JOURNAL OF BONE AND MINERAL RESEARCH
Volume 24, Number 9, 2009
Published online on March 30, 2009; doi: 10.1359/JBMR.090318


# Leukocyte Telomere Length Is Not Associated With BMD, Osteoporosis, or Fracture in Older Adults: Results From the Health, Aging and Body Composition Study

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**ABSTRACT:** Short leukocyte telomere length (TL), low BMD, and osteoporosis have been associated with increased inflammation. Previous reports suggest an association between TL, BMD, and osteoporosis in women. We sought to verify these associations and to determine whether TL is related to fracture in a cohort of older men and women. Participants included 2750 community-dwelling older persons from the longitudinal Health, Aging, and Body Composition Study (Health ABC) in who average leukocyte TL was measured at baseline using qPCR. We used unconditional logistic regression to determine the association of TL with prevalent fracture, Cox proportional hazards regression for the association with 7-yr incident fracture, and mixed linear models for the association with BMD, change in BMD, and the number of incident fractures. TL was negatively correlated with age, weight, fasting insulin, and fasting glucose in men and women, and additionally, with C-reactive protein and IL-6 in men. TL was not associated with BMD; change in BMD over 1, 3, or 5 yr; osteoporosis; baseline fracture; or 7-yr incident fracture, before or after adjustment for age, race, smoking, and health characteristics. TL is not associated with BMD, osteoporosis, or fracture in older men or women in this sample.
**J Bone Miner Res 2009;24:1531–1536. Published online on March 30, 2009; doi: 10.1359/JBMR.090318**

**Key words: aging, BMD, fracture, osteoporosis, telomere**

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## INTRODUCTION

Fracture is a leading cause of morbidity and mortality in older individuals, although the pathogenesis of fracture remains incompletely understood. There is growing evidence that inflammation and bone cell senescence play major roles in the development of osteoporosis, the most important risk factor for fracture.[1,2] Telomere length (TL) is a mediator of cell senescence in normal and accelerated aging and is shorter in older individuals compared with younger ones,[3,4] but may also reflects the body's inflammatory experience independent of chronological age.[5] Thus, TL could be a marker of osteoporosis.

Murine models also show that telomere maintenance directly impacts bone homeostasis. $Wrn^{-}/Terc^{-}$ knockout mice that mimic human Werner syndrome (where telomere dysfunction induces features of premature aging) exhibit a low bone mass phenotype.[6] These mice develop age-related osteoporosis as a result of impaired osteoblast differentiation. Furthermore, telomerase reverse transcriptase prevents bone loss in these mice after transfection into in vitro aged (presenescent) osteoblasts that are grafted into the murine model. Altogether, these observations suggest that shorter TL might play a role in fracture risk.

Human studies support this hypothesis. Patients with mutations in telomerase, such as in the condition dyskeratosis congenita, show increased osteoporosis.[7] Shorter TL has been associated with fracture-specific physiologic precursors including decreased BMD and osteoporosis in white women, although the magnitudes of the associations were quite modest.[8] Moreover, longer TL in women has been related to higher serum vitamin D,[9] a proponent of bone strength. Reduced white blood cell TL is also associated with other risk factors for fracture, such as age,[5,10]

The authors state that they have no conflicts of interest.

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cardiovascular disease,[11,12] obesity,[13] smoking,[13] and sedentary lifestyle.[14] Despite these shared associations, a study directly examining the relationship between TL and fracture has yet to be published to our knowledge. Consequently, we analyzed data from the Health, Aging, and Body Composition Study (Health ABC), a longitudinal cohort study of community-dwelling men and women 70–79 yr of age, to assess the relationship of leukocyte TL to both prevalent and incident fracture. A secondary aim was to replicate a previous finding that leukocyte TL is associated with BMD and osteoporosis.

## MATERIALS AND METHODS

### *Health ABC Study population*

Our analysis of the association between TL and fracture used all participants in the prospective Health ABC cohort in whom mean TL was measured at baseline ($N$ = 2750; 89.4% of the Health ABC baseline cohort) in 1997–1998. Briefly, participants were identified from the Medicare-eligible population in the Memphis, TN, and Pittsburgh, PA, areas. Eligibility criteria included no reported difficulty in walking for 0.25 mi, walking up 10 steps, getting in and out of bed or chairs, bathing or showering, dressing, or eating; no need of using a cane, walker, crutches, or other special equipment to get around; not enrolled in a lifestyle intervention trial; free of life-threatening illness; and plans to stay in the geographic area for $\geq$3 yr. The University of Pittsburgh and the University of Tennessee Institutional Review Boards approved all procedures related to Health ABC. The Health ABC sample ($N$ = 2750) was 41% black and 48% male, 70–79 yr of age.

### *Telomere measurement*

DNA was extracted from isolated peripheral blood mononuclear leukocytes. We measured average TL using a validated qPCR method, as described previously.[15] This method measures the relative average TL in genomic DNA by determining the ratio of telomere repeat copy number to single copy gene copy number (T/S ratio) in experimental samples relative to a reference sample. The CV for the relative T/S ratios was 5.8%. Assuming a normal distribution for relative T/S ratios in repeated measurements of the same sample, samples differing in average TL by as little as 11.4% should be distinguishable by this method at the 95% confidence level. One TL ''ratio unit'' measured by the qPCR method is equivalent to a mean TL of 4270 bp in the leukocytes. Thus, the leukocyte TL unit presented was converted to base pairs using this conversion factor.

### *BMD, osteoporosis, and fracture outcomes*

Total hip and femoral neck BMD (g/cm$^2$) was assessed at both field centers by DXA (Hologic 4500A, software version 9.03; Hologic, Bedford, MA, USA). Quality assurance measurements of DXA at both study sites ensured scanner reliability and identical scanning protocols. Baseline osteoporosis was coded as prevalent if total hip BMD T-score was $< -2.5$ using race- and sex-specific thresholds from the NHANES III reference database.

Baseline fracture status was ascertained by trained interviewers at the baseline questionnaire administration between April 1997 and May 1998. Subjects were coded as having a prevalent fracture if they reported ever having a fracture after age 45. Incident nontraumatic fracture was ascertained every 6 mo by self-report and verified by radiology reports. Adjudication was available for 7 yr of follow-up, and mean (SD) length of follow-up was 5.9 (1.6) yr. Nontraumatic fractures were defined as those occurring spontaneously or from modest trauma. Fractures were excluded if they were caused by excessive trauma, a pathologic condition, stress, or were of unknown/other cause.

### *Covariates*

Trained interviewers administered the baseline Health ABC questionnaire to assess demographic and socioeconomic characteristics, health behaviors, health status, and medical history. Within 2 wk of the interview, subjects visited the University of Pittsburgh or University of Tennessee clinics for baseline biological, anthropometric, and functional measures and a blood draw. We selected possible confounders measured at baseline based on those documented in the literature. Demographic characteristics included age, sex, race (white or black), and study site. Height was measured using a stadiometer, and weight was measured with a calibrated balance beam scale. Health behaviors included smoking (never/ever), current drinking, and weekly physical activity (kcal). Health status included losing $\geq$2.25 kg in the past year or reporting a fall in the past year. The prevalence of diabetes (defined by an elevated fasting glucose [$\geq$126 mg/dl] or 2-h glucose [$\geq$200 mg/dl] in an oral glucose tolerance test), cerebrovascular disease (transient ischemic attack or stroke), coronary heart disease (bypass or coronary artery bypass graft, carotid endarterectomy, myocardial infarction, angina, or congestive heart failure), kidney disease, and retinopathy/retinal disease was determined using algorithms based on self-reporting and medication use. Blood pressure was measured with the participant seated for 5 min and then 1 min after standing. Participants were asked to bring all prescription and over the counter medications used in the previous 2 wk. Medication and supplement use were coded using the Iowa Drug Information System,[16] and we specifically examined use of thiazide diuretic, statins, estrogens, osteoporosis drugs, vitamin D, and calcium as covariates. IL-6 and high-sensitivity C-reactive protein (CRP) were measured with ELISA kits using stored samples collected at baseline and analyzed using a blind duplicate system to ensure reliability. The HS600 Quantikine kit (R&D Systems, Minneapolis, MN, USA) was used to measure IL-6 with a detectable limit of 0.10 pg/ml and interassay CV of 10.3%. The CRP ELISA used purified protein and polyclonal anti-CRP antibodies (Calbiochem, San Diego, CA, USA) and was standardized according to the World Health Organization's First International Reference Standard, with a sensitivity of 0.08 μg/ml and a CV of 8.0%. It has been shown previously in older adults that levels of IL-6 and CRP measured at one time point are reliable, reproducible, and representative throughout time.[17]

### *Statistical analysis*

All analyses were stratified by sex because of its established modification of BMD and fracture risk. We assessed possible confounders or mediators for their association with exposure (TL) and outcome (fracture) using Pearson correlation coefficients, Student's *t*-test, and $\chi^2$ statistics. We chose the significance level (0.10) as a cut-off for inclusion of possible confounders in multivariable models. Age, height, weight, weekly physical activity (kcal), CRP, IL-6, fasting insulin, fasting glucose, BMD, and 1-, 3-, and 5-yr changes in hip BMD were treated as continuous variables, whereas race, smoking, current drinking at baseline, falling in the past year, losing ≥2.25 kg in the past year, diabetes-related complications, comorbidities, and medication and supplement use were treated categorically. CRP, IL-6, and fasting insulin levels were log-transformed to account for skewness. Transformed values were used to calculate adjusted ratio measures, although nontransformed descriptive statistics are reported.

TL was measured on 45 qPCR plates so we included a plate variable as a random effect in all mixed linear, logistic, and proportional hazards models to account for slight TL measurement variation between plates. To examine whether BMD or changes in BMD were different across quartiles of TL, we used the overall model *p* value from a mixed linear model adjusted for confounders. TL quartiles were as follows—men: Q1 = 0–3.81 kbp, Q2 = 3.81–4.54 kbp, Q3 = 4.54–5.35 kbp, Q4 = >5.35 kbp; women: Q1 = 0–4.21 kbp, Q2 = 4.21–4.95 kbp, Q3 = 4.95–5.77 kbp, Q4 = >5.77 kbp. Because of low numbers of participants in some categories for change in BMD, some values were not estimable. We used unconditional logistic regression to calculate the OR of fracture for each increase in quartile of TL. Multivariable unconditional logistic regression was used to adjust for confounding. We used a frailty model for Cox proportional hazards regression to calculate the hazard ratio for 7-yr incident fragility fracture for each increase in quartile of TL (Stata 10.0; StataCorp, College Station, TX, USA). Multivariable proportional hazards regression was used to adjust for confounding. Finally, we used the overall *p* value from an adjusted mixed linear model to examine whether TL was associated with number of incident fractures. Age, race, smoking, falling in the year previous to baseline interview, and hip BMD were included a priori in all multivariable fracture models given their established association with fracture risk. We used a significance level of 0.05 and SAS 9.1 (SAS Institute, Cary, NC, USA) for all analyses, except as noted above.

## RESULTS

Mean (SD) TL was 4670 bp (1360 bp) in men and 5040 bp (1330 bp) in women at baseline. Longer TL was correlated with lower age, lighter weight, lower fasting insulin, and lower fasting glucose in men and women (Table 1). Whereas TL was inversely correlated with CRP and IL-6 in men, it was not associated with inflammatory markers in women. TL was 190 bp greater in male never smokers ($p < 0.03$) versus ever smokers and 217 bp greater in male statin users ($p = 0.080$) versus nonusers. TL was not associated with race, health status, specific comorbidities, or other medications in both men and women.

**TABLE 1.** Correlation of Telomere Length and Continuous Covariates by Sex

| | *Men* | | *Women* | |
|---|---|---|---|---|
| *Covariate* | *r** | *p* | *r** | *p* |
| Age (yr) | −0.065 | 0.017 | −0.055 | 0.038 |
| Height (cm) | −0.023 | 0.397 | −0.027 | 0.311 |
| Weight (kg) | −0.046 | 0.094 | −0.050 | 0.062 |
| Weekly physical activity (kcal) | −0.036 | 0.188 | −0.029 | 0.278 |
| CRP | −0.051 | 0.063 | 0.004 | 0.877 |
| IL-6 | −0.074 | 0.008 | −0.008 | 0.771 |
| Fasting insulin (mg/dl) | −0.064 | 0.028 | −0.069 | 0.014 |
| Fasting glucose† (mg/dl) | −0.061 | 0.025 | −0.045 | 0.089 |
| Total hip BMD (g/cm$^2$) | −0.017 | 0.534 | −0.007 | 0.792 |
| Femoral neck BMD (g/cm$^2$) | −0.003 | 0.921 | −0.016 | 0.550 |

* Pearson coefficient.
† Spearman rank coefficient.

**TABLE 2.** Least Squares Means of BMD by TL Quartile

| *Covariate (g/cm$^2$)* | *Q1* | *Q2* | *Q3* | *Q4* | *p** |
|---|---|---|---|---|---|
| Men† | | | | | |
| Total hip BMD | 0.943 | 0.939 | 0.920 | 0.944 | 0.091 |
| Femoral neck BMD | 0.795 | 0.800 | 0.778 | 0.794 | 0.122 |
| 1-yr change hip BMD | NC | NC | NC | NC | NC |
| 3-yr change hip BMD | 0.021 | 0.012 | 0.020 | 0.032 | 0.533 |
| 5-yr change hip BMD | 0.000 | 0.002 | 0.000 | 0.012 | 0.922 |
| Women‡ | | | | | |
| Total hip BMD | 0.785 | 0.785 | 0.783 | 0.784 | 0.999 |
| Femoral neck BMD | 0.705 | 0.710 | 0.695 | 0.699 | 0.308 |
| 1-yr change hip BMD | −0.010 | 0.058 | NC | 0.019 | 0.850 |
| 3-yr change hip BMD | −0.028 | −0.038 | −0.050 | −0.015 | 0.216 |
| 5-yr change hip BMD | −0.043 | −0.039 | −0.039 | −0.024 | 0.533 |

* Mixed linear model overall *p* value for TL.
† Adjusted for age, race, smoking, weight, glucose, insulin, and statin use.
‡ Adjusted for age, race, smoking, weight, glucose, and insulin.
NC, not calculable.

### *BMD*

TL was not associated with total hip or femoral neck BMD (Tables 1 and 2). TL was also not associated with 1-, 3-, or 5-yr changes in total hip BMD in men or women (Table 2).

### *Prevalent fracture*

Sixteen percent of men and 27.0% of women reported a history of fracture after age 45 at the time of the study baseline interview. Lower BMD ($p < 0.02$) and osteoporosis ($p < 0.0001$) were associated with prevalent fracture in both women and men. A greater prevalence of fracture was associated with drinking, falling in the year before baseline, and calcium supplement use in men (all $p < 0.05$) and with increased weekly physical activity, lower IL-6, lower prevalence of diabetes, lower fasting glucose, more

**TABLE 3.** Sex-Specific Association of Prevalent Fracture With TL

| *Telomere length quartile* | *Unadjusted OR* | *Unadjusted 95% CI* | *Adjusted OR* | *Adjusted 95% CI* |
|---|---|---|---|---|
| Men* | | | | |
| Q1 | Ref | | Ref | |
| Q2 | 0.98 | 0.64–1.49 | 0.94 | 0.62–1.45 |
| Q3 | 1.05 | 0.69–1.59 | 1.04 | 0.68–1.58 |
| Q4 | 1.11 | 0.74–1.68 | 1.12 | 0.74–1.70 |
| Women† | | | | |
| Q1 | Ref | | Ref | |
| Q2 | 0.91 | 0.65–1.26 | 0.87 | 0.62–1.23 |
| Q3 | 0.73 | 0.52–1.03 | 0.76 | 0.54–1.08 |
| Q4 | 0.90 | 0.64–1.26 | 0.87 | 0.62–1.23 |

* Adjusted for age, race, smoking, falling in past year, and hip BMD.
† Adjusted for age, race, smoking, falling in past year, hip BMD, and fasting glucose.

**TABLE 4.** Sex-Specific Association of 7-yr Incident Fracture With TL

| *Telomere length quartile* | *Unadjusted HR* | *Unadjusted 95% CI* | *Adjusted HR* | *Adjusted 95% CI* |
|---|---|---|---|---|
| Men* | | | | |
| Q1 | Ref | | Ref | |
| Q2 | 0.98 | 0.57–1.68 | 1.07 | 0.61–1.88 |
| Q3 | 0.66 | 0.37–1.17 | 0.58 | 0.32–1.07 |
| Q4 | 0.88 | 0.50–1.55 | 0.93 | 0.53–1.66 |
| Women† | | | | |
| Q1 | Ref | | Ref | |
| Q2 | 1.29 | 0.89–1.86 | 1.41 | 0.96–2.08 |
| Q3 | 0.99 | 0.68–1.44 | 1.08 | 0.72–1.63 |
| Q4 | 1.03 | 0.72–1.46 | 1.07 | 0.73–1.57 |

* Adjusted for age, race, smoking, falling in past year, hip BMD, history of fracture after age 45, and weight.
† Adjusted for age, race, smoking, falling in past year, hip BMD, history of fracture after age 45, weight, and fasting insulin.

kidney disease, and use of several medications in women. In crude and adjusted logistic regression models, TL was not associated with baseline fracture in men or women (Table 3).

### *Incident fracture*

Over the 7-yr follow-up period, 97 fractures occurred among 1333 men (12.4 fractures/1000 person-years) and 235 fractures occurred among 1417 women (28.1 fractures/1000 person-years). Incident fracture was significantly associated with older age ($p < 0.05$), lower baseline weight ($p < 0.0275$), white race ($p < 0.0005$), and lower BMD ($p < 0.0001$) in men and women. Mean (SD) TL in men and women who experienced an incident fracture was 4590 bp (1570 bp) and 5070 bp (1480 bp) and in men and women free of incident fracture was 4670 bp (1340 bp) and 5040 bp (1300 bp), respectively. In both crude and multivariable proportional hazards models, TL was not associated with incident fracture (Table 4). TL was also not associated with number of incident fractures over 7 yr (Table 5).

**TABLE 5.** Mean TL by Number of Incident Fractures Over 7 yr

| *Number of fractures* | *Mean TL (bp)** | *p* |
|---|---|---|
| Men† | | |
| 0 | 4880 | 0.656 |
| 1 | 4810 | |
| ≥2 | 5210 | |
| Women‡ | | |
| 0 | 5330 | 0.155 |
| 1 | 5510 | |
| ≥2 | 5140 | |

* Least-squares means from mixed linear model.
† Adjusted for age, race, smoking, falling in past year, hip BMD, history of fracture after age 45, and weight.
‡ Adjusted for age, race, smoking, falling in past year, hip BMD, history of fracture after age 45, weight, and fasting insulin.

## DISCUSSION

In analyses of a large community-dwelling cohort of older men and women, we did not find any significant association between leukocyte TL and fracture. Additionally, we were not able to replicate previous reports that leukocyte TL is associated with BMD, change in hip BMD, or osteoporosis in women.[8]

In comparison with previous reports, our study is strengthened by several factors. First, the Health ABC study has a relatively small age range (70–79 yr). In contrast, the Twins UK study population (from which the only other population-based analysis of leukocyte TL and bone outcomes has been published) is 18–79 yr old.[8] Because age is associated with shortened TL,[5,10] low BMD, and increased fracture, the Twins UK results likely suffer from more residual confounding from age than do our results. Similarly, Health ABC participants were drawn from a more homogeneous population at baseline, and so residual confounding from other factors likely affects our results to a smaller degree. Second, we believe our results are more clinically relevant because our population was representative of those most at risk for fracture (i.e., older adults). Third, we examined race in our analysis, which is known to greatly influence fracture risk, and indeed black race was highly protective of fracture in all multivariable models. Fourth, the Health ABC study has data on other critical covariates, which we adjusted for, such as falling in the year before baseline and recent weight loss. Finally, all fractures in the Health ABC study are adjudicated, and type of fracture is documented. This allowed us to exclude fractures that were unlikely to be associated with aging, such as those from excessive trauma or pathologic conditions. Given the characteristics of our study population, ascertainment of and adjustment for important fracture-specific risk factors, and outcome adjudication, we believe our results are valid.

An additional limitation of the Twins UK study is that it did not assess change in BMD with aging. We were able to analyze the relationship between TL and change in hip BMD over 1, 3, and 5 yr and found no significant associations with TL. A recent study by Bekaert et al.[18] of 84 men 71–86 yr of age found no association between leukocyte TL measured using Southern blot and baseline BMD

but a significant association ($p < 0.05$) with 4-yr bone loss. Our much larger sample size and longer follow-up period add weight to our results, which consistently suggest that there is no association with change in hip BMD in older men or women. The sample of 84 men used by Bekaert et al.[18] was also a fraction of their original cohort ($N$ = 352, 24%), and therefore selection bias could have influenced their results.

Our consistent null results suggest a true association does not exist. Indeed, the Twins UK study showed a weakly significant association with BMD and they did not find a significant correlation between leukocyte TL and forearm or femoral neck BMD after adjustment.[8] Although there is evidence that immune aging and musculoskeletal status are linked,[1,2] there might not be a significant association between short leukocyte TL and bone pathologies because of the complex interaction of their shared risk factors. The immune system could age at a different rate than the musculoskeletal system, and so even if risk factors similarly impact both systems, their effects might be temporally or quantitatively mediated by other factors not measured in these analyses. Leukocyte TL could predominantly reflect immune aging and would thus be more strongly associated with immune-related outcomes (e.g., increased susceptibility to infection in old age)[11] rather than bone-related outcomes.

Despite our null findings, bone cell TL, rather than leukocyte TL, could be associated with BMD, osteoporosis, or fracture. This hypothesis is more readily supported by studies showing that mouse strains that mimic accelerated aging caused by telomere dysfunction have impaired osteoblast differentiation, low bone mass, and develop osteoporosis.[6] Human and animal studies, both in vitro and in vivo, show that TL differs by cell type, cell culture, and does not entirely correlate with age.[19] These discrepancies are apparent in a previous study of TL in cultured human osteoblasts and in peripheral leukocytes of young (age 20–26 yr, $N$ = 15), elderly (age 48–85 yr, $N$ = 15), and osteoporotic (age 52–81 yr, $N$ = 14) women.[20] Whereas osteoblasts in vitro exhibited marked telomere attrition, leukocyte TL was only slightly longer in young versus old women and was equivalent between osteoporotic women and age-matched controls. Subsequently, whereas it seems that bone cell TL is associated with musculoskeletal pathology, it is unclear whether leukocyte TL would be a primary cause of low bone mass or a nonetiologic marker for bone cell status.

There are several reasons why our results might differ from those reported by the Twins UK study. First, we must emphasize underlying differences in the study populations analyzed and in the methods used to measure TL. As stated previously, the Twins UK population had a much wider age range (18–79 yr) than the Health ABC population (70–79 yr). This single factor could have influenced results greatly. This was seen in the difference in overall average TL, which in the Twins UK population was 7.09 kbp, whereas in the female Health ABC population was 5.04 kbp. It seems likely that a young person in the United Kingdom has a different risk profile than an older American, and even with statistical adjustment, these individuals would be difficult to compare. The Twins UK study also used the Southern blot method to measure telomere restriction fragment (TRF) length, whereas we used qPCR to measure the relative average TL in genomic DNA. Although qPCR correlates highly with Southern blot,[15] these techniques are not exactly equivalent. Furthermore, TRF length can vary by the type of restriction enzyme used and can overestimate average TL,[15] whereas qPCR may have a larger CV, which will affect our power to detect a modest effect. Because of differences in the study populations and TL measurement techniques, it is possible that our results are not directly comparable to those from the Twins UK study.

Alternatively, it is possible that a weak association does exist between leukocyte TL and bone pathologies and that larger studies are necessary to detect this relationship. Indeed, significant correlations reported by the Twins UK study are weak before and after adjustment. One could argue that our smaller sample size of women ($N$ = 1410) compared with the Twins UK study ($N$ = 2150) was underpowered to detect a weak association, although posthoc power analysis showed that we had 80% power to detect a correlation of 0.074 with BMD, which is a weak association. Additionally, although we presented regressions using quartiles for ease of interpretation, regressions using SDs of TL (which have greater power) confirmed no association with baseline or incident fracture, with 80% power to detect an OR of 0.85. Our null results for the correlation with femoral neck BMD also agree with the Twins UK report. These analyses imply that leukocyte TL is likely not associated with BMD or fracture and that a much larger sample size would be required to detect a true, weak association. Concerning osteoporosis, we did not classify osteoporosis ordinally; rather, we examined osteoporosis as a dichotomous variable. The Twins UK report suggested that leukocyte TL is equivalent in individuals with osteoporosis at zero or one site but is shorter in individuals with osteoporosis at two or more sites compared with those without osteoporosis. Because of our classification scheme, we could have failed to detect this difference.

The major weakness in our analysis may be the selection criteria for Health ABC, which overall resulted in less phenotypic variation at the start of our study than would be seen in the general population. For example, because participants were generally free of mobility impairment at baseline, we selected individuals with better musculoskeletal aging, narrowing the range of continuous outcomes such as BMD and the number of categorical outcomes such as prevalent fractures. This also limits generalizability to the wider, less healthy population. Inclusion of blacks, who are less at risk for fracture, reduces the number of prevalent cases. All of these factors might bias our results for baseline BMD and prevalent fracture toward the null, although it would have less of an effect on change in BMD, incident fracture, and number of incident fractures, which were not associated with TL. The age range for Health ABC was narrower than that for Twins UK, which could also limit generalizability, but it nonetheless provided an adequate range of TL (range, 0.35–15.70 kbp; SD, 1.36 kbp). By using a healthier sample at baseline and not capturing more extreme aging phenotypes, our analysis

is potentially limited in scope. Validation of these results in a large cohort of older individuals with a range of musculoskeletal phenotypes is warranted, particularly using TL measurements from bone cells rather than leukocytes, as discussed previously.

In conclusion, our data do not support an association between leukocyte TL and BMD, osteoporosis, or fracture in older adults. Future studies examining this association might be aided by TL measurement in other tissues more closely associated with the musculoskeletal system. Incorporation of TL measurement into large, ongoing, bone-specific studies would be most informative.

## ACKNOWLEDGMENTS

The authors thank the Health ABC Publications Committee members for helpful comments on the manuscript; the entire Health ABC Study staff at the Pittsburgh and Memphis study sites for their assistance; and the Health ABC Study participants for their enthusiastic and longtime participation. This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging. The Health ABC study is funded by contracts N01-AG-6-2101, N01-AG-6-2103, and N01-AG-6-2106 from the National Institutes of Health, Bethesda, MD, USA. Telomere length measurement was supported by Grant 5U19AG023122-05 from the Longevity Consortium.

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Received in original form October 13, 2008; revised form December 27, 2008; accepted March 25, 2009.