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Relationships of sex hormone levels with leukocyte telomere length in Black, Hispanic, and Asian/Pacific Islander postmenopausal women

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Abstract

Background: Sex hormones may play important roles in sex-specific biological aging. In the study, we specifically examined associations between circulating sex hormone concentrations and leukocyte telomere length (TL).

Methods: A cross-sectional study was conducted among 1124 Black, 444 Hispanic, and 289 Asian/Pacific Islander women in the Women's Health Initiative Observational Cohort. Estradiol and testosterone concentrations were measured using electrochemiluminescence immunoassays; TL was measured using quantitative polymerase chain reaction.

Results: Women in the study were aged 50–79 years. Estradiol concentrations were not significantly associated with TL in this sample. The associations between total and free testosterone and TL differed by race/ethnicity ($P_{\text{interaction}} = 0.03$ and 0.05 for total and free testosterone, respectively). Total and free testosterone concentrations were not associated with TL in Black and Hispanic women, whereas in Asian/Pacific Islander women their concentrations were

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Disclosure

All authors declare no conflict of interest.

inversely associated with TL ($P_{\text{trend}} = 0.003$ for both). These associations appeared robust in multiple sub-group analyses and multivariable models adjusted for potential confounding factors. In Asian/Pacific Islander women, a doubling of serum free and total testosterone concentrations was associated with a 202-bp shorter TL (95% confidence interval [CI] 51–353 bp) and 203-bp shorter TL (95% CI 50–355 bp), respectively.

Conclusions: Serum estradiol concentrations were not associated with leukocyte TL in this large sample of postmenopausal women. Total and free testosterone concentrations were inversely associated with TL in Asian/Pacific Islander women, but not in Black and Hispanic women, although future studies to replicate our observations are warranted particularly to address potential ethnicity-specific relationships.

Keywords

aging; estradiol; sex steroid hormones; telomere length; testosterone

Introduction

Telomeres are DNA–protein complexes that prevent genomic loss during chromosome replications.^{1–3} Aging has been linked to progressive shortening of telomere length (TL), which is estimated to be at a rate of 20–60 bp per year.^{4,5} Given that female life expectancy in the US is, on average, 80.2 years, compared with a male life expectancy of 75.1 years,⁶ it is not surprising that women have longer TL than age-matched men.^{7,8} Despite these observations, the mechanisms underlying the relationship between sex and longevity have not been fully elucidated. Although many theories have been proposed to explain this sex divergence, including oxidative damage and chromosomal complement,⁹ the role of sex steroids relating to TL is not yet fully understood.

Prior studies have shown that sex hormones may affect the enzyme telomerase, which is responsible for elongating telomeres.¹⁰ Both estradiol and dihydrotestosterone, an active metabolite of testosterone, have been positively correlated with leukocyte TL in men,¹¹ and this effect may be mediated by upregulation of an estrogen-sensitive promoter in the telomerase reverse transcriptase gene, partially explaining the sex divergence in TL.¹² Interestingly, other research on testosterone concentrations in children showed a relationship between high stress levels of testosterone and decreased TL.¹³ A Phase I/II non-randomized study showed that danazol, a synthetic sex hormone with androgenic properties, increased TL in patients with abnormally short telomeres due to telomere disease.¹⁴ However, given the supposition that aromatization of androgen to estrogen may be responsible for the increase in telomerase, and thus TL, the mechanism responsible for the longer TL with danazol treatment is not clear, because danazol typically decreases circulating estradiol concentrations.

To further elucidate the potential roles of sex hormones in biological aging, we examined the associations of circulating estradiol and testosterone with leukocyte TL in Black, Hispanic, and Asian/Pacific Islander postmenopausal women participating in the Women's Health Initiative (WHI) Observational Study.

Methods

Study subjects

The Women's Health Initiative (WHI) is a long-term national health study that has focused on strategies for preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. The original WHI study included 161 808 postmenopausal women enrolled between 1993 and 1998.^{15,16} The WHI has two major components: a partial factorial randomized clinical trial (WHI-CT) and an observational study (WHI-OS). Both the WHI-CT and WHI-OS were conducted at 40 clinical centers nationwide in the US. The WHI-OS examined the relationship between lifestyle, environmental, medical and molecular risk factors and specific measures of health or disease outcomes. This component involved tracking the medical history and health habits of 93 676 women not participating in the WHI-CT. The present study reports findings from a case-control study of sex steroids, sex hormone-binding globulin (SHBG), and risk of type 2 diabetes, nested in the WHI-OS cohort.¹⁷ Briefly, incident diabetes cases were selected among women without prior history of diabetes or cardiovascular diseases, and, for each incident case, up to two controls were selected randomly among women who remained free of clinical diabetes at the time the case was identified, matched to cases by age, race/ethnicity, clinical center (geographic location), time of blood draw, and length of follow-up. The original study further restricted the population to non-White women because previous studies have already indicated a strong association between SHBG and diabetes among White men and women.¹⁸ In the present study, we included eligible cases and controls from the original case-control study (1124 Black, 444 Hispanic, and 289 Asian/Pacific Islander women) whose blood samples were assayed for sex hormones (estradiol and testosterone) and leukocyte TL. The study was reviewed and approved by human subjects review committees at each participating institution, and signed informed consent was obtained from all women enrolled.

Measurement of sex steroid hormones and SHBG

Measurements of circulating estradiol, testosterone, and SHBG concentrations have been described elsewhere.¹⁷ Briefly, fasting serum specimens were collected at baseline from each participant, and serum estradiol, testosterone, and SHBG concentrations were measured by electrochemi-luminescence immunoassays on the Elecsys 2010 immunoanalyzer (Roche Diagnostics, Indianapolis, IN, USA). Interassay imprecision (expressed as percentage coefficient of variation [CV]) was 12.4% for estradiol, 10.3% for testosterone, and 5.4% for SHBG. Free estradiol and free testosterone concentrations were calculated using the methods described by Vermeulen et al.¹⁹ and Södergard et al.,²⁰ previously validated in postmenopausal women.^{19–23}

Measurement of TL

The measurement of TL has been described elsewhere.¹⁷ Briefly, we adopted a quantitative polymerase chain reaction (qPCR) method first proposed by O'Callaghan et al.²⁴ using a high-throughput 384-well format system (7900HT PCR System; Applied Biosystems, Life Technologies, Carlsbad, CA, USA). All samples for telomere and 36B4 (a single-copy gene that served as the reference DNA sample) reactions, as well as standard curves, were

performed in duplicate on the same plates. As part of routine quality control, 10% of samples were blind duplicate samples. The overall intra- and interplate CV) was 0.8% and 5.7%, respectively.

Measurements of covariates

Self-administered questionnaires were used to collect information on demographics and lifestyle factors. Participants were categorized according to smoking status as “never smoker,” “former smoker,” or “current smoker.” Levels of alcohol intake and total energy intake were also calculated from the food frequency questionnaire (FFQ). (The FFQ was based on instruments used in the WHI feasibility studies^{25,26} and the original National Cancer Institute/Block FFQ.²⁷) Information of age at menarche and menopause was collected in the questionnaire, and the difference was calculated as a surrogate of lifetime estrogen exposure. Body weight and height were measured at baseline, and body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared. The level of physical activity in metabolic equivalent hours per week (MET-h/week) was estimated based on the self-reported duration of exercise, weighted by intensity levels. Participants were also categorized according to use of hormone-replacement therapy (HRT) as “never user,” “former user,” or “current user.” Tumor necrosis factor- α receptor 2 (TNF- α -R2) was measured by ELISA (R&D Systems, Minneapolis, MN, USA), interleukin (IL)-6 was measured using an ultrasensitive ELSIA (R&D Systems), and high-sensitivity C-reactive protein (hsCRP) was measured on Roche Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN, USA) using an immunoturbidimetric assay with reagents and calibrators (Denka Seiken, Niigata, Japan).

Statistical analysis

Baseline characteristics were summarized according to race/ethnicity. Categorical variables are shown as percentages; normally distributed continuous variables are expressed as the mean SD, whereas non-normally distributed continuous variables are given as the median with interquartile range (IQR). *P*-values for differences among ethnic groups were obtained from Chi-squared tests for categorical variables, from analysis of variance (ANOVA) for normally distributed continuous variables, and from Kruskal–Wallis tests for non-normally distributed continuous variables.

General linear models were used to estimate mean TLs and their 95% confidence intervals (CIs) for different quartiles of sex hormones while adjusting for covariates. The basic models were adjusted only for age at enrollment (years; continuous). The multivariable adjusted models were further adjusted for race/ethnicity (Black, Hispanic, or Asian/Pacific Islander), HRT use (never, former, or current user), years between menarche and menopause (years; continuous), BMI (kg/m²; continuous), cigarette smoking (never, former, or current smoker), alcohol consumption (never, former, or current drinker), diabetes case in the primary case-control study (yes or no), physical activity (0, >0 to 5, >5 to 20, or >20 MET-h/week), daily energy intake (kcal; continuous), and serum SHBG concentration (nmol/L; continuous). In addition, the models for estradiol and testosterone were mutually adjusted for each other. Concentrations of sex hormones were categorized into quartiles among all individuals, with the fourth quartile having the highest concentration. *P*-values for linear

trend were obtained by including the medians of concentration levels as continuous variables in the regression models. Regression coefficients for the change in leukocyte TL for doubling of sex steroid hormone concentrations were calculated using linear regression models with log-transformed concentrations. To further assess potential effect modification by race/ethnicity, the interaction terms between race/ethnicity and log-transformed concentrations of sex steroid hormones were included in the models. In addition, subgroup analyses stratified by race/ethnicity were conducted. In the first sensitivity analysis, we fitted models with additional adjustment of serum concentrations of inflammatory biomarkers, including IL-6, hsCRP, and TNF- α -R2. In the second sensitivity analysis, we stratified the analyses by BMI with a cut-off point at 25 kg/m². To explore potential non-linear relationships between sex hormone concentrations and TL, we used restricted cubic spline models.

All statistical analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC, USA). All *P*-values are two tailed, and the false discovery rate (FDR) was adopted to control for the effects of multitesting. *P* < 0.05 was considered significant.

Results

Characteristics of participants at baseline are summarized in Table 1. The age of women included in the study ranged from 50 to 79 years. On average, the Asian/Pacific Islanders had a lower BMI and a lower proportion of current smokers and current alcohol drinkers than Black and Hispanic women. Moreover, Asian/Pacific Islander women had lower concentrations of sex hormones (estradiol and testosterone), higher SHBG concentrations, and lower concentrations of markers of inflammation (hsCRP, IL6, and TNF- α) than Black and Hispanic women. The proportion of current HRT users and lifetime estrogen exposure were significantly higher in Asian/Pacific Islander women than Black and Hispanic women. In addition, Asian/Pacific Islander women had the shortest TL among the three ethnic groups.

Total and free estradiol concentrations appeared to be positively associated with leukocyte TL in the pooled analysis, although the linear trends were not significant ($P_{\text{trend}} = 0.14$ for free estradiol; $P_{\text{trend}} = 0.19$ for total estradiol; Table 2). In subgroup analysis by race/ethnicity, we did not observe significant associations between estradiol concentrations and TL in any of the three ethnic groups, although the associations using the continuous measure of estradiol were in the same direction as the pooled analysis. We did not observe a significant interaction between estradiol concentration and race/ethnicity ($P = 0.48$ for free estradiol; $P = 0.63$ for total estradiol).

Total and free testosterone concentrations were modestly associated with TL in the pooled analysis ($P_{\text{trend}} = 0.04$ for free testosterone; $P_{\text{trend}} = 0.02$ for total testosterone), where the mean TL appeared shorter in higher testosterone concentration quartiles (Table 3). Multivariable adjustment did not change the estimates materially, although most associations were no longer significant. The interaction between testosterone concentration and race/ethnicity was significant for both free testosterone ($P = 0.05$) and total testosterone ($P = 0.03$). In subgroup analyses, we observed significant inverse associations between free

testosterone and TL in Asian/Pacific Islander women ($P_{\text{trend}} = 0.003$, $\text{FDR} < 0.05$). In multivariable-adjusted models, Asian/Pacific Islander women in the highest quartile of serum free testosterone (median 0.241 ng/dL) had a 785-bp shorter TL (95% CI 48–1522 bp) than women in the lowest quartile (median 0.012 ng/dL). In Asian/Pacific Islander women, doubling of serum free testosterone concentration was associated with a 202-bp shorter TL (95% CI 51–353 bp). No associations between free testosterone concentration and TL were observed in Black and Hispanic women. In sensitivity analyses, neither additional adjustment for inflammation markers nor stratifying on BMI changed the results materially. When we restricted our analyses to those who had never used HRT, the association between free testosterone and TL remained in the same direction but was no longer significant ($P_{\text{trend}} = 0.08$). In cubic spline models (Fig. 1), we observed that leukocyte TL decreased substantially with higher concentrations of free testosterone in Asian/Pacific Islander women, whereas the trends among Black and Hispanic women were not apparent.

In a similar manner, we also observed a significant inverse association between total testosterone concentration and TL in Asian/Pacific Islander women ($P_{\text{trend}} = 0.008$, $\text{FDR} < 0.05$). In multivariable-adjusted models, Asian/Pacific Islander women with highest serum concentrations of total testosterone (median 22.4 ng/dL) had a 660-bp shorter TL (95% CI 45–1274 bp) than those in the lowest concentration group (median 1.9 ng/dL). In Asian/Pacific women, doubling of serum total testosterone concentration was associated with a 203-bp shorter TL (95% CI 50–355 bp). No association between total testosterone concentration and TL was observed in Black and Hispanic women. In the sensitivity analyses, neither additional adjustment for inflammation markers nor stratifying on BMI changed the results materially. When we restricted our analyses to women who had never used HRT, the association between total testosterone and TL remained in the same direction but was no longer significant ($P_{\text{trend}} = 0.07$).

Discussion

Overall, we did not find significant associations between estradiol concentrations and leukocyte TL in the women in the present study. However, in Asian/Pacific Islander women, total and free testosterone concentrations appeared to be inversely associated with TL, independent of potential confounders. In these women, TL attrition was estimated to be approximately 22 bp per year on average. In Asian/Pacific Islander women, a doubling of the free or total testosterone concentration was associated with an approximate 9.2-fold higher rate of this average annual attrition. However, among Black and Hispanic women, no associations were observed between testosterone concentrations and TL. When we restricted our analyses to women who had never used HRT, although the magnitudes of associations did not change materially, the associations were no longer significant, probably due to lower statistical power. Both sex hormones and TL were shown as important predictors of diabetes.^{17,18,28–31} Elucidating the relationship between these two factors will help better understand the etiology of diabetes and generate more personalized preventive strategies based on patient characteristics. For example, for Asian/Pacific Islander women, closer monitoring for diabetes and other aging-related chronic conditions may be considered among those with high testosterone concentrations.

Available evidence indicates that women generally live longer than men and suffer less from certain age-related diseases, such as certain cancers and cardiovascular diseases,^{32,33} which has long been attributed to sex differences in social or lifestyle factors (e.g. cigarette smoking, alcohol consumption, job stress, and utilization of medical services).³⁴ Recent work has also identified altered serum lipid levels by sex steroid hormones concentrations as potential biological mechanisms responsible for the sex difference in cardiovascular disease.^{28,35} For women after the menopausal transition, not only does endogenous estrogen plummet, but the estrogen-to-androgen ratio is also greatly altered; androgens, rather than estrogens, become the primary sex hormone in postmenopausal women who do not pursue HRT.³⁶ Although TL is a well-known indicator of biologic aging and senescence and has been associated with chronic diseases,^{37–40} few studies have investigated the relationship between sex steroid hormones and TL.

In animal studies, estrogen deficiency has been associated with telomere shortening.^{41,42} Estrogen was suggested to diminish oxidative stress,⁴³ which is fundamental to biologic aging and can accelerate telomere shortening and stimulate the transcription of the gene encoding telomerase.⁴⁴ In a human study examining the relationship between estradiol concentrations and TL, the duration of endogenous estrogen exposure (difference between age at menopause and age at menarche) was associated with greater TL and lower telomerase activity.⁴⁵ In the present study, we observed longer TL in women with higher concentrations of free or total estradiol, although these associations were not statistically significant at the conventional $\alpha = 0.05$ level. One limitation of the present study is that we could not accurately evaluate and control the lifetime exposure of sex hormones. However, we did assess the difference between age at menopause and age at menarche as a surrogate to adjust for potential confounding of lifetime exposure to estrogen.

Previous studies investigating the association between androgen concentrations and TL are scarce. One study in healthy elderly men in Belgium reported no statistically significant association between age-corrected testosterone concentrations and TL.⁴⁶ In the present study, we observed racial heterogeneity of the association between testosterone concentration and TL; the significant associations were only observed in Asian/Pacific Islander women. However, the reason for this significant interaction with race/ethnicity is still not clear. In Asian/Pacific Islander women, both higher total and free testosterone concentrations were associated with shorter TL, and the associations were robust in the sensitivity analyses. High testosterone concentrations have been associated with insulin resistance, metabolic syndrome, and cardiovascular disease in elderly women,⁴⁷ as well as with higher levels of cardiovascular risk factors in a multiethnic population of women.⁴⁸ Interestingly, the present data suggest that the Asian/Pacific Islander population generally has elevated markers typically associated with good health, including lower BMI, lower levels of inflammation, higher SHBG concentrations, and resultant lower free testosterone concentrations compared with Black and Hispanic women.

It is interesting to note that Asian/Pacific Islander women had the lowest testosterone concentrations of the ethnic groups, but also the strongest inverse relationship between elevated testosterone and TL. Some of the baseline differences are attributable to the decreased BMI and higher SHBG levels in Asian women. In addition to SHBG that can

account for the decreased total testosterone, Asian women may also experience differences in metabolic clearance rates of hormones that can also contribute to racial heterogeneity.

Another possible mechanism for the ethnic discrepancy is related to the genetic diversity of the androgen receptor. It has been demonstrated that Asians have the lowest prevalence of CAG microsatellites of exon 1 of the androgen receptor (*AR*) gene,⁴⁹ and fewer CAG repeats in the *AR* gene result in higher transcriptional activity and higher levels of serum androgens.⁵⁰ Moreover, given the shape of the relationship between testosterone and TL in Asian/Pacific Islanders seems to be linear when testosterone concentrations were higher in the spline analysis, and because women in this ethnic group generally have lower testosterone levels, we cannot rule out the possibility that more prevalent or severe metabolic abnormalities (e.g. insulin resistance) among Asian/Pacific Islander women with extremely high testosterone concentrations could explain the findings.

There are several limitations of the present study that need to be kept in mind when interpreting the findings. First, the present study is crosssectional study in nature because both sex hormone levels and TL were measured from the blood sample collected at the same time point. This makes direct causal inference difficult. Prospective studies with repeated measurements of sex hormone concentrations and TL are clearly warranted to further establish the causal relationship and investigate the role of sex hormones in affecting changes in TL among post-menopausal women. Second, although the analyses have controlled for confounding of the association to the extent possible by including potential predictors of sex hormone concentrations or TL, the possibility of residual confounding cannot be excluded. However, given the magnitude of association observed between testosterone and TL among Asian/Pacific Islander women in the multivariable-adjusted model, it is not likely that the residual confounding alone can explain the observed association. Third, given that this is a post hoc analysis of data from an existing study, the generalizability of the results is restricted by the population included in the original study. For example, the present study did not include men or White women. Because the present study shows racial heterogeneity of the association between sex hormone and TL, future studies with a broader population coverage are warranted to investigate the association in other populations. Finally, there were likely some measurement errors associated with both plasma concentrations of sex steroids and measures of leukocyte TL. Measurement errors, when they are not dependent, are likely to bias the parameter of interest towards null. In the present study, all samples for telomere reactions, as well as standard curves, were performed in duplicate on the same reaction plates. As part of quality control, 10% of samples were blind duplicate samples. The overall intraplate CV was 0.8%, and the interplate CV of the telomere assays was 5.7%.¹⁷

In conclusion, serum concentrations of estradiol were not significantly associated with leukocyte TL in the multiethnic population of postmenopausal women. However, higher total and free testosterone concentrations appeared to be associated with shorter TL in Asian/Pacific Islander women, but not in Black or Hispanic women. These findings suggest that Asian/Pacific Islander women may be susceptible to the potential detrimental effects of high testosterone concentrations on biologic aging, although prospective studies

incorporating larger numbers of ethnic minorities followed by serial hormonal and TL measurements are essential to further justify and explain the observed associations.

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References

1. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 1990; 345: 458–60. [PubMed: 2342578]
2. von Zglinicki T Role of oxidative stress in telomere length regulation and replicative senescence. *Ann N Y Acad Sci* 2000; 908: 99–110. [PubMed: 10911951]
3. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003; 361: 393–5. [PubMed: 12573379]
4. Song Y, You NC, Song Y et al. Intake-of small-to-medium-chain saturated fatty acids is associated with peripheral leukocyte telomere length in postmenopausal women. *J Nutr* 2013; 143: 907–14. [PubMed: 23616516]
5. Valdes AM, Andrew T, Gardner JP et al. Obesity, ciga-rette smoking, and telomere length in women. *Lancet* 2005; 366: 662–4. [PubMed: 16112303]
6. Arias E United States life tables, 2006. *Natl Vital Stat Rep* 2010; 58: 1–40.
7. Benetos A, Okuda K, Lajemi M et al. Telomere length as an indicator of biological aging: The gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* 2001; 37: 381–5. [PubMed: 11230304]
8. Barrett EL, Richardson DS. Sex differences in telomeres and lifespan. *Aging Cell* 2011; 10: 913–21. [PubMed: 21902801]
9. Seifarth JE, McGowan CL, Milne KJ. Sex and life expectancy. *Gend Med* 2012; 9: 390–401. [PubMed: 23164528]
10. Bayne S, Liu JP. Hormones and growth factors regulate telomerase activity in ageing and cancer. *Mol Cell Endocrinol* 2005; 240: 11–22. [PubMed: 16005142]
11. Yeap BB, Knuiman MW, Divitini ML et al. Epidemiological and Mendelian randomization studies of dihydrotestosterone and estradiol and leukocyte telomere length in men. *J Clin Endocrinol Metab* 2016; 101: 1299–306. [PubMed: 26789780]
12. Lee DC, Im JA, Kim JH, Lee HR, Shim JY. Effect of long-term hormone therapy on telomere length in post-menopausal women. *Yonsei Med J* 2005; 46: 471–9. [PubMed: 16127770]
13. Drury SS, Shirtcliff EA, Shachet A et al. Growing up or growing old? Cellular aging linked with testosterone reactivity to stress in youth. *Am J Med Sci* 2014; 348: 92–100. [PubMed: 25010187]
14. Townsley DM, Dumitriu B, Liu D et al. Danazol treatment for telomere diseases. *N Engl J Med* 2016; 374: 1922–31. [PubMed: 27192671]
15. Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. *Control Clin Trials* 1998; 19: 61–109. [PubMed: 9492970]
16. Hays J, Hunt JR, Hubbell FA et al. The Women's Health Initiative recruitment methods and results. *Ann Epidemiol* 2003; 13: S18–77. [PubMed: 14575939]
17. You NC, Chen BH, Song Y et al. A prospective study of leukocyte telomere length and risk of type 2 diabetes in postmenopausal women. *Diabetes* 2012; 61: 2998–3004. [PubMed: 22829448]
18. Ding EL, Song Y, Manson JE et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med* 2009; 361: 1152–63. [PubMed: 19657112]

19. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999; 84: 3666–72. [PubMed: 10523012]
20. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17beta to human plasma proteins at body temperature. *J Steroid Biochem* 1982; 16: 801–10. [PubMed: 7202083]
21. Endogenous Hormones and Breast Cancer Collaborative Group. Free estradiol and breast cancer risk in postmenopausal women: Comparison of measured and calculated values. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 1457–61. [PubMed: 14693737]
22. Miller KK, Rosner W, Lee H et al. Measurement of free testosterone in normal women and women with androgen deficiency: Comparison of methods. *J Clin Endocrinol Metab* 2004; 89: 525–33.
23. Rinaldi S, Geay A, Dechaud H et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. *Cancer Epidemiol Biomarkers Prev* 2002; 11: 1065–71. [PubMed: 12376508]
24. O'Callaghan N, Dhillon V, Thomas P, Fenech M. A quantitative real-time PCR method for absolute telomere length. *Biotechniques* 2008; 44: 807–9. [PubMed: 18476834]
25. Henderson MM, Kushi LH, Thompson DJ et al. Feasibility of a randomized trial of a low-fat diet for the prevention of breast cancer: Dietary compliance in the Women's Health Trial Vanguard Study. *Prev Med* 1990; 19: 115–33. [PubMed: 2193306]
26. Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: The Women's Health Trial Feasibility Study in Minority Populations. *Am J Epidemiol* 1997; 146: 856–69. [PubMed: 9384206]
27. Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. *J Clin Epidemiol* 1990; 43: 1327–35. [PubMed: 2254769]
28. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: A systematic review and meta-analysis. *JAMA* 2006; 295: 1288–99. [PubMed: 16537739]
29. Ding EL, Song Y, Manson JE, Rifai N, Buring JE, Liu S. Plasma sex steroid hormones and risk of developing type 2 diabetes in women: A prospective study. *Diabetologia* 2007; 50: 2076–84.
30. Li C, Ford ES, Li B, Giles WH, Liu S. Association of testosterone and sex hormone-binding globulin with metabolic syndrome and insulin resistance in men. *Diabetes Care* 2010; 33: 1618–24. [PubMed: 20368409]
31. Chen BH, Brennan K, Goto A et al. Sex hormone-binding globulin and risk of clinical diabetes in American Black, Hispanic, and Asian/Pacific Islander postmenopausal women. *Clin Chem* 2012; 58: 1457–66. [PubMed: 22908136]
32. Wingard DL, Suarez L, Barrett-Connor E. The sex differential in mortality from all causes and ischemic heart disease. *Am J Epidemiol* 1983; 117: 165–72. [PubMed: 6829546]
33. Cook MB, McGlynn KA, Devesa SS, Freedman ND, Anderson WF. Sex disparities in cancer mortality and survival. *Cancer Epidemiol Biomarkers Prev* 2011; 20: 1629–37. [PubMed: 21750167]
34. Wingard DL. The sex differential in morbidity, mortality, and lifestyle. *Annu Rev Public Health* 1984; 5: 433–58. [PubMed: 6372818]
35. Hazzard WR, Applebaum-Bowden D. Why women live longer than men: The biologic mechanism of the sex differential in longevity. *Trans Am Clin Climatol Assoc* 1990; 101: 168–88. [PubMed: 2486441]
36. Fritz MA, Speroff L. *Clinical Gynecologic Endocrinology and Infertility*, 8th edn. Lippincott Williams & Wilkins, Philadelphia, 2010.
37. Harte AL, da Silva NF, Miller MA et al. Telomere length attrition, a marker of biological senescence, is inversely correlated with triglycerides and cholesterol in South Asian males with type 2 diabetes mellitus. *Exp Diabetes Res* 2012; 2012: 895185. [PubMed: 22474429]
38. Monickaraj F, Aravind S, Gokulakrishnan K et al. Accelerated aging as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with type 2 diabetes. *Mol Cell Biochem* 2012; 365: 343–50. [PubMed: 22411737]

39. Zee RY, Castonguay AJ, Barton NS, Germer S, Martin M. Mean leukocyte telomere length shortening and type 2 diabetes mellitus: A case-control study. *Transl Res* 2010; 155: 166–9. [PubMed: 20303464]
40. Salpea KD, Humphries SE. Telomere length in atherosclerosis and diabetes. *Atherosclerosis* 2010; 209: 35–8. [PubMed: 20080237]
41. Bayne S, Jones ME, Li H, Pinto AR, Simpson ER, Liu JP. Estrogen deficiency leads to telomerase inhibition, telomere shortening and reduced cell proliferation in the adrenal gland of mice. *Cell Res* 2008; 18: 1141–50. [PubMed: 18936784]
42. Bayne S, Li H, Jones ME et al. Estrogen deficiency reversibly induces telomere shortening in mouse granulosa cells and ovarian aging in vivo. *Protein Cell* 2011; 2: 333–46. [PubMed: 21574023]
43. Aviv A. Telomeres, sex, reactive oxygen species, and human cardiovascular aging. *J Mol Med (Berl)* 2002; 80: 689–95. [PubMed: 12436345]
44. Wong JM, Collins K. Telomere maintenance and disease. *Lancet* 2003; 362: 983–8. [PubMed: 14511933]
45. Lin J, Kroenke CH, Epel E et al. Greater endogenous estrogen exposure is associated with longer telomeres in postmenopausal women at risk for cognitive decline. *Brain Res* 2011; 1379: 224–31. [PubMed: 20965155]
46. Bekaert S, Van Pottelbergh I, De Meyer T et al. Telomere length versus hormonal and bone mineral status in healthy elderly men. *Mech Ageing Dev* 2005; 126: 1115–22. [PubMed: 15967485]
47. Patel SM, Ratcliffe SJ, Reilly MP et al. Higher serum testosterone concentration in older women is associated with insulin resistance, metabolic syndrome, and cardio-vascular disease. *J Clin Endocrinol Metab* 2009; 94: 4776–84. [PubMed: 19846742]
48. Sutton-Tyrrell K, Wildman RP, Matthews KA et al. Sex-hormone-binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN). *Circulation* 2005; 111: 1242–9. [PubMed: 15769764]
49. Irvine RA, Yu MC, Ross RK, Coetzee GA. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. *Cancer Res* 1995; 55: 1937–40. [PubMed: 7728763]
50. Westberg L, Baghaei F, Rosmond R et al. Polymorphisms of the androgen receptor gene and the estrogen receptor beta gene are associated with androgen levels in women. *J Clin Endocrinol Metab* 2001; 86: 2562–8. [PubMed: 11397855]

Highlights

- This study elucidates the potential roles of sex hormones in biological aging, and identified that total and free testosterone levels were inversely associated with telomere length in Asian/Pacific Islander women but not in Black and Hispanic women.
- The findings of the present study suggest that Asian/Pacific Islander women may be susceptible to the potential detrimental effects of high testosterone levels on biologic aging.

Figure 1. Cubic spline models of the association between free testosterone concentrations and leukocyte telomere length by race/ethnicity.

Table 1

Baseline characteristics of 1857 postmenopausal women by race/ethnicity

	Race/ethnicity			P-value [*]
	Black (n = 1124)	Hispanic (n = 444)	Asian/Pacific Islander (n = 289)	
Age (years)	60.9 ± 6.7	60.2 ± 6.8	63.6 ± 7.8	<0.001
Smoker (%)				<0.001
Never	49.2	68.6	71.2	
Former	38.9	26.8	25.0	
Current	11.9	4.6	3.8	
Alcohol intake (%)				<0.001
Never	16.9	22.5	40.1	
Former	31.4	24.1	21.5	
Current	51.8	53.4	38.4	
Physical activity (MET-h/week)	6.0 [0.8–15.0]	6.8 [1.2–15.2]	8.6 [3.0–18.4]	0.001
BMI (kg/m ²)	30.9 ± 7.0	28.9 ± 5.7	24.9 ± 4.6	<0.001
Lifetime estrogen exposure [‡] (years)	33.9 ± 7.4	35.2 ± 6.3	35.9 ± 6.3	<0.001
Age at menarche (years)	12.6 ± 1.6	12.6 ± 1.6	12.7 ± 1.6	0.545
Age at menopause (years)	46.5 ± 7.3	47.8 ± 6.3	48.6 ± 6.2	<0.001
Hormone replacement therapy (%)				<0.001
Never	56.4	48.0	33.7	
Former	12.5	10.1	15.3	
Current	31.1	41.9	51.0	
Biomarkers [‡]				
Free estradiol (pg/mL)	0.29 [0.15–0.46]	0.26 [0.14–0.43]	0.20 [0.08–0.39]	<0.001
Total estradiol (pg/mL)	21.4 [12.0–38.9]	19.3 [10.5–45.6]	16.6 [6.6–38.1]	0.001
Free testosterone (ng/dL)	0.095 [0.036–0.212]	0.076 [0.026–0.162]	0.061 [0.021–0.135]	<0.001
Total testosterone (ng/dL)	12.2 [5.3–22.5]	10.0 [4.4–19.0]	8.4 [2.8–16.4]	<0.001
SHBG (nmol/L)	59.1 [37.7–99.1]	64.5 [37.6–121.4]	67.5 [42.7–116.1]	0.019
Leukocyte telomere length (kb)	4.13 [3.20–5.08]	4.20 [3.37–5.24]	3.78 [2.96–4.72]	<0.001
hsCRP (mg/L)	3.00 [1.22–6.65]	2.99 [1.57–5.63]	0.92 [0.39–2.18]	<0.001
IL-6 (pg/mL)	2.19 [1.31–4.24]	2.08 [1.31–3.59]	1.29 [0.84–2.31]	<0.001

Race/ethnicity			
	Black (n = 1124)	Hispanic (n = 444)	Asian/Pacific Islander (n = 289)
TNF- α (pg/mL)	2290 [1880–2770]	2430 [1950–2890]	2190 [1820–2590]
			<i>P</i> -value*
			<0.001

Unless indicated otherwise data are given as the mean SD or as the median [interquartile range].

* *P*-values were obtained from Chi-squared tests for categorical variables, from ANOVA for normally distributed continuous variables, and from Kruskal–Wallis tests for continuous variables that were not normally distributed.

[†] Calculated as the duration between menarche and menopause.

[‡] The SI conversion factors are as follows: for estradiol, multiply concentrations in pg/mL by 3.67 to obtain concentrations in pmol/L; for testosterone, multiply concentrations in ng/dL by 0.0347 to obtain concentrations in nmol/L.

MET, metabolic equivalent; BMI, body mass index; SHBG, sex hormone-binding globulin; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α .

Table 2

Leukocyte telomere length according to serum estradiol concentrations and race/ethnicity

Model	Estradiol				P-trend	Change of TL associated with doubling of concentration
	Q1	Q2	Q3	Q4		
Free estradiol						
Pooled						
Median estradiol (pg/mL)	0.07	0.20	0.34	0.61		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	14 (-179, 207)	-123 (-316, 71)	20 (-177, 216)	0.99	-23 (-72, 27)
Multivariable	0 (Reference)	149 (-80, 378)	109 (-130, 347)	223 (-34, 480)	0.14	21 (-46, 89)
Blacks						
Median estradiol (pg/mL)	0.09	0.22	0.36	0.62		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	-64 (-306, 179)	-139 (-382, 104)	-27 (-273, 219)	0.85	-35 (-101, 30)
Multivariable	0 (Reference)	-4 (-290, 282)	76 (-224, 376)	97 (-224, 417)	0.48	9 (-79, 96)
Hispanics						
Median estradiol (pg/mL)	0.06	0.20	0.34	0.62		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	99 (-319, 518)	34 (-389, 458)	103 (-324, 529)	0.72	31 (-73, 135)
Multivariable	0 (Reference)	356 (-158, 870)	223 (-303, 749)	359 (-214, 931)	0.36	79 (-72, 230)
Asians/Pacific Islanders						
Median estradiol (pg/mL)	0.04	0.14	0.28	0.56		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	-232 (-719, 254)	-307 (-791, 178)	-385 (-874, 103)	0.15	-74 (-185, 37)
Multivariable	0 (Reference)	-212 (-803, 380)	-110 (-712, 491)	-167 (-832, 498)	0.77	6 (-147, 158)
Total estradiol						
Pooled						
Median estradiol (pg/mL)	6.3	15.3	27.4	59.1		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	-70 (-264, 123)	-75 (-267, 118)	-24 (-220, 172)	0.99	-17 (-64, 30)
Multivariable	0 (Reference)	-26 (-255, 203)	134 (-112, 381)	161 (-112, 435)	0.19	20 (-46, 87)

Model	Estradiol				P-trend	Change of TL associated with doubling of concentration
	Q1	Q2	Q3	Q4		
Blacks						
Median estradiol (pg/mL)	7.5	16.3	27.5	58.6		
Telomere length (bp) [*]						
Age-adjusted	0 (Reference)	51 (−191, 293)	−60 (−301, 181)	−11 (−257, 236)	0.78	−34 (−97, 29)
Multivariable	0 (Reference)	114 (−171, 400)	166 (−143, 474)	212 (−123, 547)	0.28	7 (−80, 94)
Hispanics						
Median estradiol (pg/mL)	6.3	14.3	28.9	67.6		
Telomere length (bp) [*]						
Age-adjusted	0 (Reference)	148 (−275, 571)	48 (−374, 469)	144 (−282, 570)	0.66	28 (−69, 125)
Multivariable	0 (Reference)	166 (−355, 687)	−7 (−557, 544)	320 (−299, 938)	0.29	72 (−78, 222)
Asians/Pacific Islanders						
Median estradiol (pg/mL)	2.5	11.9	24.3	54.5		
Telomere length (bp) [*]						
Age-adjusted	0 (Reference)	−178 (−663, 306)	−291 (−777, 194)	−89 (−574, 396)	0.88	−51 (−154, 53)
Multivariable	0 (Reference)	−122 (−723, 478)	29 (−614, 671)	138 (−580, 856)	0.61	11 (−140, 163)

* Data show adjusted differences in telomere length relative to the reference group, with 95% confidence intervals in parentheses. Multivariable models were adjusted for age, race/ethnicity, hormone-replacement therapy, years between menarche and menopause, case/control, body mass index, physical exercise, total energy intake, smoking, alcohol consumption, sex hormone-binding globulin, and testosterone.

Q1–Q4, Quartiles 1–4, respectively.

Table 3

Leukocyte telomere length according to serum testosterone concentrations and race/ethnicity

Model	Testosterone				P_{trend}	Change of TL associated with doubling of concentration
	Q1	Q2	Q3	Q4		
Free testosterone						
Pooled						
Median testosterone (ng/dL)	0.015	0.053	0.124	0.304		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	-49 (-242, 143)	-138 (-331, 54)	-199 (-391, -7)	0.04	-39 (-78, -1)
Multivariable	0 (Reference)	-37 (-260, 185)	-158 (-395, 79)	-269 (-547, 8)	0.04	-56 (-114, 1)
Blacks						
Median testosterone (ng/dL)	0.017	0.063	0.142	0.329		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	-187 (-428, 54)	-240 (-481, 1)	-88 (-329, 153)	0.85	-19 (-68, 29)
Multivariable	0 (Reference)	-138 (-417, 141)	-179 (-479, 121)	-14 (-359, 331)	0.71	-17 (-90, 55)
Hispanics						
Median testosterone (ng/dL)	0.015	0.045	0.115	0.270		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	241 (-177, 660)	54 (-367, 476)	14 (-406, 434)	0.64	-23 (-110, 64)
Multivariable	0 (Reference)	396 (-97, 889)	182 (-334, 698)	-2 (-597, 594)	0.52	-19 (-148, 110)
Asians/Pacific Islanders						
Median testosterone (ng/dL)	0.012	0.034	0.095	0.241		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	201 (-279, 682)	-18 (-498, 462)	-541 (-1027, -55)	0.003 [†]	-150 (-245, -55)
Multivariable	0 (Reference)	415 (-159, 989)	-180 (-789, 430)	-785 (-1522, -48)	0.003 [†]	-202 (-353, -51)
Total testosterone						
Pooled						
Median testosterone (ng/dL)	1.9	7.6	15.3	29.9		
Telomere length (bp)*						
Age-adjusted	0 (Reference)	-41 (-233, 151)	-220 (-411, -28)	-198 (-391, -6)	0.02	-54 (-101, -7)

Model	Testosterone				P_{trend}	Change of TL associated with doubling of concentration
	Q1	Q2	Q3	Q4		
Multivariable	0 (Reference)	-17 (-238, 204)	-233 (-457, -9)	-182 (-420, 56)	0.08	-57 (-115, 1)
Blacks						
Median testosterone (ng/dL)	1.9	8.5	16.6	32.1		
Telomere length (bp) [*]						
Age-adjusted	0 (Reference)	-123 (-365, 119)	-277 (-518, -37)	-49 (-290, 193)	0.78	-30 (-89, 29)
Multivariable	0 (Reference)	-148 (-426, 129)	-209 (-492, 73)	-25 (-325, 274)	0.93	-18 (-91, 55)
Hispanics						
Median testosterone (ng/dL)	1.9	7.0	13.1	28.2		
Telomere length (bp) [*]						
Age-adjusted	0 (Reference)	193 (-228, 615)	9 (-407, 426)	-110 (-531, 311)	0.35	-30 (-135, 75)
Multivariable	0 (Reference)	324 (-170, 817)	68 (-444, 581)	-22 (-540, 497)	0.55	-21 (-150, 109)
Asians/Pacific Islanders						
Median testosterone (ng/dL)	1.9	5.5	11.6	22.4		
Telomere length (bp) [*]						
Age-adjusted	0 (Reference)	5 (-476, 486)	-283 (-763, 197)	-681 (-1161, -202)	0.001 [†]	-206 (-323, -88)
Multivariable	0 (Reference)	178 (-397, 753)	-190 (-775, 395)	-660 (-1274, -45)	0.008 [†]	-203 (-355, -50)

^{*} Data show adjusted differences in telomere length relative to the reference group, with 95% confidence intervals in parentheses.

[†] False discovery rate < 0.05.

Multivariable models were adjusted for age, race/ethnicity, hormone-replacement therapy, years between menarche and menopause, case/control, body mass index, physical exercise, total energy intake, smoking, alcohol consumption, sex hormone-binding globulin, and estradiol. Q1–Q4: Quartiles 1–4, respectively.