European Journal of Endocrinology, 2025, **192**, 744–753
https://doi.org/10.1093/ejendo/lvaf102
Advance access publication 9 June 2025
Original Research

Association between premature ovarian insufficiency and biological aging

Jinting Zhou,[1,†] Menglin Fan,[2,3,†] Aaron M. Lett,[4] Geling Jin,[5] Qiqi You,[2,3] Jingjing Zeng,[2,3] Bo Chen,[2,3] Yucen Wu,[4] Hui Xing,[1,*] and Shaoyong Xu[2,3,*]

[1]Department of Obstetrics and Gynecology, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang, Hubei 441021, China
[2]Department of Endocrinology, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang, Hubei 441021, China
[3]Center for Clinical Evidence-Based and Translational Medicine, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang, Hubei 441021, China
[4]Department of Metabolism, Digestion and Reproduction Imperial College London, Du Cane Rd, London W12 0NN, United Kingdom
[5]College of Medicine, Wuhan University of Science and Technology, Wuhan, Hubei 430000, China

*Corresponding authors: Department of Endocrinology, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, No. 136, Jingzhou Street, Xiangyang, Hubei 441021, China. Email: yoji_xu@hotmail.com (S.X.); Department of Obstetrics and Gynecology, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, No. 136, Jingzhou Street, Xiangyang, Hubei 441021, China. Email: huixing1969@163.com (H.X.)

Abstract

Objective: This study aimed to analyze whether premature ovarian insufficiency (POI) is associated with accelerated biological aging, whether the degree of biological aging is exacerbated by an earlier age at menopause, and whether menopausal hormone therapy (MHT) in the POI population is associated with reduced biological aging.

Design: This is a cross-sectional study. A total of 229 779 participants aged 40 years and older in the UK Biobank (2006-2010) and NHANES (1999-2018) were included in the study.

Methods: Menopause information was collected through questionnaires. Biological age acceleration was defined by the Klemera–Doubal method, which is calculated through biomarkers, in reference to chronological age. Biological age acceleration > 0 was defined as biological aging. Association between POI and biological aging analyzed using multivariate linear regression and logistic regression models.

Results: The results showed that participants with POI had an increased risk of biological aging (UK Biobank: OR = 1.50 [95% CI: 1.24-1.82]; NHANES: OR = 1.20 [95% CI: 1.07-1.34]) and decrease in leukocyte telomere length compared with those without POI (UK Biobank: 0.0109 [95% CI: 0.0079-0.0109]). Participants with POI who underwent MHT had reduced risk of aging compared with those who did not (UK Biobank: OR = 0.63 [95% CI: 0.43-0.92]; NHANES: OR = 0.75 [95% CI: 0.61-0.92]).

Conclusion: This study showed that participants with POI had a significantly increased risk of biological aging compared with those without POI. Participants with POI who received MHT had a reduced risk of aging compared with those who did not.

Keywords: premature ovarian insufficiency, biological aging, biological age, menopausal hormone therapy

Significance

This study conducted the first comprehensive evaluation of premature ovarian insufficiency (POI) and biological aging across two large population-based cohorts (UK Biobank and NHANES). Biological aging was quantified using a multi-system biomarker approach through the Klemera–Doubal method. The results revealed a significant association between POI and biological aging, and the aging process increases as the age of menopause becomes earlier. Notably, menopausal hormone therapy (MHT) may partially offset the adverse outcomes caused by POI. These findings elucidated the association between POI and biological aging while emphasizing the clinical imperative of early MHT intervention to mitigate long-term health adversities in affected populations.

Introduction

Population aging has emerged as a major global concern, with the number of individuals 60 years of age or older predicted to quadruple between 2020 and 2050, making up 22% of the world's population.[1] Despite increasing chronological age (CA), there is a growing interest in strategies to delay biological aging and prevent the onset and advancement of age-related disorders. Biological aging is a complex physiological process that gradually destroys the integrity and regenerative capacity

† J.Z. and M.F. contributed equally to this work and share first authorship.

Received: January 9, 2025. **Revised:** April 19, 2025. **Editorial Decision:** May 6, 2025. **Accepted:** May 6, 2025

of cells, tissues, and organs, leading to a progressive decline in effective physiological function.[2] Biological aging can lead to a dysfunction of important organ systems, leading to age-related diseases, such as neurodegenerative disorders and cardiovascular diseases, which can greatly affect an individual's quality of life.[3-5]

The average age of natural menopause in the general population is 50-52 years, and menopause occurring before the age of 40 years is called premature ovarian insufficiency (POI), which is a phenomenon of reproductive aging.[6] Premature menopause lowers estrogen levels, causing the body to lose estrogen's protective function. This lack causes physiological changes in organs and psychological and emotional disorders, such as cardiovascular disease, osteoporosis, and neurocognitive disorders.[7-9]

Aging is a state of low-grade inflammation, and estrogen as an anti-inflammatory agent plays a role in the aging process.[10,11] The issue of whether patients with POI have accelerated aging is important. But unfortunately, there has been only one study that utilized telomere length to assess biological aging and found that premature menopause was associated with a shortened telomere length.[12] Telomere length shortening is useful as a marker of biological aging, but telomeres may not accurately reflect aging of multiple systems and are hardly feasible for application in large-scale epidemiological studies. Klemera and Doubal proposed a new assessment metric that applies biomarkers from multiple organs and systems to assess biological age (BA), such as systolic blood pressure (SBP), albumin, alkaline phosphatase, C-reactive protein, total cholesterol, the lymphocyte percentage, mean sphered cell volume, red blood cell distribution width, and so on.[13] This method is now widely used in epidemiologic studies related to aging, providing new opportunities for biological aging research.[14,15] However, no other study has yet demonstrated the association between POI and BA.

This study aimed to evaluate biological aging through two large-scale observational studies, UK Biobank and National Health and Nutrition Examination Survey (NHANES), and to analyze whether POI is associated with accelerated biological aging in female. We also analyzed whether the degree of biological aging is exacerbated by an earlier age at menopause and whether menopausal hormone therapy (MHT) in the POI population is associated with reduced biological aging.

Materials and methods

Study population and design

The study included participants from two national databases, the UK Biobank (2006-2010) and the NHANES (1999-2018). The UK Biobank was a prospective cohort study that enrolled approximately 500 000 participants aged 40-70 years between 2006 and 2010. Participants were interviewed once during the period 2006-2010. Participants' data on demographics, lifestyle, and medical history were collected through questionnaires, as well as by physical measurements and biological sample collection. The UK Biobank research was approved by the North West Multicenter Research Ethical Committee, and all participants provided written informed consent. The present study was performed under application number 92014. There were 273 314 (54.4%) female participants aged over 40 years in the UK Biobank. A total of 41 920 individuals with missing menopausal status data and 64 707 individuals with missing BA data were excluded. A total of 166 687 participants were included in the final analysis (Figure 1).

The NHANES was a nationwide epidemiological survey study that was conducted in the United States and aimed to assess the health and nutritional status of the US population. The NHANES obtained a nationally representative sample through stratified multistage sampling, and information on demographics, socioeconomics, diet, and health, as well as physical measurements and laboratory examinations, was collected. The NHANES recorded 10 cross-sectional surveys from 1999 to 2018, and this study included individuals who participated in at least one of these surveys. The NHANES was approved by the Research Ethics Review Board of the National Center for Health Statistics, and all participants provided informed consent. There were 28 607 female participants aged over 20 years in the NHANES. A total of 16 792 participants aged <40 years with missing data on menopausal status and BA were excluded (Figure 1).

This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (Table S1).

Identification of POI

Premature ovarian insufficiency was determined by the participants' self-reported age of menopause. The participants of the UK Biobank were asked "Have you had your menopause (periods stopped)?" and "How old were you when your periods stopped?" In the NHANES, the menopausal status was assessed by asking "What is the reason why you did not have a period in the past 12 months?" and "Approximately how old were you when you had the last menstrual period?" Premature ovarian insufficiency was defined as menopause before the age of 40. Additionally, natural POI was defined as menopause before the age of 40 without bilateral oophorectomy, and surgical POI was defined as menopause before the age of 40 with bilateral oophorectomy.[7,16]

Assessment of BA and biological age acceleration

Biological age is calculated using the Klemera–Doubal method. This new method was proposed by Klemera and Doubal[13] to construct BA according to age-related biomarkers, based on the assumption of the relationship between BA, CA, and biomarkers. This assumption suggests that the biological characteristics of the human body may be affected by the degree of individual aging, and in people with the same CA, the difference in BA reflects the difference in the degree of individual aging. Based on previous research and the availability of data in the UK Biobank and the NHANES,[14,15] 12 biomarkers including SBP, albumin, alkaline phosphatase, C-reactive protein, total cholesterol, glycated hemoglobin, glucose, creatinine, urea, the lymphocyte percentage, mean sphered cell volume, red blood cell distribution width, and white blood cell count were used as indicators for assessing BA (Table S2).

Biological age was calculated as a series of regressions of individual biological markers in the reference population on CA as follows:

$$BA = \frac{\sum_{i=1}^{n} (x_i - q_i)\frac{k_i}{s_i^2} + \frac{CA}{s_{BA}^2}}{\sum_{i=1}^{n} \left(\frac{k_i}{S_i}\right)^2 + \frac{1}{s_{BA}^2}}$$

The value of the biological marker x is an individual measurement. k, q, and s are the intercept, slope, and root mean square

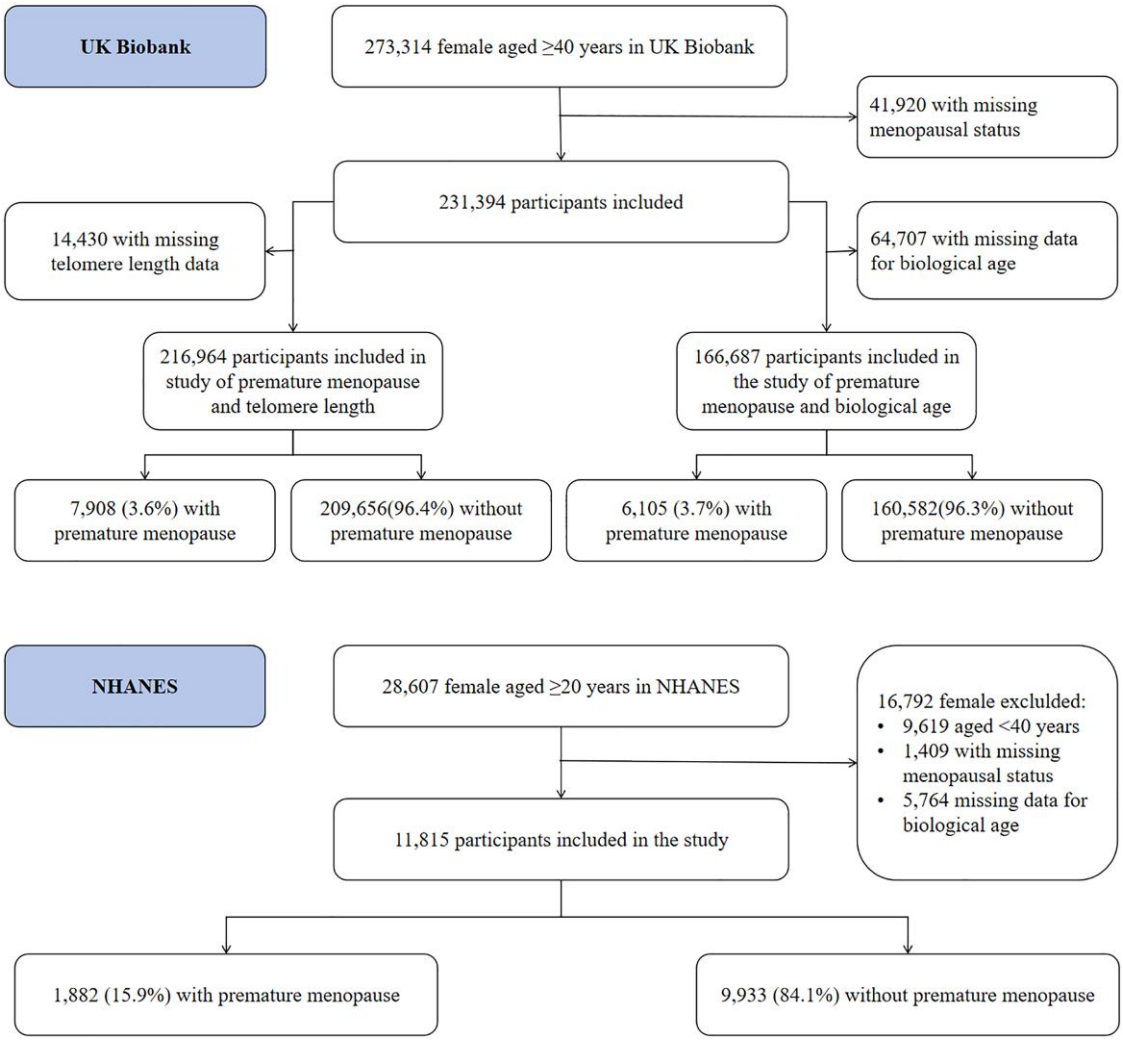

Figure 1. Study flowchart.

error, respectively, of the regression of the biological marker *i* on the actual age in the reference sample. S_{BA}^2 is a scaling factor, which is the square root of the proportion of the variance in actual age explained by the set of biological markers in the sample. Computation of BA was conducted using the R package "BioAge".[17]

Biological age increases with CA. Therefore, BA alone cannot be used to assess differences in aging between individuals of different ages. To quantify the biological aging of participants and exclude the effect of actual age, age acceleration (AA) was used to represent the difference between BA and CA (AA = BA−CA), and AA > 0 was defined as biological aging.

Assessment of leukocyte telomere length

This study used the leukocyte telomere length (LTL) in the UK Biobank population to further verify the relationship between POI and biological aging (LTL data are not available in the NHANES). The length of leukocyte telomeres reflects the degree of cellular aging, and a shortened telomere length is considered a biological marker of aging.[12,18] In the UK Biobank, DNA was extracted from peripheral blood leukocytes. Research staff conducted LTL measurements of the quantitative polymerase chain reaction method, which is the ratio of the telomere repeat copy number (T) to the single-copy gene copy number (S). This study used the technically adjusted LTL (ie, UK Biobank data field 22191).

Covariates

Data on demographics (age, education, and race), socioeconomic status (income and Townsend Deprivation Index), lifestyle factors (smoking, alcohol consumption, and physical activity), and health status were collected through a questionnaire survey in the UK Biobank and NHANES. The smoking status was categorized as never, former, and current smokers. Alcohol consumption was classified as never, occasional (1-3 times/month or on special occasions), weekly (1-4 times/week), and daily. Physical activity was self-reported and expressed as the metabolic equivalent (MET-minutes/week). The health status is based on self-reporting and is categorized as excellent, good, fair, or poor. Height and weight were measured during a physical examination, and the body mass index was calculated as weight (kg) divided by height (m).[2] Additionally, to evaluate the potential protective effect of sex hormones on biological aging in female with POI, information was collected regarding whether the participants

were using MHT. On the basis of self-reporting, hospital records, and primary healthcare data, we obtained information on relevant medical histories (ovarian dysfunction: E28.1, E28.2, E28.3, E28.8, and E28.9; pituitary hypofunction: E23.0, E23.1). Missing covariate values were imputed using multiple imputation methods.

Statistical analysis

The frequency (%) of categorical variables and the mean ± standard deviation of continuous variables were used to describe the population characteristics. The χ^2 test and *t*-test were used to compare the statistical differences between the POI group and the non-POI group. The *t*-test and analysis of variance were used to examine the differences in AA in relation to the presence of POI, natural menopause, surgical menopause, age at menopause, and MHT use.

Multivariate linear regression (AA as a continuous variable) and logistic regression models (AA as a categorical variable, AA > 0 indicates biological aging) were used to analyze the relationship between POI, causes of POI (surgical or natural), and AA. Additionally, a comparison of the LTL of female with or without POI and different causes of POI was further verifying the association between POI and biological aging. To examine whether the use of MHT can delay biological aging, the effect of MHT use and duration on biological aging in female with POI was analyzed. Additionally, the association between age at menopause (categorized as <40 years, 40-49 years, 50-55 years, and >55 years) and AA was also analyzed. Restricted cubic splines were used to examine the dose–response relationship between the age at menopause and AA. The multivariate models were adjusted for age, education, ethnicity, income, the Townsend Deprivation Index (only in the UK Biobank), smoking, alcohol, physical activity, health status, and MHT.

Multiple sensitivity analyses were conducted to evaluate the robustness of the research results. First, the study population was limited to relatively healthy female and excluded those with ovarian dysfunction, pituitary hypofunction, or a poor self-reported health status. Furthermore, chained equation multiple imputation is used to impute missing values of biomarkers. Table S3 shows the missing information on the biomarkers. Last, the analysis was repeated using an alternative definition of AA, which regressed BA on CA and used the residuals to measure biological aging.

Data were cleaned and statistically analyzed by using SAS 9.4 (SAS Institute, Cary, NC, USA) and R (version 4.3.2). A 2-sided $P < 0.05$ was considered statistically significant.

Results

Table 1 and Table S4 showed the basic characteristics of the participants according to whether they had POI. The mean CA and BA of the population in the UK Biobank were 56.2 ± 8.1 and 50.2 ± 8.1 years, and 6105 (3.7%) of the participants had POI. In the NHANES populations, the mean age was 60.2 ± 12.8 years, and the mean BA was 57.0 ± 13.7 years. A total of 1882 (15.9%) of the participants had POI. SBP, alkaline phosphatase, C-reactive protein, glycated hemoglobin, glucose, creatinine, urea, the lymphocyte percentage, mean sphered cell volume, and the white blood cell count were significantly higher in the POI than the non-POI population in both the UK Biobank and NHANES ($P < 0.05$).

In the UK Biobank and NHANES populations, the POI group had a higher BA, with greater AA of 0.30 (95% CI: 0.24-0.36) years and 0.59 (95% CI: 0.95-0.22) years, respectively, than the non-POI group (Figure 2). In the UK Biobank population, surgical and natural POI increased AA by 0.39 (95% CI: 0.28-0.50) years and 0.25 (95% CI: 0.16-0.33) years, respectively, compared with non-POI. In the NHANES population, surgical and natural POI increased AA by 0.36 (95% CI: −0.26-0.99) and 0.72 (95% CI: 0.24-1.20) years, respectively, compared with non-POI. In the POI population, the use of MHT reduced AA by 0.27 (95% CI: 0.09-0.45) years and 1.77 (95% CI: 1.11 2.45) years, respectively, compared with not using MHT (Figure S1).

Premature ovarian insufficiency was associated with a higher AA when accelerated biological aging was analyzed as a continuous variable (UK Biobank: β = 0.24 [95% CI: 0.18-0.30]; NHANES: β = 0.74 [95% CI: 0.10-1.08]). When AA was dichotomized, participants with POI had an increased risk of biological aging (AA > 0) compared with those without POI (UK Biobank: odds ratio [OR] = 1.50 [95% CI: 1.24-1.82]; NHANES: OR = 1.20 [95% CI: 1.07-1.34]). Natural and surgical POI increased the risk of biological aging compared with no POI. Participants with POI who underwent MHT had a 37% and 25% reduced risk of biological aging compared with those who did not undergo MHT (UK Biobank: OR = 0.63 [95% CI: 0.43-0.92]; NHANES: OR = 0.75 [95% CI: 0.61-0.92]), respectively (Figure 3).

Age acceleration in the group with a menopausal age of 40-49 years was 0.16 (95% CI: 0.13-0.20) and 0.33 (95% CI: −0.13-0.79), respectively, compared with that in the group with a menopausal age of 50-55 years. Additionally, AA in the group with a menopausal age < 40 years was increased, with values of 0.42 (95% CI: 0.35-0.50) years and 1.02 (95% CI: 0.49-1.56) years in UK Biobank and NHANES populations, respectively (Figure S1). The linear regression and logistic regression analyses showed that the risk of biological aging in the groups with a menopausal age of 40-49 years and <40 years was significantly increased compared with that in the group with a menopausal age of 50-55 years in the UK Biobank population. The ORs were 1.14 (95% CI: 1.02-1.28) and 1.45 (95% CI: 1.18-1.78), respectively. In the NHANES population, the group with a menopausal age of 40-49 years did not have a significantly increased risk of biological aging, while the group with a menopausal age < 40 years had a 16% increased risk of biological aging (OR = 1.16 [95% CI: 1.00-1.33]). With every 5-year increase in menopausal age, the risk of biological aging decreased by 11% and 2% (UK Biobank: OR = 0.89 [95% CI: 0.85-0.93]; NHANES: OR = 0.98 [95% CI: 0.95-1.00]), respectively (Figure 3; Table S5). The dose–response relationship between menopausal age and AA showed similar results (Figure S2).

The LTL decreased with increasing actual age (Figure 4). The LTL in the POI group (0.8330 ± 0.1301) was smaller than that in the non-POI group (0.8439 ± 0.1325), and the difference was significant (difference: 0.0109 [95% CI: 0079-0.0139]). Compared to the non-POI group, there was no significant difference in the telomere length in participants with surgical menopause, while natural menopause was associated with a 0.0168 (95% CI: 0.0125-0.0210) reduction in LTL (Figure 4).

Three sensitivity analyses were performed, all of which showed that POI was associated with biological aging (Table S6-S8).

Table 1. Characteristics of the participants in the UK Biobank and NHANES.

Characteristic[a]	UK Biobank				NHANES			
	N = 166 687	Premature ovarian insufficiency		*P*	*N* = 11 815	Premature ovarian insufficiency		*P*
		No *N* = 160 582 (96.3)	Yes *N* = 6105 (3.7)			No *N* = 9933 (84.1)	Yes *N* = 1882 (15.9)	
Age (years)	56.2 ± 8.1	56.2 ± 8.1	58.1 ± 7.4	<0.001	60.2 ± 12.8	59.8 ± 13.0	62.1 ± 11.8	<0.001
TDI	−1.4 ± 3.0	−1.4 ± 3.0	−1.0 ± 3.2	<0.001				
Smoking status, *n* (%)				<0.001				<0.001
Never	99 051 (59.4)	95 967 (59.8)	3084 (50.5)		204 (1.7)	175 (1.8)	29 (1.5)	
Previous	52 593 (31.6)	50 501 (31.4)	2092 (34.3)		2547 (21.6)	2106 (21.2)	441 (23.4)	
Current	14 490 (8.7)	13 585 (8.5)	905 (14.8)		1820 (15.4)	1424 (14.3)	396 (21.0)	
Not clear	553 (0.3)	529 (0.3)	24 (0.4)		7244 (61.3)	6228 (62.7)	1016 (54.0)	
Alcohol consumption, *n* (%)				<0.001				<0.001
Never	15 296 (9.2)	14 388 (9.0)	908 (14.9)		2171 (18.4)	1719 (17.3)	452 (24.0)	
Occasionally	46 137 (27.7)	44 118 (27.5)	2019 (33.1)		3319 (28.1)	2766 (27.8)	553 (29.4)	
Weekly	77 891 (46.7)	75 511 (47.0)	2380 (39.0)		1428 (12.1)	1248 (12.6)	180 (9.6)	
Daily	27 250 (16.3)	26 456 (16.5)	794 (13.0)		477 (4.0)	412 (4.1)	65 (3.5)	
None	113 (0.1)	109 (0.1)	4 (0.1)		4420 (37.4)	3788 (38.1)	632 (33.6)	
Physical activity (MET-min/week)	2520.7 ± 2466.7	2515.7 ± 2457.0	2658.2 ± 2716.7	<0.001	2149.1 ± 3824.1	2154.8 ± 3827.4	2115.3 ± 3806.6	0.76
BMI (kg/m^2)	26.9 ± 5.1	26.9 ± 5.1	28.2 ± 5.6	<0.001	29.6 ± 7.0	29.5 ± 7.1	30.2 ± 6.9	<0.001
Healthy status, *n* (%)				<0.001				<0.001
Excellent	29 484 (17.7)	28 854 (18.0)	630 (10.3)		796 (6.7)	692 (7.0)	104 (5.5)	
Good	99 897 (59.9)	96 840 (60.3)	3057 (50.1)		6395(54.1)	5411(54.5)	976(51.9)	
Fair	30 696 (18.4)	28 901 (18.0)	1795 (29.4)		2304 (19.5)	1827 (18.4)	477 (25.3)	
Poor	5951 (3.6)	5352 (3.3)	599 (9.8)		471 (4.0)	357 (3.6)	114 (6.1)	
Not clear	659 (0.4)	635 (0.4)	24 (0.4)		1849 (15.6)	1638 (16.5)	211 (11.2)	
Biological age	50.2 ± 8.1	50.1 ± 8.2	52.4 ± 7.5	<0.001	57.0 ± 13.7	56.5 ± 13.8	59.4 ± 12.7	<0.001
Biological age acceleration	−6.0 ± 2.3	−6.1 ± 2.3	−5.8 ± 2.6	<0.001	−3.2 ± 7.1	−3.3 ± 7.0	−2.7 ± 7.5	0.00
Components of biological ages								
SBP (mmHg)	135.0 ± 19.2	135.0 ± 19.2	136.9 ± 19.4	<0.001	131.0 ± 22.0	130.8 ± 22.0	132.5 ± 22.1	0.002
Albumin (g/L)	45.0 ± 2.6	45.0 ± 2.6	44.8 ± 2.7	<0.001	41.5 ± 3.1	41.5 ± 3.1	41.4 ± 3.2	0.05
ALP (U/L)	84.6 ± 27.1	84.3 ± 27.1	90.2 ± 27.6	<0.001	74.9 ± 23.8	74.3 ± 23.7	78.3 ± 23.9	<0.001
CRP (mg/dL)	0.3 ± 0.4	0.3 ± 0.4	0.4 ± 0.5	<0.001	0.5 ± 0.6	0.5 ± 0.6	0.5 ± 0.6	<0.001
TC (mg/dL)	226.9 ± 43.4	226.8 ± 43.3	228.8 ± 45.7	<0.001	206.7 ± 40.2	206.4 ± 40.0	208.2 ± 41.2	0.09
HbA1c (%)	5.4 ± 0.5	5.4 ± 0.5	5.5 ± 0.6	<0.001	5.8 ± 0.8	5.8 ± 0.8	5.9 ± 0.9	<0.001
Glucose (mmol/L)	5.1 ± 1.0	5.1 ± 1.0	5.2 ± 1.3	<0.001	5.6 ± 1.5	5.6 ± 1.5	5.7 ± 1.6	0.05
Creatinine (μmol/L)	64.3 ± 13.4	64.2 ± 13.0	65.8 ± 20.4	<0.001	71.1 ± 19.1	70.4 ± 18.8	74.5 ± 20.8	<0.001
Bun (mg/dL)	14.6 ± 3.7	14.6 ± 3.7	15.2 ± 4.1	<0.001	14.2 ± 5.4	14.2 ± 5.4	14.7 ± 5.7	<0.001
LYMPH (%)	29.8 ± 7.3	29.7 ± 7.3	30.4 ± 7.6	<0.001	31.1 ± 8.4	31.0 ± 8.4	31.7 ± 8.7	0.00
MCV (fL)	82.9 ± 5.3	82.9 ± 5.3	83.2 ± 5.5	<0.001	89.4 ± 5.8	89.3 ± 5.9	89.9 ± 5.3	<0.001
RDW (%)	13.5 ± 1.1	13.5 ± 1.1	13.5 ± 1.0	0.330	13.3 ± 1.3	13.3 ± 1.3	13.2 ± 1.2	0.21
WBC (1000 cells/μL)	6.8 ± 1.9	6.8 ± 1.9	7.1 ± 1.9	<0.001	7.1 ± 2.0	7.1 ± 2.0	7.3 ± 2.1	<0.001

Abbreviations: ALP, alkaline phosphatase; BMI, body mass index; Bun, urea; CRP, C-reactive protein; HbA1c, glycated hemoglobin; LYMPH, lymphocyte percentage; MCV, mean sphered cell volume; RDW, red blood cell distribution width; SBP, systolic blood pressure; TC, cholesterol; TDI, Townsend Deprivation Index; WBC, and white blood cell count.
[a]See Table S4 for information on education level, ethnicity, and income.

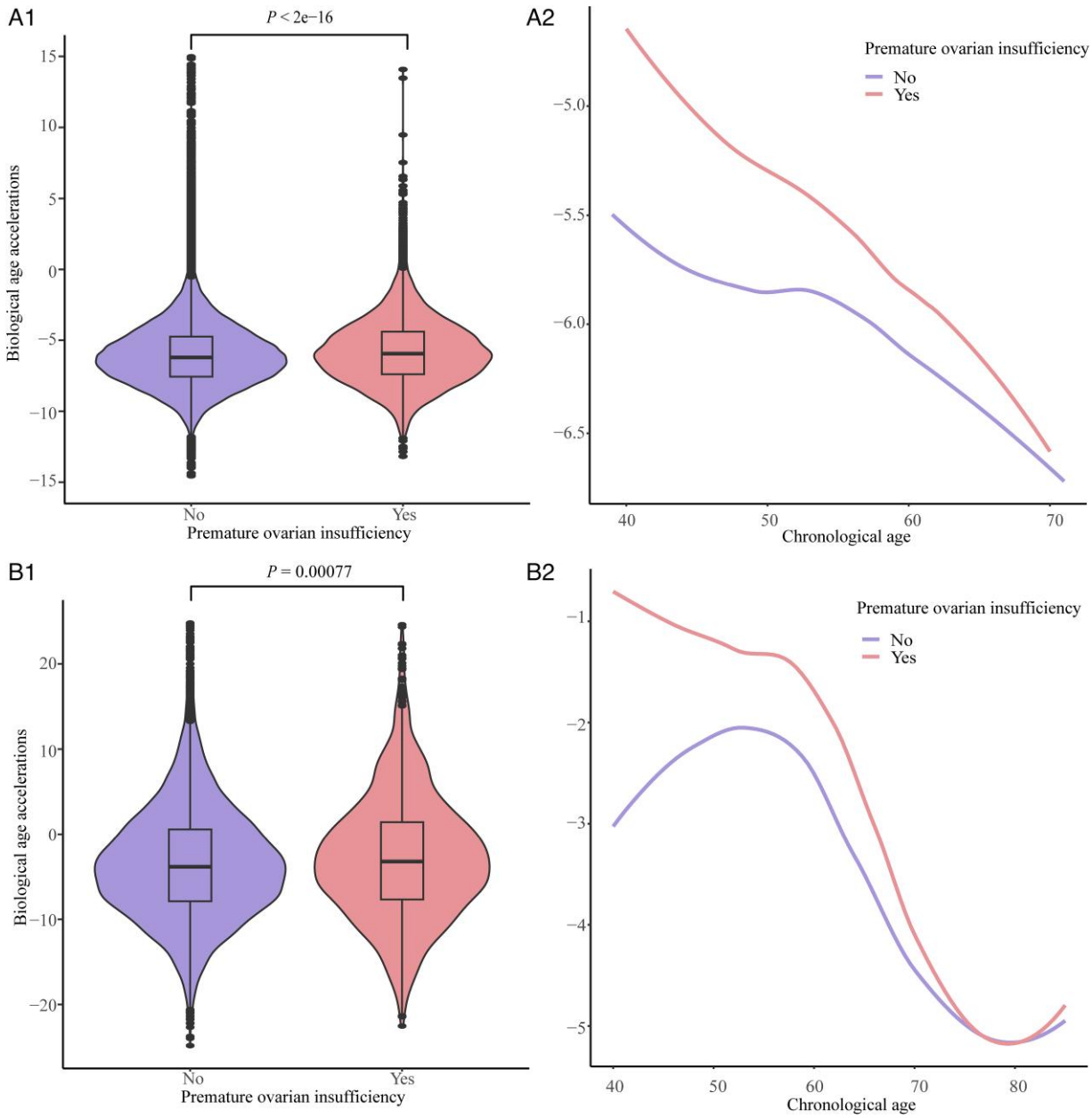

Figure 2. Differences in biological age acceleration and association with chronological age in the presence or absence of premature ovarian insufficiency in the UK Biobank (A1 and A2) and NHANES (B1 and B2). The smoothed curves were generated with the ggplot 2 package in R version 4.1.3 using locally weighted polynomial smoothing.

Discussion

To the best of our knowledge, this is the first study to comprehensively evaluate POI and biological aging in a large population-based study. The main findings of this study are as follows: (1) female with POI had an increased risk of biological aging compared with those without POI; (2) the degree of biological aging increased as the age at menopause decreased; and (3) the use of MHT in female with POI had a protective effect on biological aging. A strength of this study is the use of the validated Klemera–Doubal method to calculate BA and assess the degree of biological aging. We combined data from the UK Biobank and NHANES, which are two large population-based studies with an adequate sample size, and validated the findings in two different populations. By comparing the LTL, we further examined the association between POI and biological aging at the genetic level. We also focused on the effect of MHT use on biological aging in female with POI.

Our results showed that the prevalence of POI differed between the UKB and NHANES populations. Many factors may be responsible for the difference in the POI prevalence, such as ethnicity, physical activity, dietary habits, and so on. However, our results inferred the correlation between biological aging and POI in both databases, demonstrating the stability of the findings.

Our study showed that POI was accompanied by biological aging, which is consistent with the results of an epidemiological study, which showed that earlier menopause was associated with shorter telomere length.[12] Telomere length was recognized as a biomarker of biological aging.[19] Previous studies have demonstrated links between telomere shortening and elevated risks of cardiovascular aging and related diseases.[20,21]

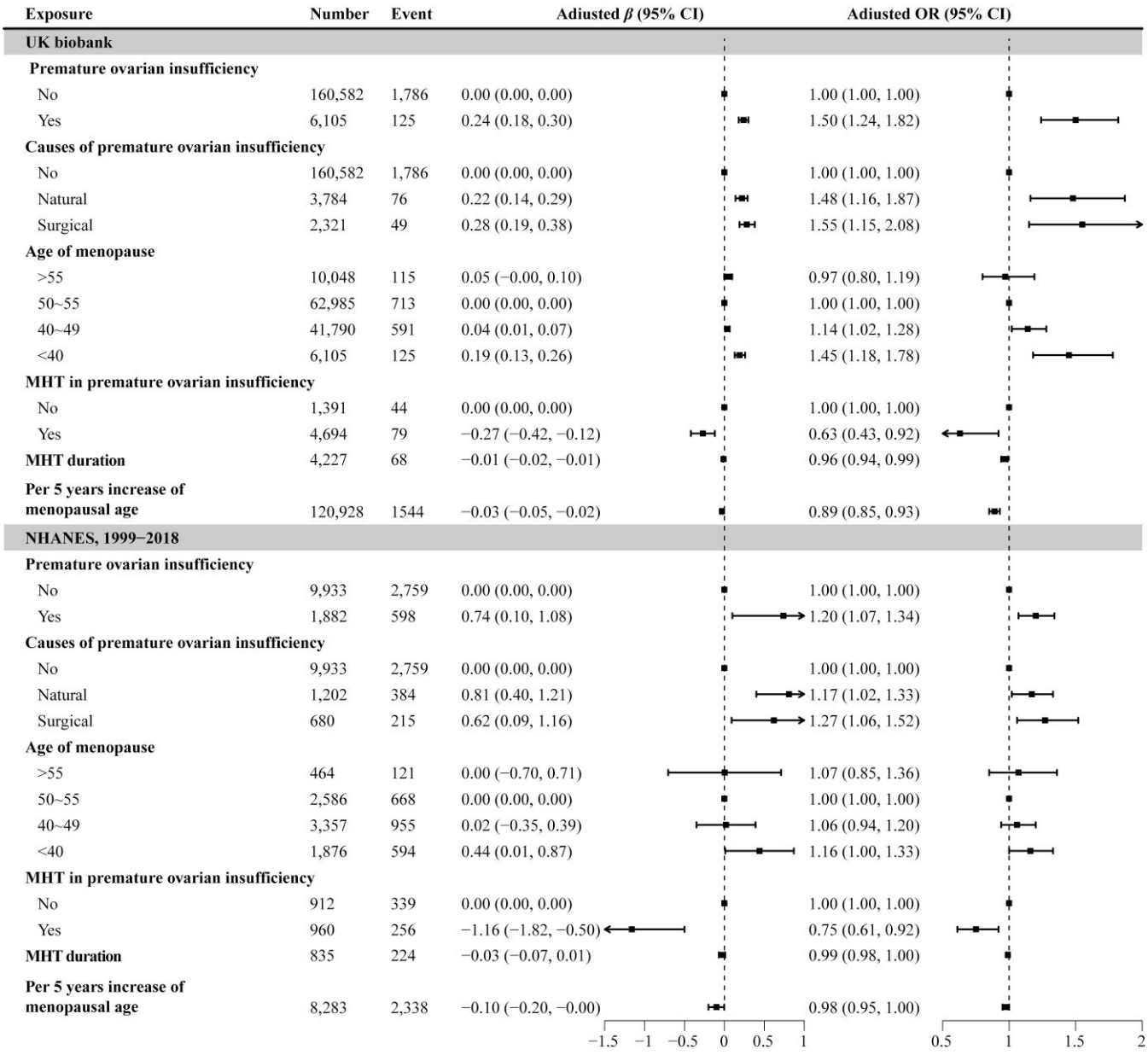

Figure 3. Forest plot of the associations between the menopausal status and biological age acceleration in the UK Biobank and NHANES. β (95% CI) and OR (95% CI) were derived from linear regression models, logistic regression models, respectively. The model was adjusted for age, ethnic, education, income, TDI (only in UK biobank), smoking status, alcohol consumption, MET, BMI, and MHT.

Our study further revealed significantly shorter telomeres in POI patients compared to non-POI controls. Furthermore, we comprehensively considered factors, such as inflammation, immunity, and metabolism, from the perspective of BA, and found that POI was related to biological aging. Studies have reported that POI is associated with other age-related adverse health outcomes, such as an increased risk of cardiovascular disease, osteoporosis, cognitive impairment, and dementia.[7-9,22,23] A study based on the Korean National Health System showed that female with a history of premature menopause had an increased risk of heart failure and atrial fibrillation by 33% and 9%, respectively.[24] These studies provide some evidence to support our conclusions. Furthermore, we found that an earlier age of menopause was associated with a higher risk of biological aging, and similar associations were observed between menopause age and other adverse health outcomes.[12,25] Honigberg[7] and Schuermans[12] found that a younger age at menopause was associated with a higher risk of cardiovascular disease. Another study showed that female who experience menopause before 40 years of age have a higher risk of dementia than those aged 46-50 years, while menopause after 55 of age is associated with a lower risk of dementia.[25] These findings suggested a time-dependent relationship between estrogen deficiency and adverse health outcomes and may indirectly reflect the relationship between menopause age and biological aging.

The early loss of estrogen is currently recognized as the main cause of adverse outcomes of POI.[26] Maioli *et al.*[27] found that a decrease in estrogen receptors was directly related to brain aging, and estrogen had a powerful neuroprotective effect in mouse brain sections. The rapid decline in estrogen concentrations in the circulation after menopause increases female's vulnerability to adverse neurological events. Russell *et al.*'s[28] study suggested that during the normal brain aging process, the decline in estrogen is associated with many changes in

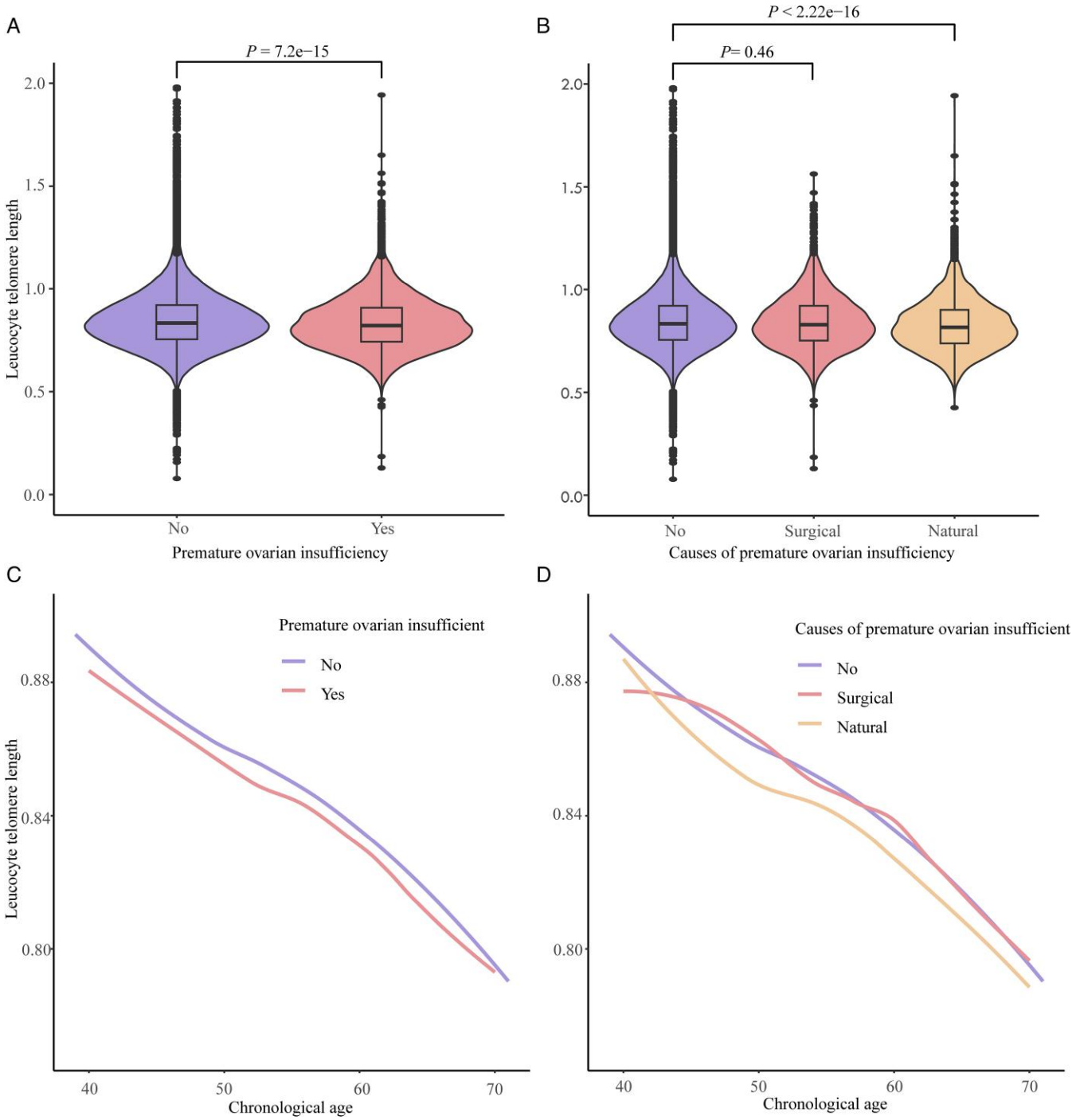

Figure 4. Differences in the leucocyte telomere length (A, B) and association with chronological age (C, D) according to premature ovarian insufficiency and the cause of premature ovarian insufficiency in the UK Biobank.

the brain, including cognitive changes, effects on sleep, and effects on emotions. These effects have been confirmed in rodents and non-human preclinical models.[29,30] Jacobs *et al.*'s[31] study showed that the sudden loss of estrogen during menopause was a risk factor for Alzheimer's disease. Additionally, basic research has shown that estrogen can not only increase neuroplasticity and improve various aspects of cognition but can also protect neurons from cell death. The above-mentioned animal experiments provide a theoretical basis for the association between POI and biological aging.

Interestingly, we found that in female with POI, using MHT significantly reduced the risk of biological aging compared with not using MHT. A recent population-based study by Liu and Li[32] found that MHT slows down biological aging, which were consistent with the findings of our study. Similar results were also found in studies of other disease outcomes, where MHT improved the adverse effects caused by POI, such as cardiovascular diseases and cognitive dysfunction.[31,33,34] These findings suggest that MHT is an effective measure for female with POI, which is consistent with previous treatment guidelines for POI. In all female with POI,

hormone therapy is recommended before the expected cessation of ovarian function because this treatment has greater benefits than risks for most female.[35] Therefore, in female with POI, hormone therapy may be greatly beneficial in delaying the aging of such patients. However, MHT may be highly heterogeneity (estrogens-only vs estrogens–progestogen combinations, oral treatment vs transdermal). Different hormonal compounds and routes of administration have been demonstrated to exert different effects on vascular and metabolic health, which may influence aging.[36,37] Future prospective studies should be rigorously designed to elucidate the therapeutic effects of specific MHT regimens.

We specifically used the Klemera–Doubal method, which has been applied in several studies.[14,15] The selection and quantification of aging biomarkers mainly depend on blood-based aging biomarkers collected in observational studies, which is the most active research area for validating aging biomarkers. In addition, we measured the telomere length to calculate aging. The telomere length shortens with each cell division, and when a certain threshold is reached, it triggers cellular senescence. Therefore, telomere attrition is considered a major hallmark of aging.[12,18]

Although our research design was relatively rigorous, there are still some limitations. First, our study was cross-sectional and only evaluated the status of POI, BA, and LTL at baseline and lacked a clear time sequence. This situation limited further investigation of the causal relationship between POI and biological aging. In addition, POI was assessed according to the self-reported age of menopause by the study participants, which may have been subject to recall bias. Third, this study only obtained LTL data in the UK Biobank, and we regretted that this part of the results could not be validated in NHANES. Although we used data from two large-scale studies to validate our conclusions in different populations, the majority of the participants were Caucasian, especially in the UK Biobank. Therefore, further research is required to verify whether our results can be generalized to other populations.

In summary, this study shows that biological aging may be associated with POI, and the aging process increases as the age of menopause becomes earlier. Additionally, our study implied that MHT may partially offset the adverse outcomes caused by POI. Our research suggests that patients with POI should be informed about the risks of aging and the potential benefits of early MHT. Future studies are required to verify these findings.

Acknowledgments

We thank the UK Biobank and NHANES participants. We thank Ellen Knapp, PhD, from Liwen Bianji (Edanz) (www.liwenbianji.cn/), for editing the English text of a draft of this manuscript.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

Funding

The study was partly supported by the Hubei Provincial Natural Science Foundation (Key Project of Joint Fund) (no. 2023AFD031).

Authors' contributions

Jinting Zhou (Conceptualization [equal], Investigation [lead], Methodology [supporting], Writing—original draft [lead], Writing—review & editing [supporting]), Menglin Fan (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Methodology [supporting], Visualization [lead], Writing—original draft [supporting], Writing—review & editing [equal]), Aaron M. Lett (Writing—review & editing [supporting]), Geling Jin (Writing—review & editing [supporting]), Qiqi You (Data curation [supporting], Visualization [supporting], Writing—review & editing [supporting]), Jingjing Zeng (Data curation [supporting], Writing—review & editing [supporting]), Bo Chen (Writing—review & editing [supporting]), Yucen Wu (Writing—review & editing [supporting]), Hui Xing (Project administration [equal], Supervision [equal], Writing—review & editing [supporting]), and Shaoyong Xu (Conceptualization [equal], Funding acquisition [lead], Project administration [lead], Supervision [equal], Writing—review & editing [supporting])

Conflict of interest: The authors declare no conflict of interests.

Ethics approval

The UK Biobank research was approved by the North West Multicenter Research Ethical Committee, and all participants provided written informed consent based on the principles of the Declaration of Helsinki before enrollment in the study. The present study was performed under application number 92014. The NHANES was approved by the Research Ethics Review Board of the National Center for Health Statistics, and all participants provided informed consent.

Data availability

Data are available in the public, open-access repository. This research has been conducted using the UK Biobank (application number 92014) and NHANES resource. The UK Biobank data are available on application to the UK Biobank (www.ukbiobank.ac.uk/) with access fees. The NHANES data are publicly available (https://www.cdc.gov/nchs/nhanes/).

References

1. WHO. Ageing and health. Accessed August 20, 2024. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health
2. Khan SS, Singer BD, Vaughan DE. Molecular and physiological manifestations and measurement of aging in humans. *Aging Cell*. 2017;16(4):624-633. https://doi.org/10.1111/acel.12601
3. Evans MA, Sano S, Walsh K. Cardiovascular disease, aging, and clonal hematopoiesis. *Annu Rev Pathol*. 2020;15(1):419-438. https://doi.org/10.1146/annurev-pathmechdis-012419-032544
4. Hou Y, Dan X, Babbar M, *et al*. Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol*. 2019;15(10):565-581. https://doi.org/10.1038/s41582-019-0244-7
5. Kritsilis M, VR S, Koutsoudaki PN, Evangelou K, Gorgoulis VG, Papadopoulos D. Ageing, cellular senescence and neurodegenerative disease. *Int J Mol Sci*. 2018;19(10):2937. https://doi.org/10.3390/ijms19102937
6. Pal L, Santoro N. Premature ovarian failure (POF): discordance between somatic and reproductive aging. *Ageing Res Rev*. 2002;1(3):413-423. https://doi.org/10.1016/s1568-1637(02)00009-0
7. Honigberg MC, Zekavat SM, Aragam K, *et al*. Association of premature natural and surgical menopause with incident

cardiovascular disease. *JAMA*. 2019;322(24):2411-2421. https://doi.org/10.1001/jama.2019.19191
8. Karamitrou EK, Anagnostis P, Vaitsi K, Athanasiadis L, Goulis DG. Early menopause and premature ovarian insufficiency are associated with increased risk of dementia: a systematic review and meta-analysis of observational studies. *Maturitas*. 2023;176:107792. https://doi.org/10.1016/j.maturitas.2023.107792
9. Xu X, Jones M, Mishra GD. Age at natural menopause and development of chronic conditions and multimorbidity: results from an Australian prospective cohort. *Hum Reprod*. 2020;35(1):203-211. https://doi.org/10.1093/humrep/dez259
10. Horstman AM, Dillon EL, Urban RJ, Sheffield-Moore M. The role of androgens and estrogens on healthy aging and longevity. *J Gerontol A Biol Sci Med Sci*. 2012;67(11):1140-1152. https://doi.org/10.1093/gerona/gls068
11. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. *Cell*. 2023;186(2):243-278. https://doi.org/10.1016/j.cell.2022.11.001
12. Schuermans A, Nakao T, Uddin MM, *et al.* Age at menopause, leukocyte telomere length, and coronary artery disease in postmenopausal women. *Circ Res*. 2023;133(5):376-386. https://doi.org/10.1161/circresaha.123.322984
13. Klemera P, Doubal S. A new approach to the concept and computation of biological age. *Mech Ageing Dev*. 2006;127(3):240-248. https://doi.org/10.1016/j.mad.2005.10.004
14. Gao X, Huang N, Guo X, Huang T. Role of sleep quality in the acceleration of biological aging and its potential for preventive interaction on air pollution insults: findings from the UK Biobank cohort. *Aging Cell*. 2022;21(5):e13610. https://doi.org/10.1111/acel.13610
15. Gao X, Geng T, Jiang M, *et al.* Accelerated biological aging and risk of depression and anxiety: evidence from 424,299 UK Biobank participants. *Nat Commun*. 2023;14(1):2277. https://doi.org/10.1038/s41467-023-38013-7
16. Nash Z, Davies M. Premature ovarian insufficiency. *BMJ*. 2024;384:e077469. https://doi.org/10.1136/bmj-2023-077469
17. Kwon D, Belsky DW. A toolkit for quantification of biological age from blood chemistry and organ function test data: BioAge. *Geroscience*. 2021;43(6):2795-2808. https://doi.org/10.1007/s11357-021-00480-5
18. Shay JW. Role of telomeres and telomerase in aging and cancer. *Cancer Discov*. 2016;6(6):584-593. https://doi.org/10.1158/2159-8290.Cd-16-0062
19. De Meyer T, Nawrot T, Bekaert S, De Buyzere ML, Rietzschel ER, Andrés V. Telomere length as cardiovascular aging biomarker: JACC review topic of the week. *J Am Coll Cardiol*. 2018;72(7):805-813. https://doi.org/10.1016/j.jacc.2018.06.014
20. Li C, Stoma S, Lotta LA, *et al.* Genome-wide association analysis in humans links nucleotide metabolism to leukocyte telomere length. *Am J Hum Genet*. 2020;106(3):389-404. https://doi.org/10.1016/j.ajhg.2020.02.006
21. Peng H, Mete M, Desale S, *et al.* Leukocyte telomere length and ideal cardiovascular health in American Indians: the Strong Heart Family Study. *Eur J Epidemiol*. 2017;32(1):67-75. https://doi.org/10.1007/s10654-016-0199-6
22. Goh VH, Hart WG. Aging and bone health in Singaporean Chinese pre-menopausal and postmenopausal women. *Maturitas*. 2016;89:16-21. https://doi.org/10.1016/j.maturitas.2016.04.004
23. Sochocka M, Karska J, Pszczołowska M, *et al.* Cognitive decline in early and premature menopause. *Int J Mol Sci*. 2023;24(7):6566. https://doi.org/10.3390/ijms24076566
24. Shin J, Han K, Jung JH, *et al.* Age at menopause and risk of heart failure and atrial fibrillation: a nationwide cohort study. *Eur Heart J*. 2022;43(40):4148-4157. https://doi.org/10.1093/eurheartj/ehac364
25. Hao W, Fu C, Dong C, *et al.* Age at menopause and all-cause and cause-specific dementia: a prospective analysis of the UK Biobank cohort. *Hum Reprod*. 2023;38(9):1746-1754. https://doi.org/10.1093/humrep/dead130
26. Isola JVV, Ko S, Ocañas SR, Stout MB. Role of estrogen receptor α in aging and chronic disease. *Adv Geriatr Med Res*. 2023;5(2):e230005. https://doi.org/10.20900/agmr20230005
27. Maioli S, Leander K, Nilsson P, Nalvarte I. Estrogen receptors and the aging brain. *Essays Biochem*. 2021;65(6):913-925. https://doi.org/10.1042/ebc20200162
28. Russell JK, Jones CK, Newhouse PA. The role of estrogen in brain and cognitive aging. *Neurotherapeutics*. 2019;16(3):649-665. https://doi.org/10.1007/s13311-019-00766-9
29. Ramírez-Hernández D, López-Sanchez P, Lezama-Martínez D, *et al.* Early estrogen replacement therapy attenuates cardiac dysfunction caused by aging and ovariectomy in female Wistar rats. *Front Biosci (Landmark Ed)*. 2024;29(1):46. https://doi.org/10.31083/j.fbl2901046
30. Fang YY, Zeng P, Qu N, *et al.* Evidence of altered depression and dementia-related proteins in the brains of young rats after ovariectomy. *J Neurochem*. 2018;146(6):703-721. https://doi.org/10.1111/jnc.14537
31. Jacobs EG, Kroenke C, Lin J, *et al.* Accelerated cell aging in female APOE-ϵ4 carriers: implications for hormone therapy use. *PLoS One*. 2013;8(2):e54713. https://doi.org/10.1371/journal.pone.0054713
32. Liu Y, Li C. Hormone therapy and biological aging in postmenopausal women. *JAMA Netw Open*. 2024;7(8):e2430839. https://doi.org/10.1001/jamanetworkopen.2024.30839
33. Liu F, Day M, Muñiz LC, *et al.* Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. *Nat Neurosci*. 2008;11(3):334-343. https://doi.org/10.1038/nn2057
34. Hodis HN, Mack WJ. Menopausal hormone replacement therapy and reduction of all-cause mortality and cardiovascular disease: it is about time and timing. *Cancer J*. 2022;28(3):208-223. https://doi.org/10.1097/ppo.0000000000000591
35. Webber L, Davies M, Anderson R, *et al.* ESHRE guideline: management of women with premature ovarian insufficiency. *Hum Reprod*. 2016;31(5):926-937. https://doi.org/10.1093/humrep/dew027
36. Løkkegaard E, Andreasen AH, Jacobsen RK, Nielsen LH, Agger C, Lidegaard Ø. Hormone therapy and risk of myocardial infarction: a national register study. *Eur Heart J*. 2008;29(21):2660-2668. https://doi.org/10.1093/eurheartj/ehn408
37. Rossouw JE, Anderson GL, Prentice RL, *et al.* Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA*. 2002;288(3):321-333. https://doi.org/10.1001/jama.288.3.321