Journal of Bone and Mineral Metabolism (2019) 37:1004–1012
https://doi.org/10.1007/s00774-019-01004-0

ORIGINAL ARTICLE

The age modification to leukocyte telomere length effect on bone mineral density and osteoporosis among Chinese elderly women

Lailin Tao[1] · Qin Huang[2] · Rui Yang[3] · Yu Dai[4] · Yun Zeng[5] · Can Li[1] · Xiaolong Li[1] · Junchao Zeng[3] · Qi Wang[1]

Received: 14 January 2019 / Accepted: 9 April 2019 / Published online: 20 April 2019

Abstract

Critically short telomeres indicate cellular senescence. Leukocyte telomere length (LTL) is regarded as an aging predictor. Osteoporosis is an age-related disease. The purpose of our study is to examine the association between LTL, and BMD and osteoporosis among an elderly Chinese population. A total of 1017 participants (584 postmenopausal women) with a mean age of 66.4 years were recruited from April 2016 to August 2017. Dual-energy X-ray absorptiometry was used for BMD measurement at skeleton sites of lumbar spine (LS), femoral neck (FN), and total hip (TH). LTL was measured using quantitative real-time polymerase chain reaction. Among women, age significantly modified the effect of LTL on BMD at FN. Additionally, significant age modification was observed for the association between LTL and LS BMD category (indicative of control or osteopenia or osteoporosis), and the number of osteoporotic sites at LS or TH. The corresponding estimates (95% CI) for the relative excess risk due to interaction (RERI) were − 0.07 (− 0.11, − 0.01) and − 0.11 (− 0.16, − 0.03) sequentially in ordinal logistic regression models. The estimated RERIs (95% CI) were − 0.11 (− 0.25, − 0.02) and − 0.23 (− 0.39, − 0.10) in multinomial logistic regression models for LS/FN/TH BMD category, and − 0.20 (− 0.31, − 0.09) and − 0.34 (− 0.49, − 0.21) for FN BMD category. However, similar findings did not show in men. The effect of LTL on BMD and osteoporosis risk is modified by age in elderly women but not in men, suggesting that the predictive role of LTL in bone loss differs by sex.

Keywords Bone mineral density · Osteoporosis · Telomere length · Elderly population · Aging

Lailin Tao and Qin Huang contributed equally to this article and share first authorship.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00774-019-01004-0) contains supplementary material, which is available to authorized users.

✉ Qi Wang
lwq95@126.com; wangqi_tj@hust.edu.cn

1 MOE Key Lab of Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

2 Department of Rehabilitation Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

3 Department of Health Checkup, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

4 Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

5 Wuhan No. 1 Hospital, Wuhan 430030, China

Introduction

Osteoporosis is a systemic condition characterized by low bone mass and microarchitectural deterioration of bone tissue, resulting in increased bone fragility and fracture susceptibility [1]. Osteoporotic fracture is a serious age-related complication for the elderly, especially among menopausal women. Bone mineral density (BMD) assessment is preferred in predicting primary osteoporotic fracture [2], while bone turnover markers (BTMs) can also add valuable information for osteoporosis diagnosis and therapy [3]. Osteoporotic fractures occur frequently at skeleton sites of the hip, vertebra, and wrist, contributing to the high risk of disabilities and low quality of life of patients and increasing the mortality, morbidity, and economic cost [4]. Aging and estrogen deficiency are two leading causes of osteoporosis that is a multifactorial disease [5, 6]. Moreover, aging can impair bone formation, such as inducing the dysfunction of osteoprogenitor cells [7] and osteoblastic cells [8]. Studies have shown that telomere length can be considered as a

biomarker of cellular aging due to the indication of oxidative stress and replicative senescence with critically short length [9, 10].

Telomeres consist of repeats of the sequence TTAGGG/CCCTAA at the ends of chromosomes, which are essential for chromosomal stability in humans [11]. The ribonucleoprotein enzyme telomerase adds telomeric DNA independent of normal DNA synthesis. In most somatic cells, telomerase is inactive; thus, telomeres shorten progressively at each round of replication, resulting in chromosomal instability and senescence with critically short length. Findings that associate the age-related impaired homeostasis of bone cells with telomere shortening, as well as telomere dysfunction, have been reported. Murine models have demonstrated that telomerase deficiency and telomere shortening in *Terc*$^{-/-}$ mice (telomerase-deficient mice) lead to age-related bone loss due to intrinsic osteoblastic defects and increased osteoclastogenesis by inflammatory microenvironment [12] and premature aging [13]. In humans, osteoporosis commonly occurs with premature aging diseases including dyskeratosis congenita and Werner syndrome which are characterized by telomere dysfunction [14, 15].

Considering that bone cells are not easily available in clinical settings and shortened leukocyte telomere length (LTL) is associated with oxidative stress [16] and cumulative inflammation [17], LTL may be a candidate that reflects bone cell aging. Therefore, we hypothesize that LTL may be associated with BMD and osteoporosis and can be a predictor of osteoporosis. The association of LTL with BMD and osteoporosis has been reported [18–23], but inconsistencies need to be addressed. Given that study population characteristics play important roles in the association between LTL with BMD and osteoporosis, and the interaction between age and LTL is rarely discussed, we examined the association with interactions considered among an elderly population in both men and women.

Materials and methods

Study population

All participants were enrolled from a health check-up center and two community centers in Wuhan City, central China, from April 2016 to August 2017. We recruited the individuals aged about 60 years or above. Women had been non-surgically menopausal (menopause was defined as menstruation that had not occurred for a year) for approximately 10 years. The exclusion criteria for participants were implemented as follows: (1) ever diagnosed with hypothyroidism/hyperthyroidism, (2) ever taking medications regularly for treating these mentioned diseases, (3) ever treated by hormonal drugs (e.g., glucocorticoid and estrogen) for a period of more than 6 months, and (4) self-reporting a previous osteoporosis diagnosis by a physician.

A self-made questionnaire was used to collect sociodemographic data of the participants (e.g., age, gender, and occupation), lifestyle (e.g., smoking and alcohol drinking), and menopause status and reproductive history of women.

This study was approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology. All participants presented written informed consent prior to enrollment.

BMD measurement

Dual-energy X-ray absorptiometry (DXA, GE Healthcare Lunar Prodigy), weekly calibrated, was used to measure BMD at skeleton sites of lumbar spine (LS) (L1–L4) and left hip of all participants. BMD measurements of the right hip were used in case of a previous fracture or surgery in the left hip. Specially, all DXA images were reviewed by a radiologist. Consistent with the WHO criteria [24], a normal BMD, osteopenia, and osteoporosis were defined as *T* scores ≥ -1.0, between -2.5 and -1.0, and ≤ -2.5, respectively. A diagnosis for LS, femoral neck (FN), and total hip (TH) was separately considered based on the representative *T* scores.

DNA extraction

A peripheral blood sample was taken from every participant during the visit of DXA screening. Genomic DNA (gDNA) was extracted from peripheral blood cells using RelaxGene blood DNA system DP319-02 (Tiangen, Beijing, China). The concentration and purity of gDNA were tested using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). For gDNA samples, the concentration was 50 ng/μl or above, and the 260/280 and 260/230 optical densities ranged from 1.75 to 2.0 and 2.0 or above, respectively. Successfully extracted gDNA was stored at − 80 °C until the preparation of a working solution of 20 ng/μl and quantitative real-time polymerase chain reaction (qPCR).

LTL measurement

LTL was measured using the qPCR method described by Cawthon [25] with slight modifications. In brief, the T/S ratio was calculated by the sample difference from a reference DNA sample in its ratio of telomere repeat copy number to single copy gene copy number. This ratio should be proportional to the average LTL. When calculating the T/S ratio, the amplification efficiency was considered [26] using the standard curve method. In particular, telomere (T) and single copy gene (S) PCRs were conducted in separate 384 well plates, wherein each sample was assayed in

triplicate on an ABI 7900HT Sequence Detection System (Applied Biosystems). For both T PCRs and S PCRs, the 10 µl reaction consisted of 5 µl of SYBR green PCR master mix (A25742, Applied Biosystems), 1 µl of DNA temple (or 1 µl of ddH_2O), and a pair of primers. For each sample in triplicate, the median was obtained, and the acceptable standard deviation was 0.4 and less; otherwise, a potential outlier was excluded. Any sample with cycle threshold value beyond the range of mean ± 4 SD was excluded as an outlier. Reproducibility was considered and tested. The intra-assay coefficient of variation in T and S PCRs was 1.77% and 0.94%, respectively. The inter-assay coefficient of variation was 13.56%.

Statistical analysis

All analyses were separately performed among men and women. The mainly used methods were one-way ANOVA, Student's *t* test, and Chi-square tests. Correlation analysis was conducted to explore the relationship between LTL and other continuous covariates. In this study, the putative interaction between age and LTL was considered. Multiple linear and ordinal/multinomial logistic regressions [27] were modeled to explore the dependency of outcome variables on age, log-transformed LTL, and the interaction between age and log-transformed LTL adjusting for covariates including BMI, age at menarche (only for women), age at menopause (only for women), smoking status, alcohol drinking. Each of LS, FN and TH BMD measurements was the dependent variable in the multiple linear regression modeling. Total of six models fitted with categorized BMD (indicating control, osteopenia, and osteoporosis) at skeleton sites of LS/FN/TH and each of LS, FN and TH, and with the number (0–2) of osteoporotic sites at skeleton sites of LS/TH and LS/FN as dependent variables in ordinal/multinomial logistic regressions, sequentially. The interaction between age and LTL was measured on an additive scale with the relative excess risk due to interaction (RERI) provided [28]. All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA), and a two-tailed *p* value was considered statistically significant at 0.05.

Results

The sex-specific demographic data are shown in Table 1. A total of 433 men and 584 women were included in our study. Compared with women, men were older and possessed a shorter LTL. BMD at the three skeletal sites of LS, FN, and TH among women was lower than that among men. Additionally, age was significantly and negatively correlated with LTL among men (Pearson's $r = -0.1202$; $p = 0.0302$) and among women (Pearson's $r = -0.1158$; $p = 0.0055$).

Table 1 Descriptive characteristics of the participants by sex

	Men ($N = 433$)	Women ($N = 584$)	*p* value
Age, year	68.7 (6.3)	66.4 (5.7)	< 0.0001
Height, cm	166.2 (6.1)	154.8 (5.5)	< 0.0001
Weight, kg	67.7 (10.1)	58.3 (8.6)	< 0.0001
BMI, kg/cm^2	24.5 (3.2)	24.3 (3.3)	0.4561
Reproductive history	308 (2.3%)	403 (4.7%)	
Age at menarche, year	NA	14.1 (1.9)	NA
Age at menopause, year	NA	49.6 (3.8)	NA
Lifestyle			
Cigarette smoking			
Ever or current	161 (37.8%)	12 (2.1%)	< 0.0001
Never	265 (62.2%)	557 (97.9%)	
Alcohol drinking			
Ever or current	145 (34.1%)	10 (1.8%)	< 0.0001
Never	280 (65.9%)	557 (98.2%)	
LTL	0.94 (0.28)	1.01 (0.30)	0.0003
BMD			
LS (L1–L4), g/cm^2	1.13 (0.20)	0.95 (0.17)	< 0.0001
Left FN, g/cm^2	0.85 (0.12)	0.75 (0.12)	< 0.0001
Left TH, g/cm^2	0.93 (0.14)	0.82 (0.12)	< 0.0001
Diagnosis (control/OPA/OP)			
LS (L1–L4)	236/133/56	141/215/203	< 0.0001
Left FN	108/231/88	109/285/174	0.0003
Left TH	204/165/56	171/288/97	< 0.0001
LS (L1–L4)/FN/TH	100/271/59	63/340/171	< 0.0001

The total numbers in the cells may vary slightly due to missing data

The data were reported as mean (SD), *N* (percentage) or *N*

All continuous and categorical data were analyzed using Student's *t* test and Chi-square test, respectively

BMI body mass index, *LTL* leukocyte telomere length, *BMD* bone mineral density, *LS* lumbar spine, *TH* total hip, *FN* femoral neck, *OP* osteoporosis, *OPA* osteopenia, *SD* standard deviation, *NA* not applicable

Nevertheless, no significant correlations were observed between LTL and the other continuous variables.

Results of multiple linear regression analysis are displayed in Table 2. Significant associations were found between the log-transformed LTL ($\beta = 0.346$; 95% CI, 0.095–0.598) and BMD at FN among women. Age modification ($\beta = -0.008$; 95% CI, −0.010–0.007) was found in the association. Short LTL contributed to decreased FN BMD among women under 60 years old, and the effect slowly declined with increasing ages (Fig. 1a). However, such findings did not show among men. In addition, a significant effect of age at menarche on FN BMD was observed, and age at menopause was significantly and positively associated with BMD at the three skeletal sites. In particular, BMI was significantly (all $p < 0.0001$) and positively (all $\beta > 0$)

Table 2 Sex-specific multiple linear regression analysis of associations between LTL and BMD measurements at skeleton sites of LS, FN, and TH

	LS Estimate (95% CI)	FN Estimate (95% CI)	TH Estimate (95% CI)
Men			
Age, year	0.003 (− 0.001, 0.006)	− 0.004 (− 0.006, − 0.001)**	− 0.001 (− 0.004, 0.002)
BMI, kg/cm^2	0.025 (0.019, 0.031)***	0.013 (0.009, 0.017)***	0.017 (0.012, 0.021)***
Cigarette smoking[a]	− 0.029 (− 0.075, 0.016)	− 0.021 (− 0.051, 0.009)	− 0.013 (− 0.046, 0.019)
Alcohol drinking[a]	− 0.023 (− 0.068, 0.022)	− 0.005 (− 0.034, 0.025)	− 0.007 (− 0.040, 0.025)
Log LTL	− 0.012 (− 0.525, 0.502)	− 0.119 (− 0.455, 0.217)	− 0.193 (− 0.563, 0.176)
Age × Log LTL	0.000 (− 0.007, 0.007)	0.002 (− 0.003, 0.006)	0.003 (− 0.003, 0.008)
	Model R^2 = 0.18	Model R^2 = 0.16	Model R^2 = 0.16
Women			
Age, year	− 0.005 (− 0.007, − 0.002)***	− 0.008 (− 0.010, − 0.007)***	− 0.008 (− 0.009, − 0.006)***
BMI, kg/cm^2	0.019 (0.015, 0.023)***	0.010 (0.008, 0.013)***	0.015 (0.012, 0.017)***
Age at menopause, year	0.005 (0.002, 0.009)**	0.003 (0.001, 0.006)**	0.004 (0.002, 0.007)***
Age at menarche, year	0.001 (− 0.006, 0.009)	0.007 (0.002, 0.012)**	0.004 (− 0.001, 0.009)
Cigarette smoking[a]	− 0.003 (− 0.093, 0.086)	− 0.020 (− 0.078, 0.039)	− 0.011 (− 0.073, 0.051)
Alcohol drinking[a]	− 0.088 (− 0.191, 0.016)	− 0.030 (− 0.097, 0.038)	− 0.016 (− 0.087, 0.056)
Log LTL	0.369 (− 0.011, 0.750)	0.346 (0.095, 0.598)**	0.209 (− 0.061, 0.478)
Age × Log LTL	− 0.005 (− 0.011, 0.000)	− 0.006 (− 0.009, − 0.002)**	− 0.003 (− 0.007, 0.001)
	Model R^2 = 0.18	Model R^2 = 0.24	Model R^2 = 0.27

BMD bone mineral density, *LS* lumbar spine, *FN* femoral neck, *TH* total hip, *BMI* body mass index, *LTL* leukocyte telomere length, *CI* confidence interval

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

[a]Reported in ever or current, and never; the 'never' group is referenced

associated with BMD at the three skeletal sites among men and women.

Results of ordinal logistic regression analysis among women are shown in Table 3. The effect of LTL on osteopenia/osteoporosis risk was statistically significant in the two models for women but not men. The associations were modified by age. The estimated RERIs (95% CI) were − 0.07 (− 0.11 to − 0.01) and − 0.11 (− 0.16 to − 0.03) in the two models. Short LTL was associated with increased risk of osteoporosis at LS (Fig. 1b) and the number of osteoporotic sites at LS/TH (Fig. 1c) among women below 65 years old. However, the association declined with increasing age. Results of multinomial logistic regression analysis among women are shown in Table 4. The estimated RERIs (95% CI) were − 0.11 (− 0.25 to − 0.02) and − 0.23 (− 0.39 to − 0.10) for LS/FN/TH BMD category, and were − 0.20 (− 0.31 to − 0.09) and − 0.34 (− 0.49 to − 0.21) for FN BMD category. No significant effect of LTL was observed on TH BMD category (Table S1) and number of osteoporotic sites at LS/FN (Table S2) among women. Meanwhile, all models did not achieve significance in men (Table S3–S4). In the models with BMD category at LS/FN/TH (Table 4) and TH (Table S1) as dependent variables, ORs for alcohol drinking seemed to be poorly estimated among women. Hence, we further performed the models without adjustment of alcohol drinking to observe the associations still of significance (Table S5). In addition, high BMI was a preventive factor for osteoporosis in all models among men and women. In particular, among women, increasing age at menopause decreased the risk of osteopenia/osteoporosis at the three skeleton sites except LS.

Discussion

Our study observed that age modified the effect of LTL on FN BMD, osteoporosis/osteopenia risks at skeleton sites of LS/FN/TH, LS and FN, and number of osteoporotic sites at LS/TH in elderly women. The effect of LTL was dependent on age among women. Short LTL was associated with low BMD at FN and high osteoporotic risk in women aged 60–65 years, thereby suggesting that LTL could be a predictor of osteoporosis in women only.

In the present study, with advancing age under the cutoff of 65 years, the effect of LTL on BMD and osteoporosis gradually decreased and eventually disappeared. How age modifies the effect of LTL on BMD and osteoporosis is complex. Aging is characterized as a global reduction

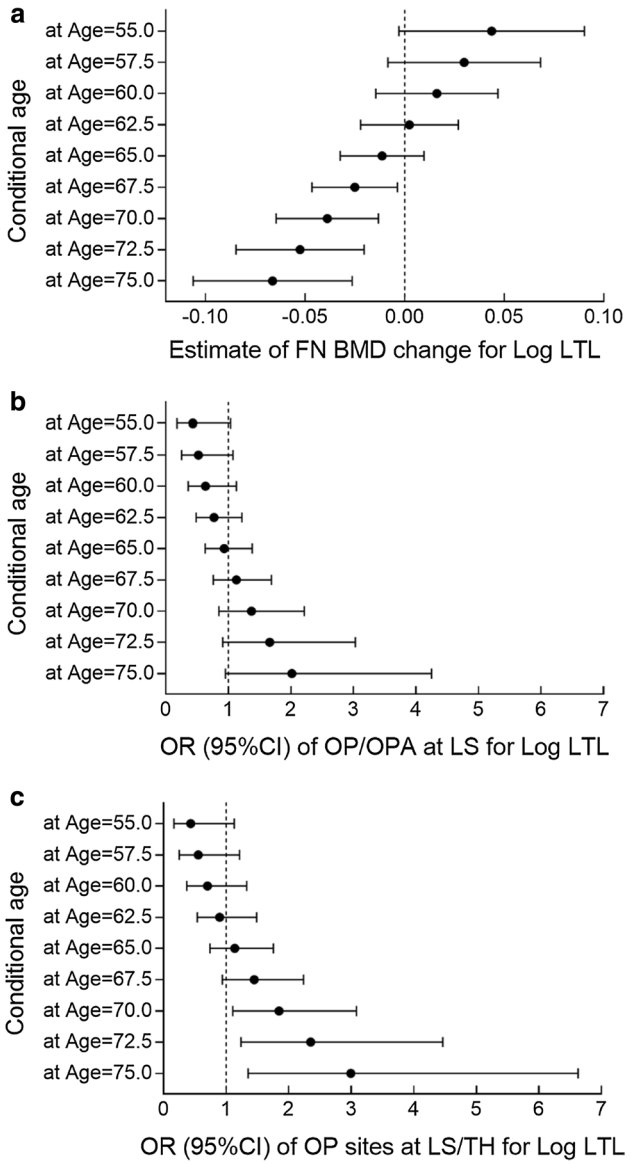

Fig. 1 LTL's effect on BMD and BMD category (indicative of control/OPA/OP) at conditional age ranging from 55.0 to 75.0 years derived from the multiple linear regression and ordinal logistic regressions, respectively, among women. **a** Estimate (95% CI) of BMD change at FN for log-transformed LTL. **b** OR (95% CI) of OP/OPA at LS for log-transformed LTL. **c** OR (95% CI) of the number of osteoporotic sites at LS/TH for log-transformed LTL. *LTL* leukocyte telomere length, *BMD* bone mineral density, *LS* lumbar spine, *FN* femoral neck, *TH* total hip, *OP* osteoporosis, *OPA* osteopenia

Table 3 Ordinal logistic regression analysis of associations between LTL and LS BMD category and the number of OP sites at LS/TH among women

	BMD category LS	Number of OP sites LS/TH
	OR (95% CI)	OR (95% CI)
Age, year	1.07 (1.03, 1.10)***	1.11 (1.07, 1.15)***
BMI, kg/cm^2	0.81 (0.76, 0.85)***	0.79 (0.74, 0.84)***
Age at menopause, year	0.96 (0.92, 1.00)	0.93 (0.89, 0.98)**
Age at menarche, year	0.99 (0.91, 1.09)	0.97 (0.88, 1.08)
Cigarette smoking[a]	0.63 (0.21, 1.90)	1.11 (0.35, 3.57)
Alcohol drinking[a]	2.26 (0.63, 8.15)	2.42 (0.63, 9.22)
Log LTL by 0.1 unit	0.60 (0.38, 0.96)*	0.54 (0.33, 0.90)*
Age × Log LTL	1.08 (1.01, 1.16)*	1.10 (1.02, 1.19)*

Measure of age and log-transformed LTL interaction on additive scale; RERIs (95% CIs) were − 0.07 (− 0.11, − 0.01) and − 0.11 (− 0.16, − 0.03), respectively

Score test of the proportional odds assumption, $p_1 = 0.4243$ and $p_2 = 0.2249$

BMD bone mineral density, *LS* lumbar spine, *TH* total hip, *FN* femoral neck, *BMI* body mass index, *LTL* leukocyte telomere length, *OR* odds ratio, *CI* confidence interval, *RERI* relative excess risk due to interaction

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

[a]Reported in ever or current, and never; the 'never' group is referenced

in the capability to cope with a variety of stressors and a concomitant progressive increase in the proinflammatory status, namely, "inflamm-aging" [29]. Such chronic low-grade inflammation can reflect the consequences of immunosenescence. Additionally, the accumulation of DNA damage caused by oxidative stress can accelerate aging [30, 31]. Chronic inflammation and oxidative stress are correlated with telomere erosion [16, 17]. Therefore, we argued that the immune system, chronic inflammation, and oxidative stress may play important roles in how age interacts with LTL to influence the development of osteoporosis. The competitive and antagonistic response in these pathological processes of osteoporosis involved age and LTL, leading to the negative interaction on an additive scale for FN BMD, BMD categories at skeleton sites of LS/FN/TH, LS and FN, and the number of osteoporotic sites at LS/TH. However, with advancing age over the cutoff, the effect of LTL on BMD and osteoporosis seemed paradoxical compared with that under the cutoff age. Although the ORs were greater than one among women aged over 65 years, the ranges of the confidence interval dramatically broadened, which weakened its reliability. This observation appeared to be related to the decline in the stability of the models among those aged over 65 years, which may be linked to the small sample size aged over 65 years in our study and high heterogeneity among elderly populations. The small sample size, aged 65 years, was derived from the relatively limited accessibility to the oldest group when we conducted the enrollment. In addition, the increasing occurrence of chronic diseases, such as cerebrovascular disease and arthritis, results in functional impairment among elderly individuals [32], contributing to high heterogeneity.

Table 4 Multinomial logistic regression analysis of association between LTL and BMD category among women

	BMD category			
	LS/FN/TH		FN	
	OR[b] (95% CI)	OR[c] (95% CI)	OR[b] (95% CI)	OR[c] (95% CI)
Age, year	1.11 (1.04, 1.19)**	1.23 (1.14, 1.32)***	1.20 (1.12, 1.28)***	1.34 (1.24, 1.44)***
BMI, kg/cm^2	0.88 (0.81, 0.96)**	0.69 (0.62, 0.77)***	0.92 (0.85, 0.99)*	0.73 (0.66, 0.81)***
Age at menopause, year	0.94 (0.87, 1.02)	0.90 (0.83, 0.99)*	0.97 (0.91, 1.03)	0.92 (0.85, 0.99)*
Age at menarche, year	0.92 (0.79, 1.08)	0.95 (0.80, 1.14)	0.86 (0.75, 0.98)	0.81 (0.69, 0.95)*
Cigarette smoking[a]	0.15 (0.02, 0.89)	0.54 (0.09, 3.13)	0.38 (0.06, 2.20)	1.02 (0.18, 5.95)
Alcohol drinking[a]	> 99 (< 0.001, > 99)	> 99 (< 0.001, > 99)	3.59 (0.39, 32.86)	7.58 (0.65, 88.56)
Log LTL by 0.1 unit	0.29 (0.12, 0.69)**	0.25 (0.10, 0.67)**	0.29 (0.13, 0.65)**	0.23 (0.09, 0.59)**
Age × Log LTL	1.21 (1.06, 1.38)**	1.24 (1.07, 1.43)**	1.22 (1.07, 1.38)**	1.26 (1.10, 1.46)**

Measure of age and log-transformed LTL interaction on additive scale; RERIs (95% CIs) were − 0.11 (− 0.25, − 0.02), − 0.23 (− 0.39, − 0.10), − 0.20 (− 0.31, − 0.09), and − 0.34 (− 0.49, − 0.21) sequentially for above models

BMD bone mineral density, *LS* lumbar spine, *TH* total hip, *FN* femoral neck, *BMI* body mass index, *LTL* leukocyte telomere length, *OR* odds ratio, *CI* confidence interval, *RERI* relative excess risk due to interaction

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

[a]Reported in ever or current, and never; the 'never' group is referenced

[b]The OR of osteopenia with the normal BMD group as the reference

[c]The OR of osteoporosis with the normal BMD group as the reference

The association between telomere and bone turnover may contribute to interpreting our findings. It has been observed that two mechanisms responsible for age-related bone loss caused by telomerase deficiency are intrinsic osteoblastic defects and creation of a proinflammatory osteoclast-activating microenvironment in telomerase-deficient mice [12]. Telomere shortening owing to telomerase deficiency results in accelerated senescence of human bone marrow stromal cells (hMSCs) in vitro [33]. On the other hand, it has been found that osteoclast-associated receptor (OSCAR), as a novel member of the leukocyte receptor complex (LRC)-encoded family, is expressed specifically in osteoclasts and its putative ligand (OSCAR-L) is expressed primarily in osteoblasts/stromal cells; moreover, OSCAR may be an important bone-specific regulator of osteoclast differentiation and involved in osteoimmunology [34]. Such evidence suggested that telomere attrition may lead to bone resorption overriding bone formation, and further to osteoporosis in elderly people. However, epidemiologic studies of the association between LTL and bone turnover markers (BTMs) are still scarce. In clinical practice, bone turnover assessment can provide essential information for untreated patients to predict osteoporosis and fracture risk, while DXA is a primary method. Although we failed to explore the association between LTL and bone turnover markers (BTMs), it deserves to be paid important attention. In one previous study, six BTMs, half for bone resorption, were assessed [19]. Only two BTMs for bone resorption [serum C-terminal telopeptides of type I collagen (S-CTX) and urinary deoxypyridinoline levels (U-DPD)] were found to be significantly and positively correlated with LTL, suggesting that the association between short telomeres and higher bone loss may be independent of bone turnover. Given the complexity of telomere biology and bone remodeling, it deserves thorough and complex research for clarification on the association of LTL and bone remodeling as well as BTMs.

The mechanisms underlying the effect of the interaction between age and LTL on BMD and osteoporosis differed by sex in our study remains unclear. This mechanism may mainly result from the differences in estrogen, BMD, and telomere dynamics. Estrogen deficiency among postmenopausal women is an important contributor to osteoporosis [5, 6], and estrogen is also involved in telomere maintenance [35]. Thus, estrogen may be linked to the mechanisms underlying sex-specific differences in osteoporosis. Moreover, osteoporosis prevalence and fracture incidence are higher in women than in men [36, 37]. Additionally, men exhibited faster telomere attrition rates than women, leading to short telomeres; whereas, no sex differences in telomere length at birth were present [38, 39]. The regulation of telomere length is multifactorial, such as genetic factors [40] and lifestyle [41, 42], and these factors are responsible for the differences in the effect of LTL on BMD and osteoporosis among men and women. However, other similar determinants of BMD and osteoporosis in men and women have been reported. Moreover, consistent with previously published results, we observed that high BMI, to some extent, was a significant preventive factor independent of age among men and women.

Explorations on the joint effect of age and LTL on osteoporosis are rare, and wide discrepancies on the issue of the association between LTL and osteoporosis have been found [43]. A prospective study revealed that LTL is correlated with BMD at FN, LS (L1–L4), and forearm without any covariates adjusted among 2150 women aged 18–80 years; however, after adjustment for confounding variables, the correlation appeared insignificant at FN and it remained significant at the other two sites [18]. By contrast, our findings revealed that LTL was associated with BMD at FN only among women after adjustment rather than without adjusted covariates. This prospective study also demonstrated that LTL decreases in women with osteoporosis, which suggests that short LTL increases the risk of osteoporosis. Another prospective study of 110 elderly men showed that LTL is associated with longitudinal bone loss rather than BMD at baseline at specific distal forearm sites [19]. Telomere length can play a crucial role as a biomarker of the aging phenotype. However, the two studies failed to provide results of the comparisons between men and women simultaneously, accounting for the limitation of populations. Nevertheless, some reports regarding the effect of LTL on the risk of osteoporosis are conflicting. Telomere from osteoblasts in vitro shortens with aging, but LTL between patients with osteoporosis and age-matched controls does not significantly differ, suggesting that generalized premature cellular aging may not occur in patients with osteoporosis [20]. Moreover, a large longitudinal Health Aging and Body Composition (ABC) Study indicated that LTL is not associated with hip BMD, osteoporosis, or fracture among older men and women [21]. Another large prospective study revealed no association between LTL and baseline BMD, as well as BMD changes at hip, among Chinese elderly men and women aged 65 years and over [22]. In a study of 460 women aged 25–93 years, although interactions between LTL and age, BMI, and menopausal status were considered, no significant associations were found between LTL and BMD at LS, FN, and TH [23]. These studies presented inconsistent results because of various reasons, including study design, populations, LTL measurements, and statistical techniques in data analysis. Considering the comparisons among these previous studies and our study, ethnics, age, and sex may be crucial factors to explore the association between LTL and osteoporosis.

The strengths of our study mainly lay in statistical methods, especially ordinal/multinomial logistic regression and interaction analyses and LTL being log-transformed when performing regression analyses. However, the present study had some limitations. First, compared with the prospective studies, our study, as a cross-sectional study, failed to directly investigate the relationships between LTL and bone loss or fracture incidence; thus, our evidence may not be sufficiently conceivable. Additionally, although we excluded the participants whose BMD condition may be potentially affected by some factor related to secondary osteoporosis, our study may also be biased due to our selection criteria because the participants we recruited were free from bone diseases, and individuals who were diagnosed with osteoporosis were oriented toward the hospital outpatient department. Moreover, this study used a small sample size and a narrow age range, which limited the generalization of results to wide populations. Furthermore, Southern blot analysis is currently considered as the standard method to obtain LTL with some compelling advantages in reliability and accuracy. Despite qPCR's relatively low cost, high throughput, and low DNA requirement, its constraints include high CV and measurements in relative and mean length. Some weaknesses in regression analysis were considered by few independent variables. More inflammatory comorbidities or lifestyle-related independent variables should be included. We may have overlooked some important confounding factors that could influence the development of osteoporosis, which undermined the power to detect the effects of LTL on osteoporosis.

In conclusion, we observed that the interaction between age and LTL was associated with BMD and osteoporosis in Chinese elderly women, suggesting that LTL could be a possible predictor of osteoporosis, and its predictive role differed by sex. Longitudinal studies with rigorous design, large sample size, and powerful data analysis could be informative to conclusively show the true effect of LTL on bone mass. Further research concerning the biological mechanisms of how age interacts with LTL in the development of osteoporosis must be conducted.

Acknowledgements The authors thank the medical staff in Qian-jin Community Health Center and Wan-song Community Health Center for helping participant recruitment. The work was funded by the Natural Science Foundation of China [Grant no. 81573235].

Author contributions LT and QW: study conception and design; LT, YZ, XL, and JZ: acquisition of data; LT, QH, and YD: analysis and interpretation of data; LT and QH: draft the manuscript; LT, QW, CL, YZ, RY, JZ, QH, YD, and XL: revise the manuscript.

Funding The work was funded by the National Natural Science Foundation of China (Grant no. 81573235).

Compliance with ethical standards

Conflict of interest LT, QH, RY, YD, YZ, CL, XL, JZ and QW declare that they have no conflict of interest.

References

1. Consensus A (1993) Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 94:646–650

2. Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, Oden A, Zethraeus N, Pfleger B, Khaltaev N (2005) Assessment of fracture risk. Osteoporosis Int 16:581–589
3. Naylor K, Eastell R (2012) Bone turnover markers: use in osteoporosis. Nat Rev Rheumatol 8:379–389. https://doi.org/10.1038/nrrheum.2012.86
4. Johnell O, Kanis J (2006) An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporosis Int 17:1726–1733
5. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M, Lewis CE, McGowan J, Neuner J (2003) Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA 290:1729–1738
6. Pacifici R (1996) Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. J Bone Miner Res 11:1043–1051
7. Stenderup K, Justesen J, Clausen C, Kassem M (2003) Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 33:919–926
8. Kassem M, Marie PJ (2011) Senescence-associated intrinsic mechanisms of osteoblast dysfunctions. Aging Cell 10:191–197
9. von Zglinicki T, Martin-Ruiz CM (2005) Telomeres as biomarkers for ageing and age-related diseases. Curr Mol Med 5:197–203
10. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153:1194–1217
11. Blackburn EH (1991) Structure and function of telomeres. Nature 350:569
12. Saeed H, Abdallah BM, Ditzel N, Catala-Lehnen P, Qiu WM, Amling M, Kassem M (2011) Telomerase-deficient mice exhibit bone loss owing to defects in osteoblasts and increased osteoclastogenesis by inflammatory microenvironment. J Bone Miner Res 26:1494–1505. https://doi.org/10.1002/jbmr.349
13. Samper E, Flores JM, Blasco MA (2001) Restoration of telomerase activity rescues chromosomal instability and premature aging in $Terc^{-/-}$ mice with short telomeres. EMBO Rep 2:800–807
14. Gray MD, Shen J-C, Kamath-Loeb AS, Blank A, Sopher BL, Martin GM, Oshima J, Loeb LA (1997) The Werner syndrome protein is a DNA helicase. Nat Genet 17:100
15. Dokal I (2011) Dyskeratosis congenita. Hematol Am Soc Hematol Educ Program 2011:480–486. https://doi.org/10.1182/asheducation-2011.1.480
16. Demissie S, Levy D, Benjamin EJ, Cupples LA, Gardner JP, Herbert A, Kimura M, Larson MG, Meigs JB, Keaney JF (2006) Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. Aging Cell 5:325–330
17. O'Donovan A, Pantell MS, Puterman E, Dhabhar FS, Blackburn EH, Yaffe K, Cawthon RM, Opresko PL, Hsueh W-C, Satterfield S (2011) Cumulative inflammatory load is associated with short leukocyte telomere length in the Health, Aging and Body Composition Study. PLoS ONE 6:e19687
18. Valdes AM, Richards JB, Gardner JP, Swaminathan R, Kimura M, Xiaobin L, Aviv A, Spector TD (2007) Telomere length in leukocytes correlates with bone mineral density and is shorter in women with osteoporosis. Osteoporosis Int 18:1203–1210
19. Bekaert S, Van Pottelbergh I, De Meyer T, Zmierczak H, Kaufman JM, Van Oostveldt P, Goemaere S (2005) Telomere length versus hormonal and bone mineral status in healthy elderly men. Mech Ageing Dev 126:1115–1122
20. Kveiborg M, Kassem M, Langdahl B, Eriksen EF, Clark BFC, Rattan SIS (1999) Telomere shortening during aging of human osteoblasts in vitro and leukocytes in vivo: lack of excessive telomere loss in osteoporotic patients. Mech Ageing Dev 106:261–271. https://doi.org/10.1016/S0047-6374(98)00114-6
21. Sanders JL, Cauley JA, Boudreau RM, Zmuda JM, Strotmeyer ES, Opresko PL, Hsueh WC, Cawthon RM, Li RL, Harris TB, Kritchevsky SB, Newman AB, Study HA (2009) Leukocyte telomere length is not associated with BMD, osteoporosis, or fracture in older adults: results from the Health, Aging and Body Composition Study. J Bone Miner Res 24:1531–1536
22. Tang NLS, Woo J, Suen EWC, Liao CD, Leung JCS, Leung PC (2010) The effect of telomere length, a marker of biological aging, on bone mineral density in elderly population. Osteoporosis Int 21:89–97. https://doi.org/10.1007/s00198-009-0948-4
23. Nielsen BR, Linneberg A, Bendix L, Harboe M, Christensen K, Schwarz P (2015) Association between leukocyte telomere length and bone mineral density in women 25–93 years of age. Exp Gerontol 66:25–31. https://doi.org/10.1016/j.exger.2015.04.004
24. Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141
25. Cawthon RM (2002) Telomere measurement by quantitative PCR. Nucleic Acids Research 30:e47. https://doi.org/10.1093/nar/30.10.e47
26. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45
27. Knol MJ, van der Tweel I, Grobbee DE, Numans ME, Geerlings MI (2007) Estimating interaction on an additive scale between continuous determinants in a logistic regression model. Int J Epidemiol 36:1111–1118
28. Rothman KJ, Greenland S, Lash TL (2008) Modern epidemiology, 3rd edn. Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia
29. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908:244–254
30. Cui H, Kong Y, Zhang H (2012) Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct 1:1. https://doi.org/10.1155/2012/646354
31. Hoeijmakers JH (2009) DNA damage, aging, and cancer. N Engl J Med 361:1475–1485
32. Boult C, Kane RL, Louis TA, Boult L, McCaffrey D (1994) Chronic conditions that lead to functional limitation in the elderly. J Gerontol 49:M28–M36
33. Simonsen JL, Rosada C, Serakinci N, Justesen J, Stenderup K, Rattan SIS, Jensen TG, Kassem M (2002) Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat Biotechnol 20:592–596. https://doi.org/10.1038/nbt0602-592
34. Kim N, Takami M, Rho J, Josien R, Choi Y (2002) A novel member of the leukocyte receptor complex regulates osteoclast differentiation. J Exp Med 195:201–209. https://doi.org/10.1084/jem.20011681
35. Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, Orimo A, Inoue M (1999) Estrogen activates telomerase. Cancer Res 59:5917–5921
36. Pietschmann P, Rauner M, Sipos W, Kerschan-Schindl K (2009) Osteoporosis: an age-related and gender-specific disease—a mini-review. Gerontology 55:3–12
37. Schuit S, Van der Klift M, Weel A, De Laet C, Burger H, Seeman E, Hofman A, Uitterlinden A, Van Leeuwen J, Pols H (2004) Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone 34:195–202
38. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A (2002) Telomere length in the newborn. Pediatr Res 52:377
39. Barrett EL, Richardson DS (2011) Sex differences in telomeres and lifespan. Aging Cell 10:913–921
40. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, Hottenga JJ, Fischer K, Esko T, Surakka I (2013) Identification

of seven loci affecting mean telomere length and their association with disease. Nat Genet 45:422
41. Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, Kimura M, Lu X, Spector TD, Aviv A (2008) The association between physical activity in leisure time and leukocyte telomere length. Arch Intern Med 168:154–158
42. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, Aviv A, Spector TD (2005) Obesity, cigarette smoking, and telomere length in women. Lancet 366:662–664
43. Herrmann M, Pusceddu I, Marz W, Herrmann W (2018) Telomere biology and age-related diseases. Clin Chem Lab Med 56:1210–1222. https://doi.org/10.1515/cclm-2017-0870