Experimental Gerontology 66 (2015) 25–31

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Association between leukocyte telomere length and bone mineral density in women 25–93 years of age ☆

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ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form 7 April 2015
Accepted 9 April 2015
Available online 11 April 2015

Section Editor: Diana Van Heemst

Keywords:
Aging
General population studies
DXA
Leukocyte telomere length

ABSTRACT

Leukocyte telomere length (LTL) and bone mineral density (BMD) are associated with health and mortality. Because osteoporosis is an age-related condition and LTL is considered to be a biomarker of aging, we hypothesized that shorter LTL could predict lower BMD. The aim of our study was to assess whether there is an association of LTL with BMD and to determine whether this possible association is independent of age.

The BMDs of the lumbar spine (LS), femoral neck (FN) and total hip (TH) were evaluated in 460 women using DXA. LTL was analyzed using quantitative polymerase chain reaction. The women completed a health and lifestyle questionnaire. The associations were estimated by regression models that considered age, body mass index (BMI), menopause, physical activity, alcohol consumption and smoking habits.

We found a statistically significant unadjusted association between LTL and age (estimate and 95% confidence interval (CI): −0.003 (−0.005; −0.002)); and between BMI adjusted age and logarithmic transformed BMD. Estimates and 95% CI were as follows: LS: −0.13 (−0.26; −0.01); right TH: −0.44 (−0.53; −0.34); left TH: −0.38 (−0.48; −0.28); right FN: −0.57 (−0.67; −0.46) and left FN: −0.51 (−0.62; −0.40).

There were no statistically significant associations between BMD and LTL (both logarithmically transformed) with or without age adjustments. The age-adjusted estimates and CI were as follows: LS: −0.10 (−0.71; 0.52); right TH: −0.13 (−0.66; 0.41); left TH: −0.13 (−0.67; 0.42); right FN: −0.03 (−0.58; 0.52) and left FN: 0.09 (−0.47; 0.66).

In conclusion, we found no statistically significant associations between BMD and LTL, although the estimates of the crude associations were all positive, indicating hypothesis consistency; that shorter LTL predict lower BMD values. This positive association was no longer apparent after adjusting for age.

As expected, age was statistically significantly associated with both telomere length and BMI adjusted BMD.

1. Introduction

As we age, fragility and morbidity increase, regardless of the considerable variation between individuals. Because descriptive statistics of life expectancy indicate that individuals are living longer, we must anticipate that the incidence of chronic disease will increase (Christensen et al., 2009). Osteoporosis is an age-related condition that primarily occurs in post-menopausal woman, although the prevalence of this condition is increasingly seen in men and in younger patients as secondary osteoporosis (Hauser et al., 2014; Meyer et al., 2001). Osteoporosis is a major public health burden due to the large

Abbreviations: LTL, leukocyte telomere length; LS, lumbar spine; FN, femoral neck; TH, total hip; BMI, body mass index; CI, confidence interval; yr, year(s); CV, variation of coefficient; T, telomere repeat copies; S, single copy gene; gDNA, genomic deoxyribonucleic acid; qPCR, quantitative polymerase chain reaction; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell.

☆ *Grant supporters*: This study was financially supported by the Lundbeck Foundation (donation of DXA scanner).

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http://dx.doi.org/10.1016/j.exger.2015.04.004
0531-5565/

number of patients who incur fractures; this disease represents not only a significant economic societal burden but also a social burden to individuals (Johnell, 1997; Kannus et al., 1999).

Aging biomarkers, such as vascular, cognitive or skeletal aging, have been developed, and some scientists have even suggested using aging equations to assess biological age (Borkan et al., 1982; Zhang et al., 2014).

For several decades, cellular aging has been investigated. The following advancements led to the hypothesis that telomere length could be a marker of biological aging (Harley, 1991): the discovery of telomeres (repetitive tandem nucleotides located in the extreme ends of every chromosome) by Muller and McClintock (McClintock, 1941, 1942; Muller, 1938); the discovery of telomerase (a cellular ribonucleoprotein reverse transcriptase that elongates telomeres de novo) by Blackburn, Greider and Szostak (Blackburn, 2010; Blackburn et al., 2006); and the description of the "End Replication Problem" by, among others, Olovnikov in 1973 (Olovnikov, 1973).

Telomeres consist of a g-rich strand (5′-TTAGGG-3′) and a complementary c-rich strand (Blackburn, 1991; Chan and Blackburn, 2004). It is believed that the telomere shortens with every genome replication, protecting essential gene sequences from the "end replication problem" (Blasco, 2005; Levy et al., 1992). When telomere shortening reaches a certain threshold, cells achieve senescence, a non-replicative but metabolically active state (Harley, 1991). Somatic cells either lack telomerase or repress its function (Chan and Blackburn, 2004).

Telomeres shorten not only due to the end replication problem but also due to environmental factors, such as oxidative stress, endogenous estrogen, inflammation and lifestyle. These factors are associated with cellular aging, thereby supporting the notion of telomere length as a potential marker of biological aging (Bendix et al., 2014a; Lin et al., 2011; O'Donovan et al., 2011; Salpea et al., 2010).

In vitro and in vivo studies of human fibroblasts and in vivo studies of human leukocytes have reported shortened telomeres with age (Allsopp et al., 1992; Harley et al., 1990; Hastie et al., 1990). Moreover, epidemiological studies support the findings of a statistically significant inverse association between age and telomere length, although with a high degree of variation within the same age group (Bendix et al., 2014b; Hoffmann et al., 2009; Kimura et al., 2007).

In bones, the osteoclasts, osteoblasts and the osteocytes that are embedded in the bone matrix are responsible for bone remodeling because bone is continually deposited by osteoblasts and continually absorbed by osteoclasts (Crockett et al., 2011). An imbalance may result in fragile porous bone. It is well known that BMD declines with age due to, among other things, enhanced oxidative stress, which increases bone cell senescence. Furthermore, it is believed that oxidative stress stimulates osteoclast function and represses the WNT pathway, which is essential for osteoblast function (Almeida et al., 2007; Garrett et al., 1990; Manolagas and Almeida, 2007). Menopausal status and lifestyle factors, such as physical inactivity, alcohol intake and smoking, also significantly influence age-related bone fragility (Englund et al., 2011; Kanis et al., 2005; Pinheiro et al., 2010; van der Klift et al., 2003, 2004).

Studies of mice have demonstrated that during aging, the telomeres of osteoblast stem cells and primary osteoblasts shorten due to cell replication, which leads to reduced osteoblast differentiation and function (Kveiborg et al., 1999; Pignolo et al., 2008; Saeed et al., 2011). Furthermore, mice with shortened telomeres and that lack telomerase showed enhanced bone resorption, which is caused by the generation of a pro-inflammatory osteoclast-activation environment (Saeed et al., 2011). Aging in mice has also revealed a switch in the differentiation of the mesenchymal stem cells from osteoblastogenesis to adipogenesis (Moerman et al., 2004).

Furthermore, to support the hypothesis of an association between bone aging and telomere length, two human genetic disorders that result in premature aging syndromes (dyskeratosis congenita and Werner syndrome) have affected LTL and osteoporosis or fragile bones (Kelly and Stelling, 1982; Ogata et al., 2001; Rubin et al., 1994; Savage and Alter, 2008).

Telomere shortening was previously studied in vitro in human osteoblasts and in vivo in human leukocytes (Kveiborg et al., 1999). A decrease in the osteoblast telomere length of approximately 100 base pairs (bp) per population doubling was found, indicating that telomere length is a useful marker of osteoblast aging. The in vivo part of the study, the case–control study of osteoporotic women, did not demonstrate a statistically significant difference in LTL (Kveiborg et al., 1999).

The association between LTL and BMD has been studied in few epidemiological surveys. Bekaert et al. (2005) and Valdes et al. (2007) both reported a statistically significant age-corrected association between LTL, as assessed by Southern blot analysis, and BMD. In contrast, two studies that analyzed LTL using qPCR did not confirm this age-corrected association (Sanders et al., 2009; Tang et al., 2010). However, the settings differed in the cited studies; therefore, we believe that a study of bone-healthy white women (who are not undergoing anti-osteoporotic treatment) with a large age span is required. Such a study should measure BMD at the LS, FN and TH and measure LTL via qPCR.

Because both LTL and BMD are affected by chronological age, endogenous estrogen oxidative stress, inflammation and lifestyle factors and because LTL is associated with other age-related conditions, such as fatigue (Bendix et al., 2014b), cardiovascular diseases (Haycock et al., 2014) and Alzheimer's disease (Damjanovic et al., 2007), we hypothesize that osteoporosis (an age-related condition), as reflected by lower BMD, is associated with shorter LTL, independently or dependently of age.

2. Material and method

2.1. Study population

The study participants were recruited via newspaper advertisements and from two population-based studies that were performed at the Research Centre for Prevention and Health in the Capital Region of Denmark. Participants from the population-based studies were selected as random samples from the background population (age 18–69 yr) (Aadahl et al., 2013; Thuesen et al., 2013). The participants who were recruited from the newspapers advertisements ranged in age from 65–91 yr.

From 2008 to 2009, 971 females from the described groups were DXA-scanned at Glostrup University Hospital in Copenhagen, Denmark. In 2011, these women were invited to participate in our cross-sectional study, which was performed at the Research Centre of Ageing and Osteoporosis, Glostrup University Hospital, Copenhagen, Denmark.

A total of 460 women (47.4%) accepted the invitation. All participants provided signed informed consent prior to undergoing the protocol procedure, and the study was approved by the Ethics Committee of the Capital Region of Denmark (H-4-2009-124/28835).

2.2. Questionnaire

All participants completed a standard questionnaire (Health 2006) about their symptoms, disease diagnoses, physical activities, smoking, alcohol intake, eating habits, mental problems, quality of life and socioeconomic variables (Thuesen et al., 2013).

2.3. Bone mineral density

BMD (g/cm^2) was evaluated with the DXA scanner (Hologic Discovery™ QDR Series scanner) at the LS and both hips (TH and FN). DXA accurately determines BMD and detects patients with fragile bones who are at a high risk of incurring an osteoporotic fracture (Theodorou and Theodorou, 2002). All analyses were performed by the same laboratory technician under the same settings.