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Association between leucocyte telomere length and erectile dysfunction in US adults: a secondary study based on 2001–2002 NHANES data

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ABSTRACT

Objective We aimed to explore the association between the leucocyte telomere length (LTL) and erectile dysfunction (ED) among a nationally representative sample of US adults.

Design Secondary population-based study.

Setting The National Health and Nutrition Examination Survey (NHANES) (2001–2002).

Participants A total of 1694 male participants were extracted from the NHANES database for 2001–2002.

Primary and secondary outcome measures The primary focus of the study was to determine the association between the LTL and ED, using multivariate logistic regression and restricted cubic spline models for examination. The secondary outcome measures involved conducting stratified subgroup analyses to exclude interactions of different variables with the LTL.

Results Participants with ED had shorter LTLs than those without ED (p<0.05). After adjusting for confounding factors, compared with the reference lowest LTL quartile, the ORs and 95% CIs for the second, third and fourth LTL quartiles were (OR 1.51; 95% CI 1.01 to 2.26), (OR 1.79; 95% CI 1.24 to 2.58) and (OR 1.25; 95% CI 0.74 to 2.11), respectively. In addition, restricted cubic splines showed an inverted J-curve relationship between the LTL and ED. At an LTL of 1.037, the curve showed an inflection point. The ORs (95% CI) of ED on the left and right sides of the inflection point were (OR 1.99; 95% CI 0.39 to 10.20; p=0.385) and (OR 0.17; 95% CI 0.03 to 0.90; p=0.039).

Conclusion Our results demonstrated an inverted J-curve relationship between the LTL and ED. When the LTL was ≥1.037, the incidence of ED decreased with increasing LTL.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study represents the pioneering investigation of the potential associations between leucocyte telomere length (LTL) and erectile dysfunction (ED).
⇒ This study used a nationally representative sample and employed rigorous statistical adjustment methods to mitigate potential confounding factors.
⇒ The findings of this secondary study do not provide sufficient evidence to establish a causal relationship between the two variables.
⇒ The diagnosis of ED was based on questionnaires and lacks specialised diagnostic tests, which may lead to selection bias.
⇒ Limited by the data, the range of the fourth LTL quartile was large, and the distribution of participants was discrete, which may affect the construction of our non-linear model.

INTRODUCTION

Inadequate penile erection or inability to maintain erectile status to complete satisfactory sexual behaviour is defined as erectile dysfunction (ED) and the degree of erection can be classified as no ED, mild, moderate and severe based on the hardness of erection.[1] About 150 million men around the world are afflicted with varying degrees of ED,[2] and a previous epidemiological study conducted in eight countries reported that the ED prevalence in middle-aged and elderly males was approximately 50%.[3] There is a growing concern about the rising prevalence of ED among young men, with estimates suggesting that it may affect up to 30% of this population.[4] ED places a significant psychological and financial burden on affected individuals. Moreover, diseases within this highly affected population can also impose a burden on society as a whole. Thus, it is critical to develop suitable biomarkers for early diagnosis and intervention for ED and to identify previously unexplored risk factors such as the COVID-19. Recently, the COVID-19 pandemic is associated with an elevated prevalence of ED.[5]

Multiple mechanisms may contribute to ED, and numerous studies have reported risk factors for the occurrence and development of ED.[6] Endothelial dysfunction, a major cause of ED, is thought to be associated with inflammatory and oxidative stress levels.[7] Many vascular endothelial damage-associated diseases, such as cardiovascular disease

(CVD)[8] and type 2 diabetes, share this common pathological mechanism with ED.[9] Therefore, ED is a harbinger of the severe development of these diseases.

Telomeres consist of repetitive sequence nucleotides and a protein complex located at the ends of chromosomes and are important for stabilising and preventing chromosome degeneration.[10] Telomeres naturally shorten as cells divide. However, telomerase fills telomeres lost by DNA replication and lengthens and repairs telomeres so that telomeres are not depleted by cell division.[11] Differentiated human cells generally lack telomerase activity, so cells are unable to replicate a small fraction of telomeres.[12] This is why telomeres have been described as biological markers of cellular senescence. Oxidative stress and inflammatory factors have been demonstrated to further contribute to telomere attrition.[13] The leucocyte telomere length (LTL) has also been shown to be inversely associated with inflammation-related chronic diseases.

Previous studies have noted the role of the LTL in a range of diseases associated with endothelial dysfunction. Codd *et al*[14] found a huge association between diabetes and the LTL in the largest LTL study thus far, and a Mendelian randomisation analysis indicated that reductions in LTL per unit were associated with a 1.38-fold higher risk of diabetes progression in patients with type 2 diabetes.[15] In addition, the negative relationship between the LTL and diseases associated with vascular endothelial disorders has been demonstrated in studies of CVD, hyperlipidaemia and stroke.[16–18] These findings collectively suggest a possible association between ED and the LTL. However, the relationship between LTL and ED has not been clearly established.

METHODS

Study population and data collection

This study represents our analysis of participants from the 2001 to 2002 cycles of the National Health and Nutrition Examination Survey (NHANES) because only this cycle contained LTL (https://wwwn.cdc.gov/Nchs/Nhanes/2001-2002/TELO_B.htm.) and ED (https://wwwn.cdc.gov/Nchs/Nhanes/2001-2002/KIQ_P_B.htm) information. NHANES uses a stratified, multistage, clustered probability sampling design, which allows it to be a sample that fits the stratification of the US population. In addition, this study was designed according to the guidelines for reporting cross-sectional studies as specified in the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[19]

A total of 11 039 participants(5331 male and 5708 female participants) were included in the 2001–2002 NHANES cycles. We considered 5331 male participants and sequentially excluded participants aged <20 years (n=2795) and individuals with missing LTL data (n=479) or missing ED data (n=186). In addition, we excluded cancer participants (n=177). Ultimately, 1694 participants were involved in our study, as shown in online supplemental figure 1.

ED ascertainment

The Massachusetts Male Aging Study single-question self-assessment for evaluation of ED was used.[20] The participants were provided the following information and asked the following question: 'Many men experience problems with sexual intercourse. How would you describe your ability to get and keep an erection adequate for satisfactory intercourse?' The answer options were as follows: 'never able', 'sometimes able', 'usually, able' and 'always or almost always able'. Participants who responded 'sometimes able' or 'never able' were defined as having ED. Participants who provided the latter two responses were defined as not having ED.

Telomere measurements

Blood samples from all surveyed individuals were collected as part of the NHANES. The telomere length assay was performed in the laboratory of Dr. Elizabeth Blackburn at the University of California, San Francisco, and PCR was used to measure the telomere length relative to the length of standard reference DNA (T/S ratio). Each blood sample was assayed three times on three different days. Assay runs included control values beyond two SDs of the mean of all runs (<6% of runs). Outliers within samples were identified and excluded (<2% of samples). A detailed description of the LTL quantification procedure and analytical methods can be found on the official website (http://www.cdc.gov/nchs/nhanes.htm).

Covariates

Information on covariates was collected as per the NHANES examinations and questionnaires administered by highly trained medical personnel. Participants reported the following information: demographic characteristics (age, race/ethnicity, education level, poverty income ratio (PIR)), lifestyle (body mass index(BMI), smoking status, alcohol consumption status, vigorous activity and moderate activity) and medical history (hypertension, diabetes, CVD, chronic kidney disease (CKD), hyperlipidaemia and ED medications). In the 2001–2002 NHANES cycles, race/ethnicity was grouped into five categories based on race and Hispanic origin. Income levels were divided into three categories (< 1.3, 1.3–1.8 and>1.8) based on PIRs. In addition, BMI was calculated as weight divided by the square of height and divided into two groups according to ≥30 and <30. Participants were divided into former smokers, never smokers and current smokers according to the number of cigarettes smoked during their lifetime and current smoking status. Alcohol consumption status was categorised as yes or no based on at least 12 drinks per year. Moderate or vigorous activity was based on the participant's exercise during the past 30 days. Hypertension was defined as a mean systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥80 mm or the use of hypertension medication or diagnosis by

a physician or other healthcare professional.[21] Random blood glucose ≥11.1 mmol/L, Hemoglobin A1c>6.5% or using diabetes pills or insulin were defined as having diabetes mellitus, as diagnosed by a physician or other professional.[22] CKD was defined as an estimated glomerular filtration rate <60 mL/min 1.73 m^2 or an albumin creatinine ratio>30 mg/g.[23] Triglycerides≥150 mg/dL, Low-density lipoprotein≥130 mg/dL, High-density lipoprotein<40 mg/dL and the use of lipid-lowering agents were defined as hyperlipidaemia.[24] ED medications were defined as having taken any medication for ED (including sildenafil citrate and yohimbine)

Statistical analysis

We used appropriate sample weights to recover population-level data across the US for statistical analysis according to Centers for Disease Control and Prevention (CDC) analysis guidelines.[25] The LTL was classified into quartiles based on the weighted sample distribution. Continuous variables are presented as the weighted mean±SD and were compared using the one-way analysis of variance. Categorical variables are presented as weighted percentages (95% CI) and were compared using the Rao-Scott χ^2 test. In addition, we used weighted logistic regression models to estimate ORs and CIs between LTL quartiles and ED. Multivariate models included model 1 (without any adjustment), model 2 (adjusted for age, race/ethnicity, education level, smoking status, PIR, vigorous activity, moderate activity, alcohol consumption status) and model 3 (adjusted for model 2 plus hypertension, diabetes, CVD, CKD, hyperlipidaemia and ED medications). Subgroup analysis was conducted using stratified multivariate logistic regression analysis and stratified by age, BMI, hypertension, diabetes, CVD, CKD, hyperlipidaemia. Multiplicative interactions between subgroups were assessed using likelihood ratio tests, which assess the heterogeneity of associations between subgroups. Moreover, non-linear relationships between the LTL and ED were explored at restricted cubic splines with 3 knots at the 10th, 50th and 90th percentiles in fully adjusted models. If non-linearity was detected, we constructed a two-piece logistic regression model to calculate the threshold effect of the LTL on ED.

We addressed a few missing values for model covariates using the missForest package in R software.[25] The algorithm can handle categorical and continuous variables and has demonstrated robust performance, with the number and percentage of missing covariate data shown in online supplemental table 1. Sensitivity analyses excluded missing covariate data to assess the impact on attribution methods, and data from the analyses are presented in online supplemental tables 2 and 3. In addition, we presented the results again using the quartile of telomere length (Q3) where the inflection point was located as a reference, and the result was added to online supplemental table 4.

To ensure that our study was nationally representative, all analyses incorporated sample weights, strata and clustering for complex sampling designs.[25] R software (V.4.1.3) was used for the analysis. All statistical tests were two tailed, and p values less than 0.05 were considered statistically significant.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

RESULTS

Participants' baseline characteristics

Descriptive statistics of our study sample according to quartiles of LTL and ED/non-ED participants are shown in table 1 and online supplemental table 5. Of 1694 males were included in this study, namely, 399 participants with ED and 1295 participants without ED, which represented 71 910 801 non-institutionalised adult males in the USA.

The mean age of the population surveyed was 43.56±0.59 years, and the mean LTL was 1.08±0.02. Among the participants, 73.92% (65.78–82.07) were non-Hispanic white, and 56.02% (50.28–61.76) had an above high school education. The average LTL was shorter in the ED participants than in the non-ED participants (0.97±0.02 vs 1.09±0.02, p<0.0001). In contrast, compared with the non-ED participants, the ED participants were on average older (58.32±1.00 vs 40.83±0.45, p<0.0001). In addition, compared with the shorter LTL groups (Q1 and Q2), the longer LTL groups (Q3 and Q4) were associated with younger age and more vigorous physical activity. In contrast, the shorter LTL groups had a higher prevalence of hypertension, CVD, CKD and hyperlipidaemia (all p<0.001). Notably, subjects with ED had a higher prevalence of non-communicable diseases, including hypertension, diabetes, CVD, CKD and hyperlipidaemia.

LTL and ED

Table 2 presents the association of the LTL and ED based on multivariate logistic regression. The association between the LTL as a continuous covariate and ED was negative in the unadjusted model (OR 0.12; 95% CI 0.06 to 0.22; p<0.0001). In analyses where the LTL was included as a quartile, this association did not change as the telomere length increased (p for trend <0.0001). However, this association was no longer significant in models adjusted for multiple covariates.

Notably, in the fully adjusted model (model 3), compared with those in the reference first quartile, participants in the second, third and fourth quartiles of LTL had higher odds of experiencing ED (OR 1.51; 95% CI 1.01 to 2.26; p=0.044; OR 1.79; 95% CI 1.24 to 2.58; p=0.004; OR 1.25; 95% CI 0.74 to 2.11; p=0.388, respectively). No significant trends were observed (p for trend=0.340).

Table 1 Baseline characteristics of the study population in the NHANES 2001–2002

Characters	Overall (n=1694)	Q1 (n=424) [0.453–0.864]	Q2 (n=425) (0.864–1.006]	Q3 (n=420) (1.006–1.179]	Q4 (n=425) (1.179–2.429]	P value
Age	43.56±0.59	53.42±0.94	45.59±1.08	40.59±1.06	37.31±1.00	<0.001
BMI						0.066
<30	72.81 (67.21 to 78.40)	68.06 (63.10 to 73.02)	71.69 (66.98 to 76.40)	75.08 (70.93 to 79.22)	75.21 (72.31 to 78.10)	
≥30	27.19 (23.66 to 30.73)	31.94 (26.98 to 36.90)	28.31 (23.60 to 33.02)	24.92 (20.78 to 29.07)	24.79 (21.90 to 27.69)	
Race/ethnicity						0.144
Mexican American	8.02 (5.94 to 10.10)	4.40 (2.56 to 6.35)	9.80 (7.60 to 11.99)	9.50 (6.72 to 12.29)	7.92 (4.38 to 11.46)	
Non-Hispanic black	9.19 (6.82 to 11.55)	8.32 (5.39 to 11.25)	8.01 (4.94 to 11.08)	9.54 (6.23 to 12.85)	10.49 (6.44 to 14.54)	
Non-Hispanic white	73.92 (65.78 to 82.07)	80.21 (75.29 to 85.12)	73.57 (66.85 to 80.28)	70.58 (64.12 to 77.04)	72.53 (64.08 to 80.99)	
Other Hispanic	5.75 (1.56 to 9.94)	3.65 (0.00 to 7.57)	6.91 (2.02 to 11.79)	5.64 (1.55 to 9.74)	6.43 (1.56 to 11.29)	
Other race/ethnicity	3.11 (1.74 to 4.48)	3.42 (0.91 to 5.93)	1.72 (0.26 to 3.19)	4.73 (1.30 to 8.16)	2.62 (0.39 to 4.85)	
Education						0.386
Less than high school	17.82 (14.79 to 20.84)	20.69 (16.36 to 25.03)	17.23 (13.93 to 20.54)	19.55 (14.53 to 24.56)	29.01 (22.01 to 36.02)	
High school graduate	26.17 (22.94 to 29.39)	28.07 (21.05 to 35.09)	26.85 (21.01 to 32.70)	23.56 (18.49 to 28.62)	26.48 (22.46 to 30.50)	
Above high school	56.02 (50.28 to 61.75)	51.23 (43.68 to 58.79)	55.91 (50.29 to 61.54)	56.90 (48.56 to 65.23)	58.82 (56.91 to 60.73)	
Poverty income ratio						0.536
<1.3	15.67 (12.84 to 18.51)	15.58 (11.49 to 19.66)	11.62 (8.30 to 14.59)	16.96 (12.80 to 21.13)	17.94 (11.61 to 24.27)	
1.3–3.5	37.21 (32.19 to 42.23)	37.88 (31.13 to 44.62)	39.51 (35.06 to 43.97)	34.79 (26.03 to 43.54)	36.92 (31.99 to 41.85)	
≥3.5	47.12 (42.24 to 52.00)	46.55 (38.86 to 54.23)	48.86 (44.25 to 53.47)	48.25 (38.37 to 58.13)	45.13 (39.66 to 50.61)	
Smoking status						0.069
Now	28.07 (24.02 to 32.13)	27.14 (20.47 to 33.80)	25.95 (18.78 to 33.12)	29.82 (23.90 to 35.74)	28.98 (21.98 to 35.98)	
Former	28.15 (24.10 to 32.19)	34.13 (28.53 to 39.73)	33.51 (26.44 to 40.58)	23.23 (19.67 to 16.78)	23.67 (19.83 to 27.51)	
Never	43.78 (38.57 to 48.99)	38.73 (31.87 to 45.60)	40.54 (34.00 to 47.08)	46.96 (42.44 to 51.47)	47.35 (40.04 to 54.67)	
Alcohol consumption status						0.543
Yes	84.76 (75.39 to 94.14)	81.68 (74.15 to 89.21)	84.45 (76.82 to 92.09)	85.78 (79.11 to 92.45)	86.38 (78.17 to 94.59)	
No	15.24 (8.80 to 21.67)	18.32 (10.79 to 25.85)	15.55 (7.91 to 23.18)	14.22 (7.55 to 20.89)	13.62 (5.41 to 21.83)	
Vigorous activity						0.009
Yes	44.63 (39.71 to 49.55)	35.35 (29.64 to 41.06)	39.87 (32.60 to 47.14)	46.73 (38.57 to 54.90)	53.44 (47.38 to 59.50)	
No	55.37 (49.87 to 60.87)	64.65 (58.94 to 70.36)	60.13 (52.86 to 67.40)	53.27 (45.10 to 61.43)	46.56 (45.50 to 52.62)	
Moderate activity						0.877
Yes	52.46 (47.08 to 57.85)	53.63 (46.84 to 60.43)	54.14 (48.07 to 60.12)	51.73 (43.65 to 59.90)	50.88 (42.03 to 59.73)	
No	47.54 (42.17 to 52.90)	46.37 (39.57 to 53.16)	45.86 (39.79 to 51.93)	48.27 (40.10 to 56.44)	48.91 (39.92 to 57.91)	
Hypertension						

Continued

Table 1 Continued

Characters	Overall (n=1694)	Q1 (n=424) [0.453–0.864]	Q2 (n=425) (0.864–1.006]	Q3 (n=420) (1.006–1.179]	Q4 (n=425) (1.179–2.429]	P value
Yes	52.30 (47.31 to 57.28)	62.21 (57.18 to 67.24)	56.13 (49.53 to 62.74)	49.91 (42.59 to 56.86)	44.02 (39.80 to 48.24)	<0.001
No	47.70 (42.07 to 53.34)	37.79 (32.76 to 42.82)	43.87 (37.26 to 50.47)	50.09 (43.14 to 57.05)	49.12 (40.27 to 57.97)	
Diabetes						0.140
Yes	8.68 (6.90 to 10.47)	11.83 (8.30 to 15.36)	9.60 (6.28 to 12.91)	8.09 (5.83 to 10.36)	6.17 (2.63 to 9.71)	
No	91.31 (83.82 to 98.81)	88.17 (84.64 to 91.70)	90.40 (87.09 to 93.72)	91.91 (89.64 to 94.17)	93.83 (90.29 to 97.37)	
Cardiovascular disease						
Yes	7.03 (5.91 to 8.16)	13.94 (10.27 to 17.62)	6.96 (4.41 to 5.51)	5.22 (3.31 to 7.14)	3.64 (1.45 to 5.83)	<0.001
No	92.97 (85.48 to 100.46)	86.06 (82.38 to 89.73)	93.04 (90.49 to 95.59)	94.78 (92.86 to 96.69)	96.36 (94.17 to 98.55)	
Chronic kidney disease						<0.001
Yes	9.73 (8.19 to 11.26)	18.44 (13.87 to 23.00)	9.57 (6.52 to 12.63)	7.02 (4.63 to 9.40)	5.86 (2.70 to 9.02)	
No	90.28 (82.94 to 97.61)	81.56 (77.00 to 86.13)	90.43 (87.37 to 93.48)	92.98 (90.60 to 95.37)	94.14 (90.98 to 97.30)	
Hyperlipidaemia						0.005
Yes	73.61 (67.20 to 80.02)	78.27 (74.43 to 82.10)	77.04 (72.78 to 81.31)	74.75 (70.66 to 78.84)	66.43 (60.30 to 72.56)	
No	26.39 (23.63 to 29.15)	21.73 (17.90 to 25.57)	22.96 (18.69 to 27.22)	25.25 (21.16 to 29.34)	33.57 (27.44 to 39.70)	
ED medications						0.463
Yes	0.44 (0.04 to 0.85)	0.91 (0.00 to 2.18)	0.69 (0.00 to 1.77)	0.33 (0.00 to 1.00)	0.00 (0.00 to 0.00)	
No	99.56 (91.89 to 107.22)	99.09 (97.82 to 100.37)	99.31 (98.23 to 100.38)	99.67 (99.00 to 100.33)	100.00 (100.00 to 100.00)	
ED						<0.0001
Yes	15.602 (12.778 to 18.426)	23.897 (20.319 to 27.474)	18.178 (14.646 to 21.710)	14.630 (9.820 to 19.439)	8.306 (5.666 to 10.947)	
No	84.398 (77.998 to 90.798)	76.103 (72.526 to 79.681)	81.822 (78.290 to 85.354)	85.370 (80.561 to 90.180)	91.694 (89.053 to 94.334)	

Values are weighted mean±SDor weighted % (95% CI). P values are weighted.
BMI, body mass index; ED, erectile dysfunction; NHANES, National Health and Nutrition Examination Survey.

Table 2 Multivariable logistic regressions analysis between LTL and ED

Exposure	Model 1	Model 2	Model 3
Continuous	0.12 (0.06 to 0.22) p<0.0001***	0.97 (0.50 to 1.91) p=0.928	1.01(0.50 to 2.04) p=0.976
Quartile			
Q1	Reference	Reference	Reference
Q2	0.71 (0.54 to 0.93) p=0.017*	1.43 (1.01 to 2.02) p=0.042*	1.51 (1.01 to 2.26) p=0.044*
Q3	0.55 (0.38 to 0.79) p=0.004**	1.64 (1.07 to 2.49) p=0.025*	1.79 (1.24 to 2.58) p=0.004**
Q4	0.29 (0.19 to 0.44) p<0.0001***	1.19 (0.72 to 1.98) p=0.477	1.25 (0.74 to 2.11) p=0.388
P for trend	p<0.0001***	0.430	0.340

Model 1 without any adjustment; model 2 adjusted for age, race/ethnicity, education level, smoking status, PIR, vigorous activity, moderate activity, alcohol consumption status; model 3 adjusted for age, race/ethnicity, education level, smoking status, PIR, vigorous activity, moderate activity, alcohol consumption status, hypertension, diabetes, CVD, CKD, hyperlipidaemia and ED medications. Bold fonts indicate P value < 0.05; * indicate P value < 0.05; ** indicate P value < 0.01, *** indicate P value < 0.001.
CKD, chronic kidney disease; CVD, cardiovascular disease; ED, erectile dysfunction; LTL, leucocyte telomere length; PIR, poverty income ratio.

The restricted cubic spline plot displayed an inverted J-curve association between the LTL and the prevalence of ED (p for non-linearity=0.011) (figure 1). We further performed a threshold effect analysis of the association between the LTL and the prevalence of ED (table 3). We fit a logistic regression model and a two-piece logistic regression model to test the relationship between the LTL and ED. The results showed that the two-piece logistic regression model was superior to the logistic regression model for the association between the LTL and ED (p for the log likelihood ratio test <0.001). We identified an inflection point of 1.037 for the LTL. When the LTL was less than 1.037, there was no statistically significant association between LTL as a continuous variable and ED in the logistic regression model.

However, when the LTL was ≥1.037, the incidence of ED decreased with increasing LTL (OR 0.17; 95% CI 0.03 to 0.90, p=0.039).

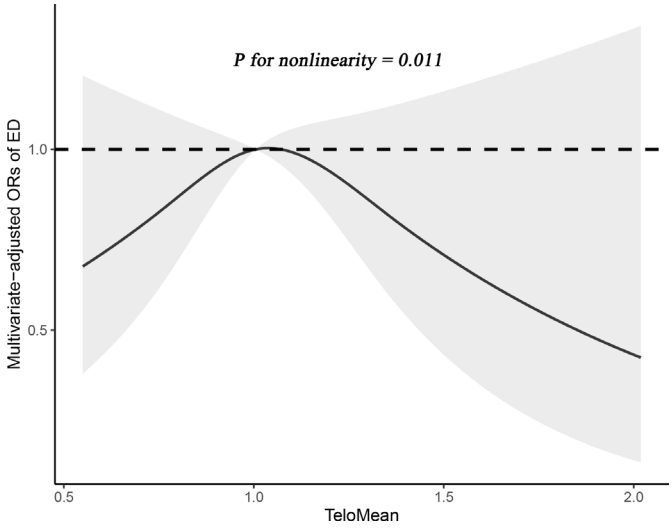

Figure 1 Restricted cubic spline of association between the LTL and the prevalence of ED (adjusted for age, race/ethnicity, education level, smoking status, PIR, vigorous activity, moderate activity, alcohol consumption status, hypertension, diabetes, CVD, CKD, hyperlipidaemia and ED medications). CVD, cardiovascular disease; CKD, chronic kidney disease; ED, erectile dysfunction; LTL, leucocyte telomere length; PIR, poverty income ratio.

Subgroup analyses

We performed subgroup analyses to assess whether the association between the LTL and ED was influenced by age, obesity level or chronic non-communicable disease (figure 2). The results showed that the interaction test was not statistically significant for age, BMI, hypertension, diabetes, CVD, CKD or hyperlipidaemia after adjusting for all potential confounders (p>0.05). The subgroup analysis results were consistent with the overall results, which indicated that there were no systematic differences in the associations between subpopulations. Thus, our main results were stable.

Sensitivity analysis

Our study imputed missing covariates using the missForest package (all variables were below 5.00% except for PIR, which had 5.25% missing data). MissForest had a seed number of 500, and data imputation was completed after six iterations. The calculated normalised root mean square error of the imputation model evaluation index was 0.0001, and the proportion of falsely classified was 0.081. The 'grubbs.test()' function function is employed to determine the presence of outliers. Our sensitivity analysis results showed that the results remained similar after removing subjects with missing data and it was determined that the findings of all models remained consistent and the overall study was not influenced by the presence of outliers (online supplemental tables 2,3,6). The

Table 3 Threshold effect analysis of LTL on prevalence of ED using the two-piecewise regression model

Leucocyte telomere length (T/S ratio)	Adjusted OR (95% CI), p value
Fitting by the standard linear model	1.01 (0.50 to 2.04), p=0.976
Fitting by the two-piecewise linear model	
Inflection point (1.037 (T/S ratio))	
Leucocyte telomere length <1.037 (T/S ratio)	1.99 (0.39 to 10.20), p=0.385
Leucocyte telomere length ≥1.037 (T/S ratio)	0.17 (0.03 to 0.90), p=0.039
Log-likelihood ratio	<0.001

Adjusted for age, race/ethnicity, education level, smoking status, PIR, vigorous activity, moderate activity, alcohol consumption status, hypertension, diabetes, CVD, CKD, hyperlipidaemia and ED medications.
CKD, chronic kidney disease; CVD, cardiovascular disease; ED, erectile dysfunction; LDL, leucocyte telomere length; PIR, poverty income ratio.

(online supplemental table 4) displays the multivariable logistic regression analysis of the association between LTL and ED, using Q3 as the reference category.

DISCUSSION

In a previous meta-analysis comprising 23 observational studies, it was observed that both sperm and LTL were notably shorter in infertile individuals compared with fertile individuals. The mean differences (95% CI) were −1.43 (−1.66 to −1.21) with a p<0.001 for sperm telomere length, and −1.67 (−2.02 to −1.31) with a p<0.001 for LTL. Moreover, significant differences in telomere length (−0.97 (−1.32 to −0.61), p<0.001) were also observed between spermatozoa from normal individuals and those with low sperm counts in semen.[26] To the best of our knowledge, this is the first study to assess the associations between the LTL and ED. A nationally representative dataset of US men was used to examine the relationship between the LTL and ED through epidemiological studies. However, although we observed that people with ED possess shorter telomeres, instead of obtaining a linear correlation, our findings suggested an inverted J-curve relationship between the LTL and ED. When the LTL was ≥1.037, the LTL was inversely associated with ED. Moreover, people in the second and third LTL quartiles had a higher chance of developing ED than those in the first LTL quartile.

Our initial hypothesis posits that longer telomere lengths are correlated with a lower prevalence of ED. This hypothesis was validated through the analysis presented in table 1, which demonstrates an increasing trend of ED prevalence with increasing telomere length. Additionally, the unadjusted models provided further support for this relationship, with a reported OR of 0.12 (95% CI 0.06 to 0.22) when considering population characteristics. Nevertheless, the relationship between age and telomere

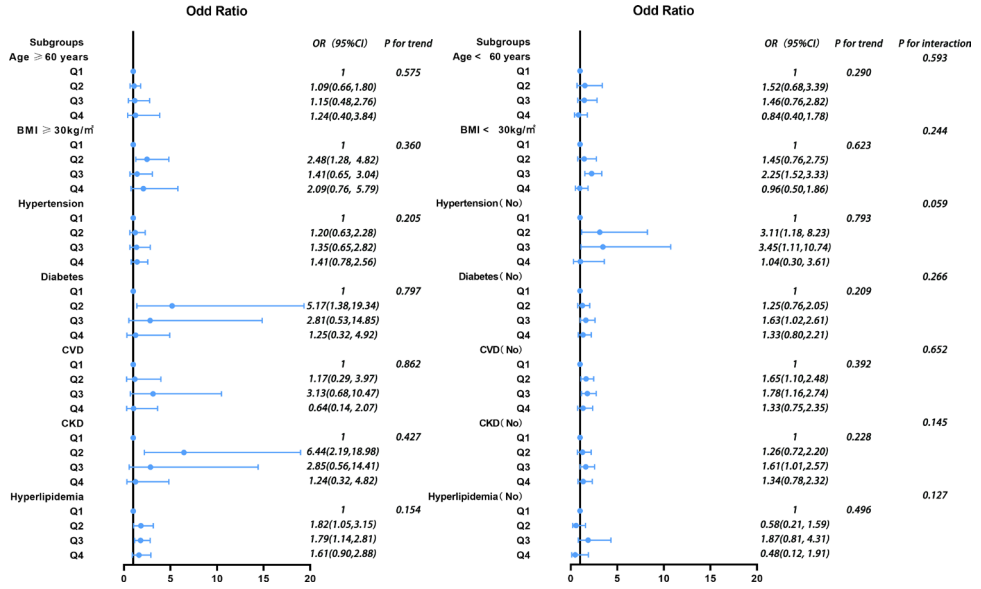

Figure 2 Multivariable logistic regression associations between LTL and ED by subgroup analysis (adjusted for age, race/ethnicity, education level, smoking status, PIR, vigorous activity, moderate activity, alcohol consumption status, hypertension, diabetes, CVD, CKD, hyperlipidemia and ED medications). When they were not the strata variables, results are survey weighted. BMI, body mass index; CVD, cardiovascular disease; CKD, chronic kidney disease; ED, erectile dysfunction; LTL, leucocyte telomere length; PIR, poverty income ratio.

length has been consistently established in prior studies. Therefore, when age was accounted for as a covariate and LTL was treated as a continuous variable in the logistic regression model, this previously observed association disappeared. This led us to speculate that the relationship between LTL and ED may transition from an initial linear association to a non-linear one under the influence of age. In the analysis considering LTL as a grouping variable, we observed statistically significant results only for Q2 and Q3, with Q1 as the reference category. To enhance our understanding of the association between LTL and ED while accounting for potential confounding factors, we conducted further analysis using non-linear regression models, specifically restricted cubic splines. The results of the RCS analysis revealed an inverted J-shaped relationship between LTL and ED, with an inflection point estimated at 1.037. To further explore this association, we performed logistic regression using LTL as a continuous variable on either side of the inflection point. The findings indicated a linear negative correlation between LTL and ED (OR 0.17, 95% CI 0.03 to 0.90) specifically on the right side of the inflection point (LTL values greater than 1.037). On the left side of the inflection point, there may be a potential risk factor for ED in terms of increasing LTL (OR 1.99). However, it is important to note that this association, while present, did not reach statistical significance in the logistic regression model. This suggests that the relationship between LTL on the left side of the inflection point and ED is not linear in nature.

The LTL is inversely associated with ED once the threshold is exceeded. This may be due to the complex aetiology of ED. In addition to vascular endothelial dysfunction, psychological factors, neural signal transmission and hormonal factors are also potential causes of ED.[27] The association between the LTL and ED is a result of multiple factors. Several possible mechanisms may support the observed relationship between the LTL and ED. First, because DNA replication at chromosome ends is incomplete, telomeres shorten at every cell division, and oxidative stress and inflammatory factors have been demonstrated to further contribute to telomere attrition.[12] During sexual or tactile stimulation, the cavernous nerve releases nitric oxide (NO), which stimulates the formation of cyclic GMP, resulting in smooth muscle relaxation and promoting erection.[28] Abnormalities in the NO/cGMP pathway resulting from oxidative stress and inflammation, including decreased NO synthase (NOS) activity and diminished NO bioavailability, may contribute to the development of ED.[29] This explains why the mean LTL of ED patients in our study was shorter than that of the control population and was inversely associated with ED. In contrast, testosterone (T), an important hormone regulating the process of penile erection and maintaining erectile status, may accelerate telomere attrition in men by increasing sensitivity to oxidative stress.[30 31] T can not only upregulate the expression of NOS but also downregulate RhoA-ROCK pathway activity, which is involved in regulating penile smooth muscle relaxation for a long time.[32 33] Thus, in our study, compared with the normal population, some ED patients may have a longer LTL due to lack of T.

In addition, psychological factors are thought to coexist in almost all ED patients[34] and have the potential to influence the association of the LTL with ED. A causal relationship between psychiatric disorders and ED remains undetermined. The results of a meta-analysis by Atlantis and Sullivan suggested a bidirectional association between depression and ED in men aged 18 years and older.[35] A recent large retrospective study further confirmed this association in more than 30 000 ED samples. Stigmatisation of sexual failure and cultural shifts in male self-images are the main explanations for this association.[36] However, we believe that the telomere length also deserves consideration. Studies have shown that people with depression and those living under social stress have shorter LTL.[37 38] LTL has also been suggested to be inversely associated with the duration and severity of severe affective episodes.[39] The ED patients in our study may have been chronically exposed to anxiety and depressed mood resulting from sexual failure, which may have accelerated their telomere attrition. Moreover, classical antidepressant drugs, represented by dopamine D2 receptor blockers, have the side effect of causing ED.[40] An in vitro study using hippocampal cell lines showed that these antidepressants increase the expression of components of shelterin, TRF1 and TRF2, thereby decreasing damage to telomeres.[41] This suggests that the effects of a depressive state and antidepressant medication use on the LTL in people with ED may be paradoxical.

Remarkably, recent Mendelian randomisation studies and prospective studies have indicated that a longer LTL is associated with a higher risk of some cancers, including prostate cancer.[42–44] The development and progression of ED is directly and indirectly influenced by a variety of cancers, particularly prostate cancer. Radical prostatectomy and prostate radiation therapy can cause ED,[45 46] and ED is a potential cause of prostate cancer.[47] Although we excluded prostate cancer patients from our study, it is not clear whether the telomere length in some ED patients is altered by the development of cancer. Moreover, the impact of other prostate diseases on the interaction between the LTL and ED deserves consideration. Lower urinary tract symptoms and benign prostatic hyperplasia are also highly associated with the development of ED.[48] However, previous studies have found no difference in the LTL between benign prostatic hyperplasia and the normal population, suggesting that benign prostatic hyperplasia has a small impact on our findings.[49]

Interestingly, our study is not the first to find a particular relationship between the LTL and disease. In a German study[50] of 4802 patients with CKD, the LTL had a U-shaped association with CKD duration. The LTL was significantly longer in patients with a CKD duration of less than 6 months or more than 5 years compared with patients with a moderate CKD duration. Similar to our hypothesis, these researchers suggested that this is a

result of inflammation and oxidative stress combined with other factors. They proposed that long-term inflammatory effects may extend the telomere length by increasing telomerase activity. However, limited by the study design, we could not verify this possibility. In addition, U-shaped curves have also been widely identified in cancer-related studies, including gastric adenocarcinoma, pancreatic cancer and glioma. Current mainstream explanations are that telomeres that are too short lead to chromosomal instability causing carcinogenesis and long telomeres upregulate cell division and increase the possibility of abnormalities causing carcinogenesis.[51–53]

Nevertheless, our study has several limitations. First, because of the limitations of the NHANES database, our secondary study could not detect a causal relationship between the LTL and ED. In addition, there were missing values in covariates, to address these missing values, we imputed the missing covariates using the missForest package in the R software. Second, the diagnosis of ED was based on questionnaires and lacks specialised diagnostic tests, which may lead to selection bias. However, the validity of single-question self-report for ED in the NHANES database has been validated previously.[54 55] Third, limited by the data, the range of the fourth LTL quartile was large, and the distribution of participants was discrete, which may affect the construction of our non-linear model. Last, despite our extensive adjustment for major confounders, we cannot completely rule out residual or non-existent confounding in the database, particularly prostate-related diseases and information on the use of drugs for ED. Thus, the results from large longitudinal studies would be the best evidence to evaluate the effect of the relationship between the LTL and ED.

CONCLUSION

In this study, we determined the association between the LTL and ED using 1694 samples from the 2001–2002 NHANES. Our study indicated an inverted J-type non-linear relationship between the LTL and ED. When the LTL was ≥1.037, the incidence of ED decreased with increasing LTL. Moreover, compared with the reference first LTL quartile, participants in the second and third LTL quartiles had a higher risk of ED. Current evidence confirms a significant link between LTL and the risk of ED and LTL may become a potential biomarker for ED diagnosis. Further studies in well-designed randomised controlled and prospective studies are needed to evaluate the association between LTL and ED.

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Patient consent for publication Not applicable.

Ethics approval The studies involving human participants were approved by the National Center for Health Statistics Research Ethics Review Board, and informed consent was obtained from all participants in this study (continuation of protocol #2001-01). Participants gave informed consent to participate in the study before taking part.

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REFERENCES

1 Muneer A, Kalsi J, Nazareth I, *et al*. Erectile dysfunction. *BMJ* 2014;348:g129.
2 Burnett AL, Nehra A, Breau RH, *et al*. Erectile dysfunction: AUA guideline. *J Urol* 2018;200:633–41.
3 Goldstein I, Goren A, Li VW, *et al*. Epidemiology update of erectile dysfunction in eight countries with high burden. *Sex Med Rev* 2020;8:48–58.
4 Nguyen HMT, Gabrielson AT, Hellstrom WJG. Erectile dysfunction in young men-a review of the prevalence and risk factors. *Sexual Medicine Reviews* 2017;5:508–20.
5 Sansone A, Mollaioli D, Ciocca G, *et al*. "Mask up to keep it up": preliminary evidence of the association between erectile dysfunction and COVID-19. *Andrology* 2021;9:1053–9.
6 Najari BB, Kashanian JA. Erectile dysfunction. *JAMA* 2016;316:1838.
7 Santoyo JM, Noguera JA, Avilés F, *et al*. Factors involved in endothelial dysfunction related to angiogenic disbalance and oxidative stress, in women at high risk of term pre-Eclampsia. *Antioxidants (Basel)* 2022;11:1409.
8 Mostafaei H, Mori K, Hajebrahimi S, *et al*. Association of erectile dysfunction and cardiovascular disease: an umbrella review of systematic reviews and meta-analyses. *BJU Int* 2021;128:3–11.
9 Kouidrat Y, Pizzol D, Cosco T, *et al*. High prevalence of erectile dysfunction in diabetes: a systematic review and meta-analysis of 145 studies. *Diabet Med* 2017;34:1185–92.
10 Blackburn EH, Epel ES, Lin J. Human Telomere biology: a contributory and interactive factor in aging, disease risks, and protection. *Science* 2015;350:1193–8.
11 Rubtsova M, Dontsova O. Human Telomerase RNA: telomerase component or more *Biomolecules* 2020;10:873.
12 Rossiello F, Jurk D, Passos JF, *et al*. Telomere dysfunction in ageing and age-related diseases. *Nat Cell Biol* 2022;24:135–47.
13 Yadav S, Maurya PK. Correlation between telomere length and biomarkers of oxidative stress in human aging. *Rejuvenation Res* 2022;25:25–9.
14 Codd V, Wang Q, Allara E, *et al*. Polygenic basis and BIOMEDICAL consequences of telomere length variation. *Nat Genet* 2021;53:1425–33.
15 Cheng F, Luk AO, Shi M, *et al*. Shortened leukocyte telomere length is associated with Glycemic progression in type 2 diabetes: a

prospective and Mendelian randomization analysis. *Diabetes Care* 2022;45:701–9.
16 Haycock PC, Heydon EE, Kaptoge S, *et al*. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* 2014;349:g4227.
17 Banach M, Mazidi M, Mikhailidis DP, *et al*. Association between phenotypic familial hypercholesterolaemia and telomere length in US adults: results from a multi-ethnic survey. *Eur Heart J* 2018;39:3635–40.
18 Wang Y, Jiao F, Zheng H, *et al*. Gender difference in associations between telomere length and risk factors in patients with stroke. *Front Aging Neurosci* 2021;13:719538.
19 von Elm E, Altman DG, Egger M, *et al*. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Surg* 2014;12:1495–9.
20 Derby CA, Araujo AB, Johannes CB, *et al*. Measurement of erectile dysfunction in population-based studies: the use of a single question self-assessment in the Massachusetts male aging study. *Int J Impot Res* 2000;12:197–204.
21 Whelton PK, Carey RM, Aronow WS, *et al*. ACC/AHA/AAPA/ABC/ACPM/AGS/Apha/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Hypertension* 2018;71:e13–115.
22 Brockamp C, Landgraf R, Müller UA, *et al*. Shared decision making, diagnostic evaluation, and pharmacotherapy in type 2 diabetes. *Dtsch Arztebl Int* 2023;120:804–10.
23 Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604–12.
24 Russo GT, De Cosmo S, Viazzi F, *et al*. Plasma triglycerides and HDL-C levels predict the development of diabetic kidney disease in subjects with type 2 diabetes: the AMD annals initiative. *Diabetes Care* 2016;39:2278–87.
25 Stekhoven DJ, Bühlmann P. Missforest--non-parametric missing value imputation for mixed-type data. *Bioinformatics* 2012;28:112–8.
26 Fernández de la Puente M, Salas-Huetos A, Valle-Hita C, *et al*. Is telomere length a biomarker of sperm quality? A systematic review and meta-analysis of observational studies. *Andrology* 2024;12:277–88.
27 De Leonardis F, Colalillo G, Finazzi Agrò E, *et al*. Endothelial dysfunction, erectile deficit and cardiovascular disease: an overview of the pathogenetic links. *Biomedicines* 2022;10:1848.
28 Mitidieri E, Cirino G, d'Emmanuele di Villa Bianca R, *et al*. Pharmacology and perspectives in erectile dysfunction in man. *Pharmacol Ther* 2020;208:107493.
29 Wang Y, Wang Y, Cong R, *et al*. Restoration of Erectile function by suppression of corporal apoptosis and oxidative stress with Losartan in aged rats with erectile dysfunction. *Andrology* 2020;8:769–79.
30 Sharpley CF, Christie DRH, Bitsika V, *et al*. Associations between reduced telomere length, depressed mood, anhedonia, and irritability in prostate cancer patients: further evidence for the presence of "male depression"? *Psychooncology* 2018;27:1072–4.
31 Montorsi F, Oettel M. Testosterone and sleep-related erections: an overview. *J Sex Med* 2005;2:771–84.
32 Filippi S, Vignozzi L, Morelli A, *et al*. Testosterone partially ameliorates metabolic profile and erectile responsiveness to PDE5 inhibitors in an animal model of male metabolic syndrome. *J Sex Med* 2009;6:3274–88.
33 Rastrelli G, Corona G, Maggi M. Testosterone and sexual function in men. *Maturitas* 2018;112:46–52.
34 Carosa E, Benvenga S, Trimarchi F, *et al*. Sexual inactivity results in reversible reduction of LH bioavailability. *Int J Impot Res* 2002;14:93–9.
35 Atlantis E, Sullivan T. Bidirectional association between depression and sexual dysfunction: a systematic review and meta-analysis. *J Sex Med* 2012;9:1497–507.
36 Manalo TA, Biermann HD, Patil DH, *et al*. The temporal association of depression and anxiety in young men with erectile dysfunction. *J Sex Med* 2022;19:201–6.
37 Birkenæs V, Elvsåshagen T, Westlye LT, *et al*. Telomeres are shorter and associated with number of suicide attempts in affective disorders. *J Affect Disord* 2021;295:1032–9.
38 Bazaz MR, Balasubramanian R, Monroy-Jaramillo N, *et al*. Linking the Triad of Telomere length, inflammation, and gut dysbiosis in the manifestation of depression. *ACS Chem Neurosci* 2021;12:3516–26.
39 Gillis JC, Chang S-C, Wang W, *et al*. The relation of telomere length at midlife to subsequent 20-year depression trajectories among women. *Depress Anxiety* 2019;36:565–75.
40 Trinchieri M, Trinchieri M, Perletti G, *et al*. Erectile and ejaculatory dysfunction associated with use of psychotropic drugs: a systematic review. *J Sex Med* 2021;18:1354–63.
41 Solek P, Koszla O, Mytych J, *et al*. Neuronal life or death linked to depression treatment: the interplay between drugs and their stress-related outcomes relate to single or combined drug therapies. *Apoptosis* 2019;24:773–84.
42 Machiela MJ, Hofmann JN, Carreras-Torres R, *et al*. Genetic variants related to longer telomere length are associated with increased risk of renal cell carcinoma. *Eur Urol* 2017;72:747–54.
43 Cigan SS, Meredith JJ, Kelley AC, *et al*. Predicted leukocyte telomere length and risk of germ cell tumours. *Br J Cancer* 2022;127:301–12.
44 Kachuri L, Saarela O, Bojesen SE, *et al*. Mendelian randomization and mediation analysis of leukocyte telomere length and risk of lung and head and neck cancers. *Int J Epidemiol* 2019;48:751–66.
45 Ju IE, Trieu D, Chang SB, *et al*. Surgeon experience and erectile function after radical prostatectomy: a systematic review. *Sex Med Rev* 2021;9:650–8.
46 Rasmusson E, Gunnlaugsson A, Wieslander E, *et al*. Erectile dysfunction and absorbed dose to penile base structures in a randomized trial comparing ultrahypofractionated and conventionally fractionated radiation therapy for prostate cancer. *Int J Radiat Oncol Biol Phys* 2020;107:143–51.
47 Dilixiati D, Kadier K, Laihaiti D, *et al*. The association between sexual dysfunction and prostate cancer: a systematic review and meta-analysis. *J Sex Med* 2023;20:184–93.
48 Calogero AE, Burgio G, Condorelli RA, *et al*. Epidemiology and risk factors of lower urinary tract symptoms/benign prostatic hyperplasia and erectile dysfunction. *Aging Male* 2019;22:12–9.
49 Graham MK, Meeker A. Telomeres and telomerase in prostate cancer development and therapy. *Nat Rev Urol* 2017;14:607–19.
50 Raschenberger J, Kollerits B, Titze S, *et al*. Do Telomeres have a higher plasticity than thought? Results from the German chronic kidney disease (GCKD) study as a high-risk population. *Exp Gerontol* 2015;72:162–6.
51 Delanaye P, Pottel H. New equation to estimate glomerular filtration rate in China: a reference issue. *Kidney Int* 2019;96:521.
52 Wang Z, Koh W-P, Jin A, *et al*. Telomere length and risk of developing gastric adenocarcinoma: the Singapore Chinese health study. *Gastric Cancer* 2018;21:598–605.
53 Wang S, Chen Y, Qu F, *et al*. Association between leukocyte telomere length and glioma risk: a case-control study. *Neuro Oncol* 2014;16:505–12.
54 Wang W, Ma Y, Chen J, *et al*. The association between 2, 4-Dichlorophenoxyacetic acid and erectile dysfunction. *Front Public Health* 2022;10:910251.
55 O'Donnell AB, Araujo AB, Goldstein I, *et al*. The validity of a single-question self-report of erectile dysfunction. Results from the Massachusetts male aging study. *J Gen Intern Med* 2005;20:515–9.