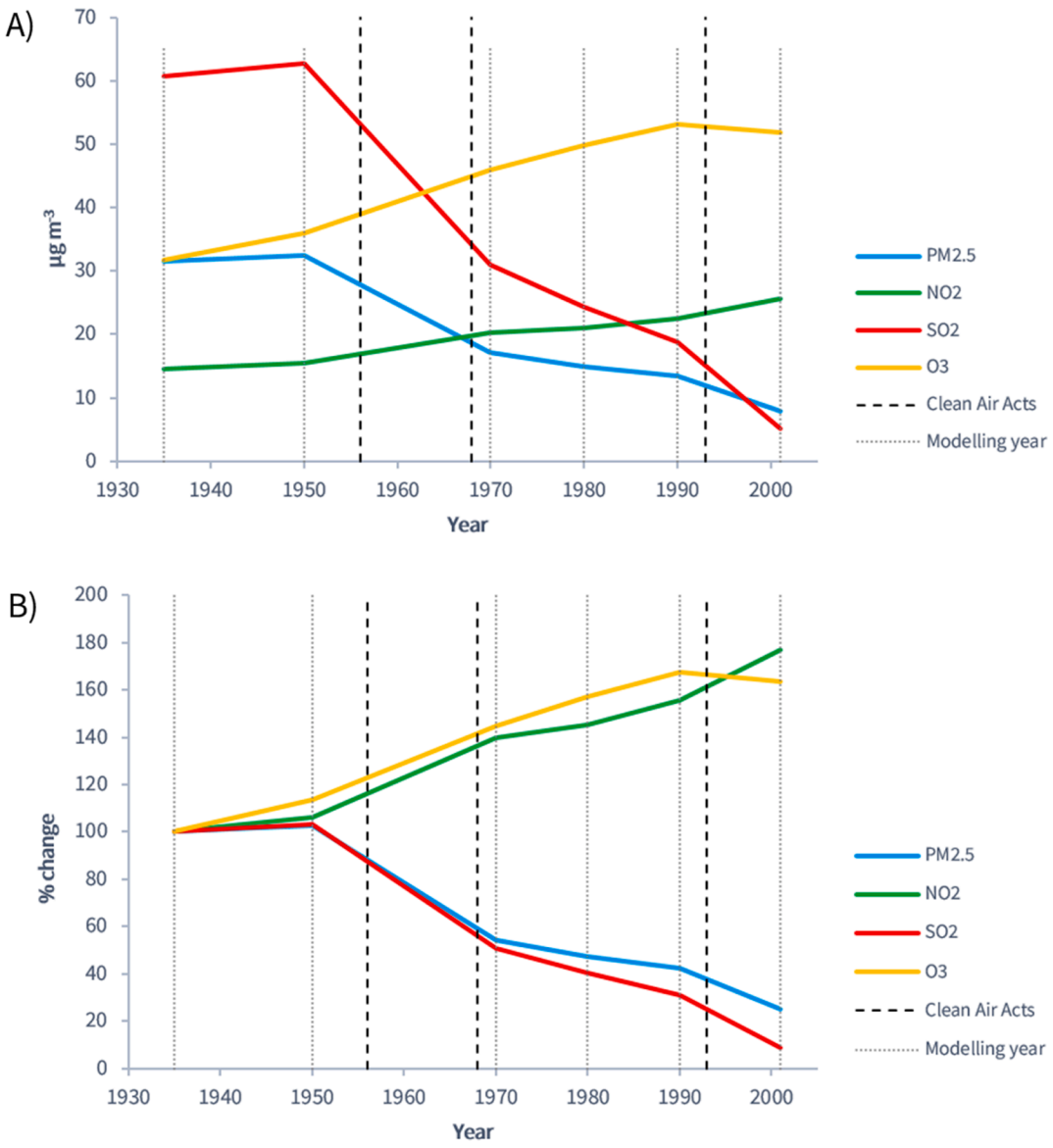

Fig. 4. Average exposure to air pollution across participants of the Lothian Birth Cohort 1936, expressed as (A) absolute values (µg m^{-3}) and as (B) relative change to 1935 levels (n = 525). Concentrations were estimated using the EMEP4UK atmospheric chemistry transport models for the modelling years of 1935, 1950, 1970, 1980, 1990, and 2001 (dotted lines). Clean Air Acts of 1956, 1968 and 1993 – key legislative triggers for the implementation of policy measures to reduce emissions of air pollutants and resulting in the reduction of ambient pollutant concentrations over the study period – are signalised as dashed lines. Recommended WHO air quality guideline levels (2021) are 5 µg m^{-3} for annual $PM_{2.5}$, 10 µg m^{-3} for annual NO_2 exposure. For SO_2 and O_3, shorter averaging times are available with the 24-hour level of 40 µg m^{-3} for SO_2, and the peak season level of 60 µg m^{-3} for O_3.

Table 2
Selecting best-fit life-course models for the association between air pollution exposure (measured with air quality index) and DNAm-based biomarkers in the Lothian Birth Cohort 1936.

Outcome	Total (n = 489)				Male (n = 265)				Female (n = 224)			
	Model	R^2	p	p_{FDR}	Model	R^2	p	p_{FDR}	Model	R^2	p	p_{FDR}
Horvath DNAmAge	SP 1970	0.009	0.046	0.230	SP 1970	0.007	0.287	0.478	SP 1980	0.003	0.734	0.953
Hannum DNAmAge	SP 1950	0.003	0.468	0.585	SP 1950	0.004	0.526	0.619	SP 1990	<0.001	0.953	0.953
DNAm GrimAge	SP 1935	0.003	0.438	0.585	Accumulation	0.007	0.274	0.478	SP 1935	0.002	0.773	0.953
DNAm PhenoAge	SP 1980	0.006	0.154	0.385	SP 1980	0.003	0.619	0.619	SP 1980	0.006	0.492	0.953
DNAmTL	SP 1980	0.001	0.873	0.873	SP 1980	0.031	0.003	0.013	SP 1935	0.026	0.009	0.044

Life-course models were progressively adjusted for confounders: SP 1935 for age, (sex,) parental occupational social class; SP 1950 additionally for childhood smoking; SP 1970 additionally for years spent in education; SP 1980 additionally for adult smoking; SP 1990 additionally for adult occupational social class; SP 2001 and Accumulation additionally for BMI. We provide false discovery rate adjusted p-values (p_{FDR}). DNAmTL = DNAm telomere length; SP = sensitive period.

Table 3
Associations between air pollution exposure and DNAm-based biomarkers for selected life-course models in the Lothian Birth Cohort 1936.

Exposure	Total (n = 525, *obs* = 1782) Horvath DNAmAge[a] Air pollution in 1970		Male (n = 277, *obs* = 950) DNAmTL[b] Air pollution in 1980		Female (n = 248, *obs* = 832) DNAmTL[c] Air pollution in 1935	
	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p
Air Quality Index	0.322 (0.088, 0.555)	0.007	−0.015 (−0.027, −0.004)	0.010	−0.017 (−0.028, −0.006)	0.003
Air pollutants (in 1-$\mu g\ m^{-3}$)						
$PM_{2.5}$	0.299 (0.046, 0.552)	0.021	−0.018 (−0.034, −0.003)	0.019	−0.002 (−0.004, −0.001)	0.003
SO_2	0.078 (0.005, 0.151)	0.038	−0.004 (−0.009, 0.000)	0.057	−0.001 (−0.002, −0.000)	0.003
NO_2	0.115 (0.010, 0.220)	0.032	−0.007 (−0.012, −0.002)	0.006	−0.006 (−0.010, −0.002)	0.007
O_3	−0.185 (−0.439, 0.069)	0.153	0.012 (0.001, 0.023)	0.041	0.015 (0.004, 0.027)	0.012

Models were fitted with mixed-effects regression with random intercepts for study participants. All models were adjusted for white blood cell proportions as fixed (CD8T, CD4T, NK, Bcell, Mono, Gran) and technical variables as random effects (set, position, plate). Abbreviations: DNAmTL = DNAm telomere length.
[a] Models were adjusted for sex, age, parental occupational social class, childhood smoking, and years spent in education.
[b] Models were adjusted for age, parental occupational social class, childhood smoking, years spent in education, and adult smoking.
[c] Models were adjusted for age and parental occupational social class.

but also with accumulated air pollution exposure ($\beta = -0.120$, 95% CI: −0.218, −0.022; $p = 0.017$; $p_{FDR} = 0.059$). In the female subsample, only air pollution exposure around 1935 was associated with DNAmTL ($\beta = -0.151$, 95% CI: −0.249, −0.053; $p = 0.003$; $p_{FDR} = 0.021$), with a standardized effect size of > 3times greater than for any other life-course model. This relatively strong association likely explained why the association became also significant in the total sample for this sensitivity analysis. Moreover, when we matched 1935 air pollution estimates with 1936 addresses only (instead of addresses between 1936 and 1942), the magnitude of association between the revised AQI around 1935 and DNAmTL increased by approximately 25% ($\beta = -0.188$, 95% CI: −0.286, −0.090; $p < 0.001$), highlighting the likelihood of a critical period around birth.

4. Discussion

Our study based on 525 older Scottish adults examined the relationship between life-course air pollution exposure and DNAm-based biomarkers. Out of a large number of tested hypotheses, our main analyses identified three key life-course associations, reporting a link between greater exposure to air pollution during sensitive time windows across the life course and older-appearing markers of biological ageing in later life. Exposure to air pollution in young-to-middle adulthood was associated with epigenetic age measured with Horvath's epigenetic clock. Shorter estimated telomere lengths were evident among males with higher exposure to air pollution in mid-adulthood, and we also found some evidence for an accumulating impact of air pollution across the life course. Among females, air pollution only around birth was linked to estimated telomere attrition. Effect estimates reported across this study were small in magnitude.

Associations between exposure to air pollution and DNAm among various age groups have been reported previously (see (Rider and Carlsten, 2019; Ferrari et al., 2019) for reviews). However, those studies focussing on epigenetic clocks mainly utilised samples capturing middle-aged and older adults alongside contemporaneous exposure measures. An investigation utilising the US-based Normative Ageing Study, an all-male cohort of 589 individuals in their 70s, found that $PM_{2.5}$ exposure was associated with Horvath DNAmAge (Nwanaji-Enwerem et al., 2016). Further analyses suggested that CpGs contributing to this association were mapping genes involved in lung pathologies (Nwanaji-Enwerem et al., 2016) and, among the heterogeneous chemical components of $PM_{2.5}$, sulphate and ammonium were most associated with accelerated ageing (Nwanaji-Enwerem et al., 2017). Other studies using the same dataset did not find an association between long-term $PM_{2.5}$ exposure and Hannum DNAmAge (Nwanaji-Enwerem et al., 2017) or DNAm PhenoAge (Wang et al., 2020); but two chemical components of $PM_{2.5}$ (i.e. lead and calcium) led to accelerated ageing measured with DNAm PhenoAge (Wang et al., 2020). The KORA study in Germany with 1777 older participants confirmed the link between higher annual $PM_{2.5}$ exposure at the time of data collection and extrinsic epigenetic age acceleration derived from Horvath's epigenetic clock; however, associations (and their direction) differed between males and females (Ward-Caviness et al., 2016). More recently, findings based on 2747 women aged 35–74 in the Sister Study from the United States indicated age acceleration using Hannum DNAmAge when exposed to higher NO_2 levels, while only clusters of $PM_{2.5}$ components were associated with Horvath DNAmAge and DNAm PhenoAge (White et al., 2019). Whereas these previous reports described the associations between air pollution exposure and faster epigenetic clocks, none has been able to look at how exposure at different epochs relates to DNAm-based biomarkers in older age. Our study not only confirms the relationship between epigenetic ageing based on Horvath DNAmAge among individuals exposed to higher air pollution (Nwanaji-Enwerem et al., 2016; Ward-Caviness et al., 2016), but also extends the literature suggesting a sensitive period in young-to-middle adulthood. In contrast to previous findings (White et al., 2019; Wang et al., 2020), we were unable to confirm associations between air pollution and DNAm PhenoAge or Hannum DNAmAge, which may be related to our comparably smaller sample size or unobserved cohort-specific characteristics.

We found shorter DNAmTL among older males exposed to higher air pollution exposure in middle adulthood, with some evidence suggesting also the detrimental impact of accumulated life course exposure. These findings corroborate and extend prior work relating to leukocyte telomere length. Long-term exposure to air pollution is associated with shorter telomere length, whereby pollution likely increases the replication rate of cells and telomere loss during cell replication (Miri et al., 2019). Findings from the KORA study indicated sex-differences in the air pollution (i.e. black carbon) and telomere length relationship, with significant attrition found only among males (Ward-Caviness et al., 2016). DNAmTL is a robust measure of telomere-associated ageing, which is related to cell replication and cellular ageing, distinct from epigenetic ageing (Lu et al., 2019). CpGs used to derived epigenetic clocks and DNAmTL are not overlapping; CpGs for DNAmTL are located near cadherin and cell signalling genes (Lu et al., 2019). DNAmTL has been shown to outperform leukocyte telomere length in predicting health-related outcomes (Lu et al., 2019); and, to our knowledge, it has not been used before to explore the air pollution-biological ageing relationship.

A key finding of this study showed that exposure to air pollution around birth was associated with shorter DNAmTL among females. This is supported by a recent systematic review concluding that prenatal exposure to air pollution is linked to global and specific alterations in DNAm levels and to telomere attrition, whereby the beginning of the pregnancy is a potentially susceptible period (Isaevska et al., 2021).

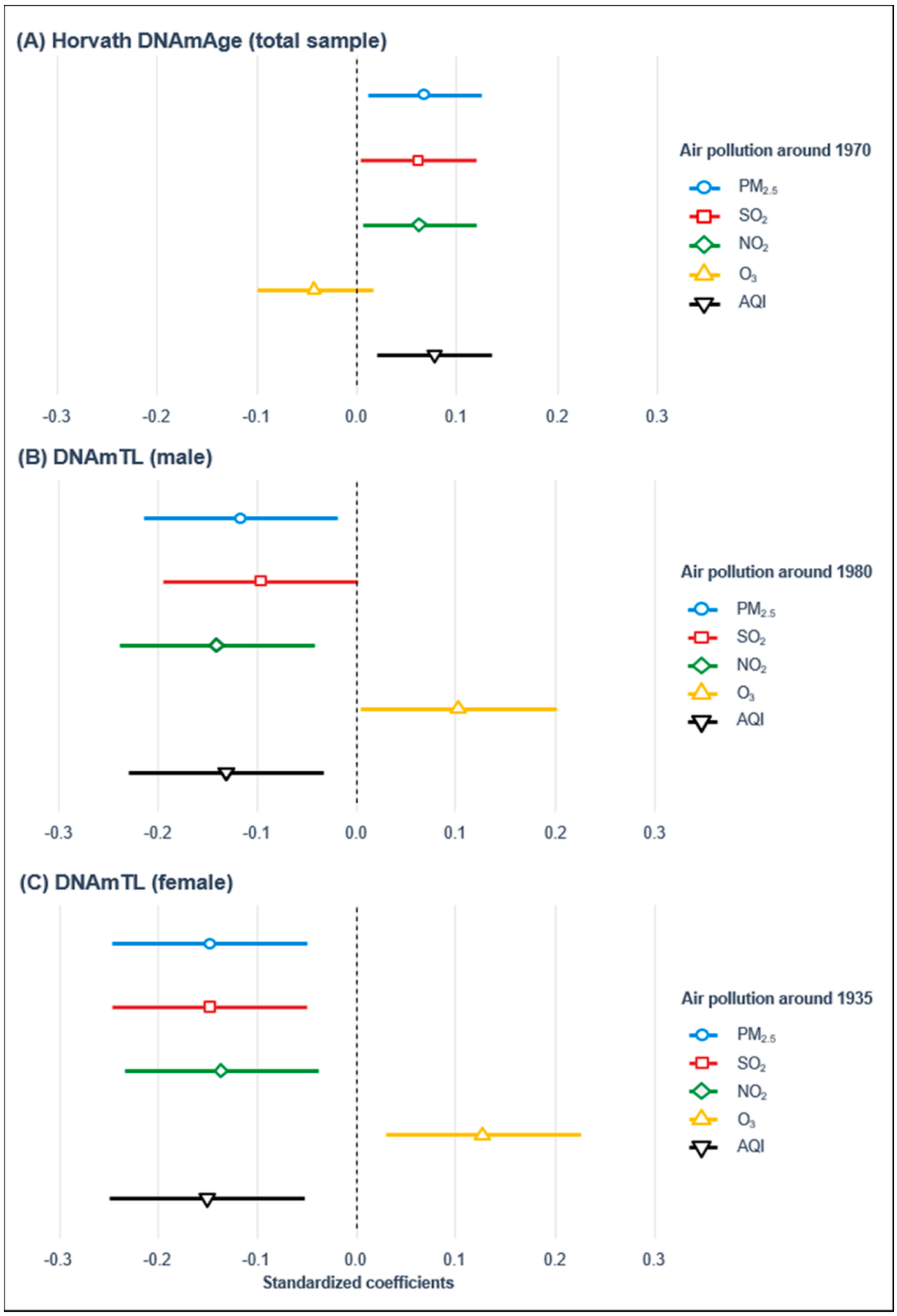

Fig. 5. Standardized effect sizes (β and their 95% CIs) providing comparable estimates for the associations between air pollution exposure and DNAm-based biomarkers in the Lothian Birth Cohort 1936 (n = 525). In addition to white blood cell proportions (CD8T, CD4T, NK, Bcell, Mono, Gran) and technical variables (set, position, plate), Model A was adjusted for sex, age, parental occupational social class, childhood smoking, and years spent in education; Model B for age, parental occupational social class, childhood smoking, years spent in education, and adult smoking; and Model C for age, and parental occupational social class. Abbreviations: AQI = air quality index; DNAmTL = DNAm telomere length.

Particles can translocate into or across the placenta and induce oxidative stress; production of reactive oxygen species leads to DNA damage and epigenetic alterations (Saenen et al., 2019). In turn, changes in methylation levels in the embryonic development are associated with abnormal development (Yin et al., 2012). Mediation analyses have confirmed the role of DNAm in the pathway between air pollution exposure and foetal growth (Zhao et al., 2021), and later life health outcomes (Sbihi et al., 2019). In our study, exposure around birth was associated with DNAmTL mainly among females (although sex differences were not significant), which is not unexpected given sex differences in the impact of air pollution on pregnancy outcomes (Ghosh et al., 2007). In line with our finding, a study from Mexico found shorter leukocyte telomere length among female but not male newborns after higher maternal $PM_{2.5}$ exposure (Rosa et al., 2019). Similarly, prenatal exposure to organic pollutants in the Shanghai Allergy Cohort was linked to shorter leukocyte telomere length at birth among females, and mediation analyses highlighted the role of elevated oxidative stress (Liu et al., 2018).

The air we breathe typically contains a mixture of multiple pollutants. Although we provided estimates for $PM_{2.5}$, NO_2, SO_2 and O_3 separately, these were highly correlated making it impossible to properly disentangle their effects on DNAm-based biomarkers, which is generally challenging in the absence of experimental data. High correlation is not surprising, given that during the earlier years for our cohort by far the largest emission sources were from coal/fossil fuel combustion, leading to high spatial and temporal correlation between $PM_{2.5}$, SO_2 and NO_2; and the significant uncertainty of emissions for these modelling years. Weakening correlation over time might be explained by the introduction of clean air measures that particularly affected large, stationary combustion sources, and by road transport sources making a

larger contribution (Carnell et al., 2019). NO_2 and SO_2, for example, are emitted as primary pollutants, whereas $PM_{2.5}$ is a mixture of primary and other secondary pollutants; fossil fuel combustion sources contribute the major share of all three pollutants. O_3 is not directly emitted into the air, but it is produced through complex chemical reactions in the atmosphere (World Health Organization, 2021). Due to the titration effect, O_3 is depleted in areas where high NO_x emissions are present, which results to O_3 being negatively correlated with NO_2 (and to a smaller degree with $PM_{2.5}$ and SO_2). While there is some evidence of a positive association between O_3 exposure and leukocyte telomere length among critically ill patients (Wang et al., 2020), our unexpected findings on O_3 and slower biological ageing might be an artefact given the above presented correlation pattern between pollutants. Two-pollutant models further suggested that overall results were mainly driven by $PM_{2.5}$, NO_2, and SO_2, making a plausible positive association between O_3 and DNAmTL less likely.

Analyses in this study were based on over 500 individuals, 1700 epigenetic samples with 5 different DNAm-based biomarkers measured in older age, and on pollution exposure estimated across the lifecourse (Taylor et al., 2018). LBC1936 is a narrow-age birth cohort from a specific region of Scotland which lowers the risk of historical events causing spurious association by only affecting part of the sample. We utilised robust and validated methods of measuring different aspects of biological ageing; this is particularly important for telomere-related ageing, where the traditional methods of estimating leukocyte telomere length can be challenging (Lu et al., 2019). A unique feature of the cohort is the presence of life-course addresses (Pearce et al., 2018), which made it possible to link individual residence to historical air pollution concentrations. Whereas retrospective recall is well-known to be prone to bias, the lifegrid approach with lightbulb prompt indicates that residential address recall among older people shows good accuracy (Berney and Blane, 2003). We are unaware of any other datasets that can allow interrogation of these relationships in the same individuals with coverage from birth to the 8th decade of life. Our analytical approach was particularly useful when competing life-course hypotheses were equally plausible (Smith et al., 2016) and we were able to test these without over-inflating coefficients and biasing the hypothesis test (Lockhart et al., 2014). To further indicate the robustness of our findings, we provided FDR adjusted p-values.

Still, several limitations need to be considered that potentially affect the interpretation of the results. First, LBC1936 compiles an ethnically homogenous sample of Scottish adults, thereby limiting the generalizability of our findings. Moreover, address data were collected when participants were in their late 70s, leaving healthier individuals in our analytical sample, introducing not only selection but also survival bias (Taylor et al., 2018). Second, residential address in 1936 indicated by the participants may not correspond with pre-birth location, leading to exposure misclassification. Third, the selection of the best-fit life-course models was based on 489 (and 455 in sensitivity analysis) out of 525 participants, as DNAm data were not available for the complete sample in any of the follow-up waves. Unavailability of data for some participants might have reduced statistical power to identify all relevant life-course models. Fourth, despite lifegrid methods being a validated way of gathering historical residential addresses (Berney and Blane, 2003), recall inaccuracy might have led to underestimating potential associations. Fifth, due to lack of data we were unable to control for the effect of several key life-course confounders (e.g. health status in young adulthood) increasing the risk of residual confounding; similarly, lack of data on DNAm measurement across the life course should be also acknowledged. Sixth, there is a large degree of uncertainty when estimating historical concentrations of air pollution. While atmospheric chemistry transport models are routinely and widely evaluated against observations in present-day conditions, showing good agreement between modelled and observed concentrations (Lin et al., 2017; Ge et al., 2021), the further back in time emissions of relevant air pollutants have to be estimated, the larger uncertainties are with regard to their spatial distribution, as well as their volume. There are few, if any, reliable observations available prior to the 1970s; for some pollutants not before the late 1980s. Also, atmospheric chemistry transport models rely on meteorological driver data to represent atmospheric transport and chemical transformation processes and for this analysis one constant set of meteorological data has been used for all calculations (Skamarock et al., 2008). While these aspects contribute to uncertainties in the estimates in ambient air pollutant concentrations and thus exposures, the consistent model setup and handling of input data (i.e. anthropogenic emissions and meteorological drivers) means that relative changes in the spatial distribution of concentrations can be considered to be sufficiently accurate. Seventh, the spatial resolution of $\sim 5 \times 6$ km (World Bank, 2016) used in this study is likely too coarse, especially for urban areas, and may lead to underestimating exposures and health impacts (Korhonen et al., 2019). This is pertinent for the 2001 air pollution exposure estimate: while during their life course LBC1936 participants resided in various places across the UK, they all lived in the Lothian region of Scotland when the cohort was established in 2004, reducing the heterogeneity of exposure. Finally, we were only able to use residential addresses to estimate air pollution exposure; incorporating school, work and other key locations could have led to more precise findings.

Due to lack of previous life-course investigations on air pollution and markers of biological ageing, our findings are provisional and should be considered as hypotheses until future studies can explore how and why air pollution during specific life stages impacts males and females differently. Alternative explanations for reported associations include different toxicity and level of pollutants, as both the composition of aerosols and their magnitude changed substantially during the study period in response to changes in pollution source (e.g. domestic heating to motor vehicles) and the regulation of emissions. On the exposure, historical air pollution data at finer scale resolution may overcome the challenges originating from the very high correlation between pollutants and provide further heterogeneity of exposure. Collecting historical data on neighbourhood indicators could further address area-level confounding. Telomere length based on DNAm proved a valuable biomarker in our study; future investigations should further explore its utility in understanding how environmental exposures can 'get under the skin'. Finally, research should aim to replicate our results in larger, nationally representative cohort studies with more diverse populations and explore more thoroughly possible life-course confounders and mediators.

5. Conclusions

This study utilised historical air pollution concentrations of $PM_{2.5}$, SO_2, NO_2 and O_3 and applied the life-course approach for the first time to contribute to the understanding of air pollution and biological ageing. We found that exposure to lower air quality at earlier stages of the life (i.e. around birth, young-to-middle adulthood) can have a modest but detectable association with epigenetic and telomere-associated ageing in later life, which likely persists across the entire life course. This study demonstrated the utility of DNAmTL in environmental research, a biomarker of cellular ageing, which seems to be particularly susceptible to air pollution exposures. Future studies should explore options to refine historical air pollution data and reinforce our findings in larger cohorts. Policy actions at national-level targeting air pollution reduction can likely have long-lasting effects on the development of future generations, especially in light of findings on effects around birth, and contribute to healthy population ageing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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IJD, DLM, NS, SR, MV, SRC and JP obtained and managed the data for the study. GB, IJD, NS, CWT, SRC and JP conceived and designed the study. GB performed the statistical analyses and led the manuscript preparations, drafting and revision. All authors participated in the interpretation of the findings, critically revised the manuscript and approved the final version.

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Data-availability statement: The LBCs' study data have been the subject of many internal (within the University of Edinburgh) and external collaborations, which are encouraged. Those who have interests in outcomes other than cognitive domains are particularly encouraged to collaborate. Both LBC studies have clear data dictionaries which help researchers to discern whether the variables they wish to use are present; these provide a simple short title for each variable, alongside a longer, common-sense description/provenance of each variable. This information is available on the study website (https://www.ed.ac.uk/lothian-birth-cohorts) alongside comprehensive data grids listing all variables collected throughout both LBC studies and the wave at which they were introduced, an 'LBC Data Request Form' and example Data Transfer Agreement. Initially, the Data Request Form is e-mailed to the Lothian Birth Cohorts Director Dr Simon R. Cox for approval (via a panel comprising study co-investigators). Instances where approved projects require transfer of data or materials outside the University of Edinburgh require a formal Data Transfer Agreement or Material Transfer Agreement to be established with the host institution. The process is facilitated by a full-time LBC database manager – there is no charge.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107501.

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