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# Paternal age at birth is associated with offspring leukocyte telomere length in the nurses' health study

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**STUDY QUESTION:** Is the association between paternal age at birth and offspring leukocyte telomere length (LTL) an artifact of early life socioeconomic status (SES)?

**SUMMARY ANSWER:** Indicators of early life SES did not alter the relationship between paternal age at birth and offspring LTL among a population of white female nurses.

**WHAT IS KNOWN ALREADY:** Telomere length is considered a highly heritable trait. Recent studies report a positive correlation between paternal age at birth and offspring LTL. Maternal age at birth has also been positively associated with offspring LTL, but may stem from the strong correlation with paternal age at birth.

**STUDY DESIGN, SIZE AND DURATION:** The Nurses' Health Study (NHS) is an ongoing prospective cohort study of 121 700 female registered nurses who were enrolled in 1976. Great effort goes into maintaining a high degree of follow-up among our cohort participants (>95% of potential person-years). In 1989–1990, a subset of 32 826 women provided blood samples from which we selected participants for several nested case–control studies of telomere length and incident chronic disease. We used existing LTL data on a total of 4250 disease-free women who also reported maternal and paternal age at birth for this study.

**PARTICIPANTS/MATERIALS, SETTING AND METHODS:** Nested case–control studies of stroke, myocardial infarction, cancers of the breast, endometrium, skin, pancreas and colon, as well as colon adenoma, were conducted within the blood sub-cohort. Each study used the following study design: for each case of a disease diagnosed after blood collection, a risk-set sampling scheme was used to select from one to three controls from the remaining participants in the blood sub-cohort who were free of that disease when the case was diagnosed. Controls were matched to cases by age at blood collection (±1 year), date of blood collection (±3 months), menopausal status, recent postmenopausal hormone use at blood collection (within 3 months, except for the myocardial infarction case–control study), as well as other factors carefully chosen for each individual study. The current analysis was limited to healthy controls. We also included existing LTL data from a small random sample of women participating in a cognitive sub-study. LTL was measured using the quantitative PCR-based method. Exposure and covariate information are extracted from biennial questionnaires completed by the participants.

**MAIN RESULTS AND THE ROLE OF CHANCE:** We found a strong association between paternal age at birth and participant LTL ($P = 1.6 \times 10^{-5}$) that remained robust after controlling for indicators of early life SES. Maternal age at birth showed a weak inverse association with participant LTL after adjusting for age at blood collection and paternal age at birth ($P = 0.01$). We also noted a stronger association between paternal age at birth and participant LTL among premenopausal than among postmenopausal women ($P_{interaction} = 0.045$). However, this observation may be due to chance as premenopausal women represented only 12.6% ($N = 535$) of the study population and LTL was not correlated with age at menopause, total or estrogen-only hormone therapy (HT) use suggesting that changes in *in vivo* estrogen exposure do not influence telomere length regulation.

**LIMITATIONS AND REASONS FOR CAUTION:** The women in our study are not representative of the general US female population, with an underrepresentation of non-white and low social class groups. Although the interaction was not significant, we noted that the

paternal age at birth association with offspring LTL appeared weaker among women whose parents did not own their home at the time of the participant's birth. As telomere dynamics may differ among individuals who are most socioeconomically deprived, SES indicators may have more of an influence on the relationship between paternal age at birth and offspring LTL in such populations.

**WIDER IMPLICATIONS OF THE FINDINGS:** As of yet, our and prior studies have not identified childhood or adult characteristics that confound the paternal age at birth association with offspring LTL, supporting the hypothesis that offspring may inherit the longer telomeres found in sperm of older men. The biological implications of the paternal age effect are unknown. A recent theory proposed that the inheritance of longer telomere from older men may be an adaptive signal of reproductive lifespan, while another theory links telomere length attrition to female reproductive senescence. However, we are unaware of any data to substantiate a relationship between paternal age at birth and daughter's fertility. Generalizability of our study results to other white female populations is supported by prior reports of paternal age at birth and offspring telomere length. Furthermore, a confounding relationship between paternal or maternal age at birth and SES was not observed in a study of SES and telomere length.

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**Key words:** epidemiology / telomere / paternal age / maternal age / social class

# Introduction

A fundamental biological mechanism present in nearly all eukaryotes is the telomere maintenance system (de Lange, 2004). The main known function of telomeres is to protect the physical integrity of linear chromosomes (Abbott, 2009), by masking chromosome ends from being recognized as double-stranded DNA breaks, which would otherwise result in chromosomal rearrangements (d'Adda di Fagagna *et al.*, 2004). These capping structures, composed from a long stretch of telomeric $(TTAGGG)_n$ DNA repeats complexed to telomere-associated proteins (de Lange, 2005), also create a buffer to prevent the loss of genomic DNA as a result of the end replication problem (Olovnikov, 1973). On average, 50–100 bp of telomeric DNA are lost per mitotic division, limiting the replicative capacity of the cell (Allsopp *et al.*, 1992). Exposure to inflammation and oxidative stress may accelerate telomere loss (von Zglinicki, 2002).

The highly conserved and tightly regulated catalytic component of the telomerase enzyme complex, telomerase reverse transcriptase (TERT), restores the length of telomeres by reverse transcribing telomeric repeats from an RNA template (Smogorzewska and de Lange, 2004). However, postnatally, only germline stem cells express sufficient levels of telomerase to maintain telomere lengths (Kim *et al.*, 1994; Wright *et al.*, 1996). Telomerase activity has not been detected in most adult somatic tissues, and where the enzyme is expressed, the levels are not sufficient to prevent attrition (Broccoli *et al.*, 1995; Counter *et al.*, 1995; Kyo *et al.*, 1997; Yasumoto *et al.*, 1996; Masutomi *et al.*, 2003) and replication-associated senescence. As a result, telomere length declines with age (Hastie *et al.*, 1990; Slagboom *et al.*, 1994; Butler *et al.*, 1998; Frenck *et al.*, 1998; Friedrich *et al.*, 2000; *Takubo et al., 2002*) and the accumulation of senescent cells is thought to contribute to age-related tissue deterioration and disease phenotypes (Campisi, 2001; Campisi *et al.*, 2001; Stewart and Weinberg, 2006). Therefore, telomeres may serve as a 'molecular clock' of biological aging (Shay and Wright, 1996).

Although considered highly heritable with estimates in the range of 36–86% (Slagboom *et al.*, 1994; Jeanclos *et al.*, 2000; Vasa-Nicotera *et al.*, 2005; Andrew *et al.*, 2006; Bakaysa *et al.*, 2007; Njajou *et al.*, 2007; Atzmon *et al.*, 2010), little is known regarding the inheritance of telomere length. Mean maternal (Nawrot *et al.*, 2004; Akkad *et al.*, 2006) and paternal (Nordfjall *et al.*, 2005, 2010; Njajou *et al.*, 2007; ) telomere lengths have been significantly correlated with offspring telomere length, with an apparent stronger paternal contribution. Several studies have also observed positive correlations between paternal age at birth, which is a surrogate for age at conception, and offspring telomere length (Unryn *et al.*, 2005; De Meyer *et al.*, 2007; Njajou *et al.*, 2007; Kimura *et al.*, 2008; Arbeev *et al.*, 2011). Longer telomere lengths have also been noted among offspring of older mothers (Unryn *et al.*, 2005; De Meyer *et al.*, 2007; Njajou *et al.*, 2007; Kimura *et al.*, 2008; Arbeev *et al.*, 2011). However, this may reflect the strong correlation between maternal and paternal age at birth (De Meyer *et al.*, 2007; Kimura *et al.*, 2008). Interestingly, cross-sectional studies have observed longer telomeres in sperm from older men compared with sperm from younger men (Allsopp *et al.*, 1992; Baird *et al.*, 2006; Kimura *et al.*, 2008). The inheritance of longer telomere lengths from the sperm of older men is considered one potential mechanism contributing to this association (Kimura *et al.*, 2008).

Low socioeconomic status (SES) has been linked to worse health outcomes, which may stem from a greater likelihood of unhealthy exposures and adverse events, while having a limited access to health-benefiting resources (Adler and Rehkopf, 2008). Several studies have found short telomere lengths associated with various factors related to low SES (Cherkas *et al.*, 2006; Batty *et al.*, 2009; Shiels *et al.*, 2011), particularly if experienced during early life (Kananen *et al.*, 2010; Kiecolt-Glaser *et al.*, 2011; Steptoe *et al.*, 2011; Needham *et al.*, 2012; Surtees *et al.*, 2012). Fathers who are older at the time of their offspring's birth may be more established financially and socially, and therefore able to contribute more resources toward the physical and psychological well-being of the child (Vigil and Geary, 2006), bestowing better health and coping behaviors for life. Prior studies indicated that the paternal age at birth association with the offspring telomere length is independent of the age of the offspring at blood collection (Unryn *et al.*, 2005; De Meyer *et al.*, 2007; Kimura *et al.*, 2008; Arbeev *et al.*, 2011), maternal age at birth (Kimura *et al.*, 2008; Arbeev *et al.*, 2011) and adult body mass index (BMI), smoking status, and household SES of the offspring (Kimura *et al.*, 2008). However,

none have considered whether early childhood or adolescent exposures may account for some or all of the observed association. To address the possibility of confounding by indicators of early life SES, we investigated the relationship within the Nurses' Health Study (NHS), which has collected an extensive array of information from female nurses since inception of the cohort in 1976. Existing leukocyte telomere length (LTL) and covariate data were available on 4250 healthy women for this analysis.

# Materials and Methods

## Study population

The NHS is a prospective cohort study of 121 700 female registered nurses in 11 US states who were 30–55 years of age at enrollment. In 1976 and biennially thereafter, self-administered questionnaires gather detailed information on lifestyle, menstrual and reproductive factors, and medical history. Self-reports of major chronic diseases are confirmed by medical records and pathology report reviews, telephone interviews or supplemental questionnaires. From 1989 to 1990, 32 826 women provided blood samples and completed a short questionnaire asking about day and time of collection, current weight and use of medications. The details of blood collection methods have been previously described (Hankinson *et al.*, 1995).

A number of nested case–control studies examining LTL associations with cancer and cardiovascular disease have been conducted in the blood sub-cohort. For the current analysis, we used existing LTL data on participants selected as controls from nested case–control studies of stroke, myocardial infarction, cancers of the breast, endometrium, skin, pancreas and colon, as well as colon adenoma (De Vivo *et al.*, 2009; Han *et al.*, 2009; Prescott *et al.*, 2010; Nan *et al.*, 2011). We also included existing LTL data from a small random sample of women participating in the NHS cognitive sub-study (Devore *et al.*, 2011).

## Ethical approval

Completion of the self-administered questionnaire and submission of the blood sample were considered to imply informed consent. The NHS protocol was approved by the Human Research Committee of Brigham and Women's Hospital, Boston, MA.

## Exposure assessment

Participants provided personal and lifestyle characteristics on the 1976 baseline questionnaire. Women reported their current weight (pounds), height (inches), age at menarche, age when menstrual periods stopped and for what reason (natural or surgery), use and duration of postmenopausal hormone therapy (HT), smoking status (never, past, current), age started smoking, number of cigarettes per day in categories (1–4, 5–14, 15–24, 25–34, 35–44 and $\geq$45 cigarettes per day) and age stopped smoking. Weight, menopausal status, HT use and smoking habits were updated on each subsequent questionnaire. Women who provided a blood sample reported current weight and HT use within the past 3 months on an accompanying short questionnaire. Height and weight at blood draw were used to calculate BMI (kg/m$^2$). Participants were defined as postmenopausal if they reported having a natural menopause or bilateral oophorectomy. Women who reported a hysterectomy with either one or both ovaries remaining were defined as postmenopausal when they were 56 years old (if a nonsmoker) or 54 years old (if a current smoker), ages at which natural menopause had occurred in 90% of the respective cohorts. Age at menopause in the NHS is reported with a high degree of reproducibility and accuracy (Colditz *et al.*, 1987). Smoking duration in years multiplied by packs of cigarettes smoked per day (20 cigarettes per pack) was used to calculate pack-years of smoking. Total pack-years of smoking were assessed in the questionnaire cycle prior to blood collection.

Women provided some basic family information. On the baseline questionnaire, women reported their mother's and father's years of birth. Parental ages at birth were calculated by subtracting the mother's or father's year of birth from the nurse's year of birth. In 1996, women were asked 'How many biological brothers and sisters do you have? (Include any deceased siblings. Do not include $\frac{1}{2}$ siblings.)'. The total number of full biological siblings was calculated by summing the number of brothers and number of sisters reported by the nurse.

Parental occupation and homeownership were used as indicators of SES in early life. On the baseline questionnaire, women were asked what their father's occupation was when the nurse was 16 years of age. Responses were grouped into the following categories: professional (10.2%), managerial (15.3%), clerical (10.9%), sales (22.1%), craftsmen (14%), service (5.1%), laborer (2.4%), farmer (10.9%), househusband ($<$1%) and unknown / retired / deceased (9.2%). In 2004, women were asked 'Did your parents own a home at the time of your birth or infancy?'. Of the 3609 women who answered, ~46% had parents who owned their home.

## Leukocyte telomere length

Genomic DNA was extracted from peripheral blood leukocytes using the QIAmp (Qiagen, Chatsworth, CA, USA) 96-spin blood protocol. PicoGreen quantitation of genomic DNA was performed using a Molecular Devices 96-well spectrophotometer (Sunnyvale, CA, USA). The ratio of telomere repeat copy number to a single gene copy number (T/S) was determined by a previously described modified, high-throughput version (Wang *et al.*, 2008) of the quantitative real-time PCR telomere assay (Cawthon, 2002) run on the Applied Biosystems 7900HT Sequence Detection System (Foster City, CA, USA). Triplicate reactions of each assay were performed on each sample. LTL is reported as the exponentiated sample T/S ratio corrected for a reference sample. In all nested case–control studies, the telomere and single-gene assay coefficients of variation (CVs) for triplicates were $<$4%. CVs for the exponentiated T/S ratio were $\leq$18%.

## Statistical analysis

Several studies have found longer LTLs among blacks than whites (Hunt *et al.*, 2008; Fitzpatrick *et al.*, 2011; Hofmann *et al.*, 2011; Zhu *et al.*, 2011). The LTL difference declines and may even reverse with aging. This may be due to a faster rate of telomere attrition among blacks (Hunt *et al.*, 2008; Aviv *et al.*, 2009; Roux *et al.*, 2009), which might stem from a higher prevalence of perceived stress and poverty (Geronimus *et al.*, 2010). Given potential racial differences in telomere length dynamics and that the NHS cohort is composed predominantly of white women (~97%), our analysis was restricted to individuals of self-reported European ancestry. Within each study, LTL values displayed a skewed distribution. Telomere data generated by non-standard assay conditions were excluded. Data were natural logarithm transformed to improve normality (lnLTL). Participants with outlier lnLTL values were identified using an extreme studentized deviate many-outlier procedure (Rosner, 1983) and excluded from the study populations. Because the distribution of lnLTL values differed slightly between studies, we generated probit scores to standardize for between-batch variability (Hosmer and Lemeshow, 1989) and created a standard normal distribution prior to pooling data. Because older paternal age at birth may be associated with adverse outcomes in offspring (Sartorius and Nieschlag, 2010; Arbeev *et al.*, 2011), we restricted our study population to the women who were selected as

controls for the nested case–control studies, and the random sample of women in the cognitive sub-study, as mentioned above ($N = 4593$). Control participants found to have had a diagnosis of cancer, diabetes, myocardial infarction, multiple sclerosis or rheumatoid arthritis prior to blood collection were excluded ($N = 39$). For the present analysis, we also excluded controls who were missing data on mother's or father's year of birth ($N = 255$), BMI at blood collection ($N = 17$) or smoking status ($N = 15$). We additionally restricted our analyses to women whose calculated maternal age at birth was in the range of 15–50 years and paternal age at birth in the range of 15–70 years of age (out of range $N = 17$). Our final study population consisted of 4250 women.

We used Spearman's partial rank correlation coefficients to examine the relationship between LTL probit scores and various factors. We used generalized linear models to estimate adjusted least-squares mean (LSM) LTL probit scores by categories of paternal age at birth (15–24, 25–29, 30–34, 35–39, 40+ years of age). All models were adjusted for the nurse's age at blood collection (continuous). We considered BMI at blood collection (continuous), smoking status (never, past, current), maternal age at birth (continuous), father's occupation when the nurse was 16 years of age (professional, managerial, clerical, sales, craftsmen, service, laborer, farmer, househusband and unknown/retired/deceased), whether the nurse's parents owned their home when the nurse was an infant (no, yes), number of full biological siblings (continuous), menopausal status (pre- and postmenopausal) and recent postmenopausal hormone use at blood collection (never, past, current) as potential confounders. The midpoints of categorical variables were used to test for trend. We conducted stratified analyses by whether the nurse's parents owned their home when the nurse was an infant, median age at blood collection (<60, 60+ years), and menopausal status. We used *F*-tests to compare nested models with and without interaction terms between paternal age categories and these variables. Generalized linear models were also used to estimate adjusted LSM LTL probit score by categories of maternal age at birth (15–19, 20–24, 25–29, 30–34, 35+ years of age). Tests used an alpha level of 0.05, and all *P*-values are two-sided. We used SAS Version 9.2 software (SAS Institute, Cary, NC, USA).

# Results

As expected, the participant's age at blood collection was inversely associated with the LTL probit score ($P = 1.1 \times 10^{-11}$; Table I). Although maternal age at birth and paternal age at birth were highly correlated ($r_s = 0.77$; Table II), we only observed a positive age-adjusted correlation for the participant's LTL probit score with paternal age at birth ($P = 0.006$), but not with maternal age at birth ($P = 0.64$; Table I). Even so, the significant positive trend in age-adjusted LSM LTL probit score ($P = 0.002$) strengthened when we included maternal age at birth in the analysis of paternal age ($P = 1.6 \times 10^{-5}$; Table III). The mutual adjustment of parental ages at birth also revealed an inverse relationship between maternal age at birth and LTL probit score ($P = 0.01$; Supplementary Table SI). Additionally adjusting for the participant's BMI and smoking status at blood collection did not change estimates.

**Table I Age-adjusted correlations between the LTL probit score and selected characteristics of women in the NHS.**

| | *N* | $r_s$ (*P*-value)[a] |
|---|---|---|
| Age at blood collection[b] | 4250 | −0.10 ($1.1 \times 10^{-11}$) |
| Paternal age at birth | 4250 | 0.04 (0.006) |
| Maternal age at birth | 4250 | 0.007 (0.64) |
| BMI at blood collection (kg/m$^2$) | 4250 | −0.02 (0.25) |
| Total pack-years of smoking[c] | 4195 | −0.02 (0.16) |
| Number of full siblings | 4058 | 0.03 (0.03) |

[a]$r_s$ = Spearman's partial rank correlation coefficient.
[b]Not adjusted for age.
[c]Never smokers assigned value of 0.

We next examined whether childhood and/or adolescent SES may have confounded the paternal age at birth association with the LTL probit score. We adjusted for father's occupation when the nurse was 16 years of age and whether or not the parents owned their home at the time of the nurse's birth as approximate measures of childhood/adolescent SES. These factors did not alter the relationship between paternal age at birth and the LTL probit score. While there was an indication that paternal age at birth was more strongly associated with the LTL probit score among nurses whose parents owned their home ($P = 0.003$) compared with those who did not ($P = 0.09$), the interaction was not significant ($P = 0.82$; Table IV).

We noted that having a larger number of full biological siblings was positively correlated with the LTL probit score ($P = 0.03$; Table I) and with paternal age at birth ($r_s = 0.15$, $P = 4.1 \times 10^{-23}$; Table II). Most of the participants in our cohort were born just before or during the great depression, a period when the fertility rates declined in the USA (Martin *et al.*, 2011). One potential explanation for the observed correlations is that men of higher SES, who may possess longer telomeres than men of lower SES, may have been more likely to continue to have children later in life. However, when we adjusted the model for number of full biological siblings, the paternal age association with the LTL probit score remained the same.

Menopause has been suggested as a potential confounder of telomere dynamics in women (Kimura *et al.*, 2008). In our dataset, menopausal status did not confound the relationship between the paternal age at birth and the LTL probit score. However, when we stratified our analysis by menopausal status, the association appeared stronger among premenopausal women ($P_{interaction} = 0.045$; Table IV). Premenopausal women with the oldest fathers had LTL probit scores 241% greater than premenopausal women with the youngest fathers (LSM of 0.48 versus −0.34, respectively). The percent difference in LTL probit score between the same categories was 188% among postmenopausal women (LSM of 0.14 versus −0.16, respectively; Table IV). The estimates remained unchanged after further adjusting for postmenopausal hormone use at blood collection among postmenopausal women.

# Discussion

Using a population of 4250 healthy women, aged 42–69 at blood collection, from the well-characterized NHS, we found a positive association between paternal age at birth and participant's LTL ($P = 1.6 \times 10^{-5}$; Table 3) that remained robust after adjusting for potential indicators of early life SES. After adjusting for age at blood collection and paternal age at birth, we also observed a weak inverse association between maternal age at birth and participant's LTL.

Overall, our results are consistent with the published literature. Taking the weighted average of estimates from prior studies suggests

**Table II Age-standardized characteristics at blood collection.**

| | Paternal age at birth | | | | |
|---|---|---|---|---|---|
| | 15–24 ($n$ = 640) | 25–29 ($n$ = 1243) | 30–34 ($n$ = 1127) | 35–39 ($n$ = 662) | 40+ ($n$ = 578) |
| Age at blood collection, years[a] | 58.7 (6.4) | 58.8 (6.8) | 58.9 (6.7) | 59.2 (6.5) | 59.6 (6.5) |
| Paternal age at birth, years | 22.5 (1.6) | 27.1 (1.4) | 31.8 (1.4) | 36.7 (1.4) | 44.1 (4.2) |
| Maternal age at birth, years | 21.6 (2.9) | 25.1 (3.2) | 28.5 (3.8) | 31.9 (4.7) | 36.0 (5.1) |
| BMI at blood collection, kg/m$^2$ | 25.6 (4.6) | 25.4 (4.7) | 25.1 (4.3) | 25.4 (4.7) | 25.2 (4.4) |
| Never smoker, % | 46 | 48 | 45 | 43 | 43 |
| Former smoker, % | 35 | 35 | 41 | 45 | 44 |
| Current smoker, % | 19 | 16 | 15 | 13 | 14 |
| Total pack-years of smoking[b] | 24.4 (19.2) | 25.4 (20.8) | 23.4 (19.9) | 21.8 (18.4) | 22.0 (18.4) |
| Number of full siblings | 2.1 (1.6) | 2.2 (1.5) | 2.3 (1.5) | 2.6 (1.6) | 3.0 (1.7) |
| Father had chronic disease, % | 54 | 54 | 57 | 59 | 53 |

Values are means (SD) or percentages and are standardized to the age distribution of the study population.
[a]Value is not age adjusted.
[b]Among ever smokers.

**Table III LSM leukocyte telomere length (LTL) probit score and 95% confidence intervals for categories of paternal age at birth.**

| | Paternal age at birth | | | | | $P_{trend}$ |
|---|---|---|---|---|---|---|
| | 15–24 | 25–29 | 30–34 | 35–39 | 40+ | |
| $N$ | 640 | 1243 | 1127 | 662 | 578 | |
| Model 1 | −0.11 (−0.18, −0.03) | 0.02 (−0.04, 0.07) | 0.02 (−0.04, 0.08) | 0.02 (−0.06, 0.09) | 0.10 (0.02, 0.18) | 0.002 |
| Model 2 | −0.19 (−0.28, −0.10) | −0.02 (−0.08, 0.04) | 0.03 (−0.03, 0.09) | 0.07 (−0.01, 0.15) | 0.20 (0.10, 0.30) | $1.6 \times 10^{-5}$ |

Model 1: results are from a generalized linear model adjusted for age at blood collection (continuous).
Model 2: results are from a generalized linear model adjusted for age at blood collection (continuous) and maternal age at birth (continuous).

an increase in telomere length of 17.7 bp (range: 10–55 bp) associated with each year of older paternal age at birth (Unryn *et al.*, 2005; De Meyer *et al.*, 2007; Njajou *et al.*, 2007; Kimura *et al.*, 2008; Arbeev *et al.*, 2011). While the PCR-based assay does not generate absolute measures of telomere length, using estimates from the age at blood collection ($\beta = -0.016$) and paternal age at birth ($\beta = 0.014$) analysis we could approximate an absolute difference in telomere length. If we assume that the telomere length declines ~23 bp on average for every 1 year increase in age at blood collection (Unryn *et al.*, 2005; De Meyer *et al.*, 2007; Kimura *et al.*, 2008; Arbeev *et al.*, 2011), we calculate that a woman in our study population has an additional ~20 bp of telomere length for every one year increase in the father's age at the time of her birth. Similar to the results of Kimura *et al.* (Kimura *et al.*, 2008), adjusting for the participant's BMI and smoking status at blood collection did not change estimates.

Individuals from disadvantaged environments are hypothesized to age more rapidly as a result of greater exposure to physiological and psychological stressors such as uncertainty, adverse events, poor housing and nutrition, lack of adequate health care and being less likely to engage in healthful behaviors (Adler and Rehkopf, 2008; Needham *et al.*, 2012). Several studies have examined the relationship between indicators of SES and telomere length. In a study of adult female twins, women classified to manual social classes had shorter telomeres than those in non-manual social classes (Cherkas *et al.*, 2006). Others did not find a relationship between adult telomere length and indicators of SES at the time of blood collection (Adams *et al.*, 2007; Batty *et al.*, 2009; Kananen *et al.*, 2010; Shiels *et al.*, 2011; Steptoe *et al.*, 2011; Surtees *et al.*, 2012). Instead two studies observed a positive association between telomere length and educational attainment, an indicator of early life SES (Steptoe *et al.*, 2011; Surtees *et al.*, 2012). Another two studies reported that a greater number of negative childhood events was independently associated with adult telomere length (Kananen *et al.*, 2010; Kiecolt-Glaser *et al.*, 2011), with chronic/serious childhood illness and parental unemployment noted as the most significant adverse events in one of the studies (Kananen *et al.*, 2010). Furthermore, among children aged 7–13, children whose parents never attended college were estimated to have telomeres 1178 base pairs shorter than children with at least one college educated parent (Needham *et al.*, 2012).

As fathers who are older at the time of their child's birth may be able to provide a more protective and health-promoting childhood environment (Vigil and Geary, 2006), we hypothesized that early life SES could account for the association between paternal age at birth and offspring LTL. However, when we adjusted for father's occupation when the nurse was 16 years old or whether parents owned their

**TableIV LSM leukocyte telomere length (LTL) probit score and 95% CI for categories of paternal age at birth by menopausal status at blood collection and parents' home-ownership strata.**

| | Paternal age at birth | | | | | $P_{trend}$ | $P_{interaction}$ |
|---|---|---|---|---|---|---|---|
| | 15–24 | 25–29 | 30–34 | 35–39 | 40+ | | |
| Premenopausal | | | | | | | |
| *N* | 71 | 162 | 144 | 91 | 67 | | |
| LSM (95% CI)[a] | −0.34 (−0.61, −0.06) | 0.10 (−0.08, 0.27) | 0.21 (0.04, 0.37) | 0.40 (0.17, 0.63) | 0.48 (0.18, 0.78) | 0.001 | |
| Postmenopausal | | | | | | | |
| *N* | 521 | 990 | 896 | 536 | 479 | | |
| LSM (95% CI)[a] | −0.16 (−0.27, −0.06) | −0.06 (−0.13, 0.006) | 0.007 (−0.06, 0.07) | −0.004 (−0.09, 0.09) | 0.14 (0.02, 0.25) | 0.002 | 0.045 |
| Parents owned home at the time of the nurse's birth | | | | | | | |
| *N* | 117 | 381 | 473 | 346 | 345 | | |
| LSM (95% CI)[a] | −0.18 (−0.38, 0.02) | −0.02 (−0.13, 0.09) | 0.05 (−0.04, 0.14) | 0.07 (−0.03, 0.18) | 0.24 (0.11, 0.36) | 0.003 | |
| Parents did not own home at the time of the nurse's birth | | | | | | | |
| *N* | 433 | 661 | 480 | 222 | 151 | | |
| LSM (95% CI)[a] | −0.14 (−0.25, −0.03) | 0.02 (−0.06, 0.10) | 0.01 (−0.08, 0.11) | 0.03 (−0.11, 0.17) | 0.11 (−0.07, 0.30) | 0.09 | 0.82 |

[a]All generalized linear models were adjusted for age at blood collection (continuous) and mother's age at birth (continuous).

home at the time of the nurse's birth as potential indicators of childhood or adolescent SES, the paternal age at birth association with LTL did not change. This is compatible with the female twin study, where maternal and paternal age at birth did not confound the association between LTL and social class (Cherkas *et al.*, 2006). Although the interaction with parental home ownership was not significant in our study ($P = 0.82$), it is noteworthy that the paternal age trend appeared stronger among women whose parents owned their home at the time of birth, suggesting stress associated with low SES may accelerate telomere shortening and reduce the paternal age effect. While area-based deprivation and employment status were not associated with telomere length in the cross-sectional study by Shiels and colleagues, the authors noted steeper age-related telomere length declines associated with low income, renting a home and poor diet (Shiels *et al.*, 2011).

A couple of novel observations were made by our study: (i) the inverse association of offspring telomere length with maternal age at birth and (ii) the effect modification of the paternal age at birth association by menopausal status. Prior studies found evidence of longer offspring telomere length associated with older maternal age at birth (*Unryn et al., 2005*; De Meyer *et al.*, 2007; *Njajou et al., 2007*; Kimura *et al.*, 2008; Arbeev *et al.*, 2011). However, this appeared to be an artifact of the very high correlation between maternal and paternal age at birth ($r = 0.72–0.85$) (De Meyer *et al.*, 2007; Kimura *et al.*, 2008). Once paternal age at birth was taken into account, maternal age at birth was no longer independently associated with offspring telomere length (De Meyer *et al.*, 2007; Kimura *et al.*, 2008; Arbeev *et al.*, 2011). Although not significant, after adjusting for paternal age at birth, inverse trends were noticeable for maternal age at birth and offspring telomere length in two of the studies (*Kimura et al., 2008*; Arbeev *et al.*, 2011). In contrast to the idea that sperm of older fathers pass on longer telomere lengths to offspring (Kimura *et al.*, 2008), oocytes ovulated late in reproductive life are hypothesized to have shortened telomeres (Keefe *et al.*, 2006), which would coincide with an inverse association between maternal age at birth and offspring telomere length.

The association of paternal age at birth with offspring telomere length was consistently observed among sons from each cohort, but was seen among daughters in only two of the four cohorts in the Kimura study (Kimura *et al.*, 2008). The authors suggested menopause, which is characterized by a decrease in estrogen levels (Burger *et al.*, 2007), may be a potential confounder given the various age distributions of the cohorts included in their study (Kimura *et al.*, 2008). While much is still unknown regarding transcriptional regulation of TERT, the rate-limiting component of the telomerase enzyme complex, *in vitro* studies have shown that estrogen treatment induces estrogen receptor binding to the promoter region with a subsequent increase in mRNA expression and telomerase activity in a variety of cell types (Kyo *et al.*, 1999; Misiti *et al.*, 2000; Nanni *et al.*, 2002; Kimura *et al.*, 2004; Sato *et al.*, 2004; Boggess *et al.*, 2006; Grasselli *et al.*, 2008; ). Furthermore, long-term HT users were found to have longer telomeres than never users (Lee *et al.*, 2005), and a recent small study found a non-significant positive relationship between age at menopause and telomere length (Lin *et al.*, 2011), suggesting that greater estrogen exposure may contribute to longer LTL. In our analytic population, menopausal status did not confound the relationship between paternal age at birth and

participant's LTL. Instead, we observed an interaction where the paternal age at birth association with participant's LTL appeared stronger among premenopausal women than postmenopausal women ($P_{interaction} = 0.045$). Menopausal status did not appear to simply be a proxy for age, as the interaction was not significant ($P_{interaction} = 0.61$) when stratified by median age at blood collection (<60, 60+ years). However, neither age at menopause, nor duration of total or estrogen only HT use up until blood collection were correlated with LTL ($P \geq 0.29$). These results are not supportive of *in vivo* estrogen regulation of telomere length in peripheral blood leukocytes. Thus, the interaction by menopausal status may suggest that non-hormonal menopausal changes influence LTL maintenance or our results could be due to chance.

Telomere dysfunction in oocytes has been proposed as a common mechanism underlying the increased rates of infertility, miscarriage and birth defects among older women (Keefe *et al.*, 2006). Eisenberg (2011) recently theorized that telomere length may serve as a marker to balance the limited energy and resources invested in maintaining a durable soma versus the potential reproductive life history of an organism. Therefore, the paternal age effect may act as an adaptive intergenerational male signal to offspring, reflecting the 'rolling average' reproductive lifespan of recent generations. Since menopause marks the end of a woman's reproductive life (Burger *et al.*, 2007), we might expect to find a positive association between paternal age at birth and daughter's age at menopause based on these hypotheses. After adjusting for menopausal status at blood draw, paternal age at birth showed a positive correlation with daughter's age at menopause in our study population ($r_s = 0.04$, $P = 0.01$). However, maternal age at birth was also positively correlated with daughter's age at menopause ($r_s = 0.05$, $P = 0.002$). When maternal age at birth was taken into account, paternal age at birth was no longer associated with daughter's age at menopause ($P = 0.88$). Since women who undergo menopause at an older age are capable of reproducing until later in life, the relationship between maternal age at birth and daughter's age at menopause may reflect the strong correlation between mother's and daughter's menopausal age (He and Murabito, 2012).

Participants in our study were all registered nurses at the time of cohort enrollment and therefore, not representative of the general US population. The distribution of father's occupation suggests that the women were raised in diverse backgrounds. Even so, the strong association observed between paternal age at birth and participant LTL suggests that SES is not a major confounder in our population, which presumably has a limited and higher range of childhood SES. As different telomere dynamics may occur among those most socioeconomically deprived, our results may not apply to lower social class groups. Generalizability of our study results to other white female populations is supported by prior reports of paternal age at birth and offspring telomere length (De Meyer *et al.*, 2007; Njajou *et al.*, 2007; Kimura *et al.*, 2008; Arbeev *et al.*, 2011). However, it is still undetermined whether a similar association exists in non-white populations, who experience a greater prevalence of perceived stress and poverty (Geronimus *et al.*, 2010). Furthermore, the paternal age effect is hypothesized to result from an overrepresentation of sperm with longer telomeres as men age (Kimura *et al.*, 2008). Acquiring sperm samples from the fathers of our participants is not feasible. Such a task would be costly and introduce survival bias as the youngest father would now be 86 years of age. Hence, we are unable to confirm whether longer telomere lengths are inherited from older fathers.

In conclusion, the paternal age association with the offspring telomere length remained robust after considering indicators of early life SES as potential confounding factors in a large, homogenous population, consistent with inheritance of longer telomere lengths from the sperm of older men. However, to-date, the observation of longer sperm telomere lengths from older compared with younger men has been made in cross-sectional studies. Longitudinal studies with repeat sperm sample collections over time are needed to confirm this observation. Further, alternate SES indicators or other confounders not measured in our study that may more directly measure childhood physiological and psychological stress may still account for the paternal age at birth association. Thus, in addition to examining the paternal age at birth relationship with offspring telomere length in non-white populations, it would be informative to assess the effect in populations with more detailed childhood SES data and of adopted individuals. We also showed for the first time an inverse correlation between maternal age at birth and telomere length as well as an interaction by menopausal status. Additional studies are needed to confirm these observations and to further investigate the significance of the paternal age effect on offspring telomere length.

## Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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## Authors' roles

J.P. and I.D.V. were involved in the conceptualization of the manuscript. J.P. performed the analyses and drafted the manuscript. All authors contributed toward data acquisition, critically revised the manuscript and approved the final manuscript.

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## Conflict of interest

None declared.

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