Relations of Sex Hormone Levels to Leukocyte Telomere Length in Black, Hispanic, and Asian/Pacific Islander Postmenopausal Women

Running title: Sex Hormone Levels and Telomere Length

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ABSTRACT

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

Methods: We conducted a cross-sectional study of 1124 black, 444 Hispanic, and 289 Asian/Pacific Islander women in the Women's Health Initiative Observational Cohort. Concentrations of estradiol and testosterone were measured using electrochemiluminescence immunoassays. TL was measured using quantitative PCR.

Results: Women included in the study were 50 to 79 years of age. Levels of estradiol were not significantly associated with TL in this sample of women. The associations between total and free testosterone and TL differed by race/ethnicity (*P* for interaction=0.03 for total testosterone and 0.05 for free testosterone). Total and free testosterone concentrations were not associated with TL in black and Hispanic women, whereas in Asian/Pacific Islanders, their concentrations were inversely associated with TL (*P*-trend=0.003 for both). These associations appeared robust in multiple subgroup analysis and multivariable models adjusted for potential confounding factors. In Asian/Pacific Islanders, doubling of serum free testosterone concentration was associated with 202 bp shorter TL (95% CI, 51 to 353 bp), and doubling of total testosterone concentration was associated with 203 bp shorter TL (95% CI, 50 to 355 bp).

Conclusions: Serum concentration of estradiol was not associated with leukocyte TL in this large sample of postmenopausal women. Total and free testosterone levels 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 relations.

The significant findings of the study: 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 study adds: The findings of this study suggest that Asian/Pacific Islander women may be susceptible to the potential detrimental effects of high testosterone level on biologic aging.

Key Words: Sex Steroid Hormones, Estradiol, Testosterone, Telomere Length, Aging

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Introduction

Telomeres are DNA-protein complexes that prevent genomic loss during chromosome replications. [1-3] Aging has been linked to progressive shortening of the telomere length (TL), which is estimated to be at a rate of 20 to 60 base pairs (bp) per year. [4,5] Given that female life expectancy is on average 80.2 years, compared to a male life expectancy of 75.1 years in the United States, [6] it is not surprising that women have longer TL than age-matched men. [7,8] Despite these observations, the mechanisms underlying the relation 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 influence the enzyme telomerase, which is responsible for elongating telomeres.[10] Both estradiol and testosterone's active metabolite dihydrotestosterone, 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 levels 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 might be responsible for the increase in telomere and thus TL, the mechanism of the longer TL with danazol is not clear, as danazol typically decreases circulating estradiol levels.

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 participated in the Women's Health Initiative Observational Study (WHI-OS).

Methods

Study Subjects

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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. The WHI has two major components: a partial factorial randomized Clinical Trial (CT) and an Observational Study (OS); both were conducted at 40 Clinical Centers nationwide. The OS examines the relationship between lifestyle, environmental, medical and molecular risk factors and specific measures of health or disease outcomes. This component involves tracking the medical history and health habits of 93,676 women not participating in the CT. The current study reported 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.[15] Briefly, incident diabetes cases were selected among women without prior history of diabetes or cardiovascular diseases, and for each incident case, up to 2 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. In the current 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

The measurements of circulating estradiol, testosterone, and SHBG have been described in Chen et al. [15] In brief, fasting serum specimens collected at baseline from each participant, and serum concentrations of estradiol, testosterone, and SHBG were measured by electrochemiluminescence immunoassays on the Elecsys 2010 immunoanalyzer (Roche Diagnostics, Indianapolis, IN, USA). Inter-assay imprecision (expressed as %CV) was 12.4% for estradiol, 10.3% for testosterone, and

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5.4% for SHBG. Free estradiol and free testosterone were calculated using the methods described by Vermuelen et al. [16] and Sodergard et al., [17] previously validated in postmenopausal women.[16-20]

Measurement of telomere length

The measurement of TL has been described elsewhere. [15] In brief, we adopted a qPCR method first proposed by O'Callaghan et al. [21] using a high-throughput 384-well format Applied Biosystems' 7900HT PCR System (Applied Biosystems by Life Technologies Corporation, Carlsbad, CA). All samples for TEL and 36B4 reactions, as well as standard curves, were performed in duplicate on the same plates. As part of routine quality control, 10% of the samples were blind duplicate samples. The overall intra-plate coefficient of variation (CV) was 0.8% and the inter-plate CV was 5.7%.

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", and "current smoker". Levels of alcohol intake and total energy intake were also calculated from the food frequency questionnaire (The FFQ was based on instruments used in the WHI feasibility studies [22,23] and the original National Cancer Institute/Block FFQ [24]). 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 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/wk) 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 as "never-user", "former user", and "current user". Tumor necrosis factor ± receptor 2 (TNF-±-R2) was measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota). Interleukin 6 (IL-6) was measured by an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems). High-sensitivity C-reactive protein (hsCRP) was measured on Roche Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, Indiana) using an immunoturbidimetric assay with reagents and calibrators (Denka Seiken Co Ltd, Niigata, Japan).

Statistical Analysis

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Baseline characteristics were summarized according to race/ethnicity. Categorical variables were shown as percentages; normal-distributed continuous variables were expressed as the mean (standard deviation); non-normal-distributed continuous variables were shown as median (interquartile range). *P* values for differences among ethnic groups were obtained from chi-square tests for categorical variables, from ANOVA for normal-distributed continuous variables, and from Kruskal-Wallis tests for non-normal 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 additionally adjusted for race/ethnicity (Black, Hispanic, or Asian/Pacific Islander), hormone replacement therapy (HRT) use (never, former, or current user), years between menarche and menopause (years, continuous), BMI (kg/m^2, 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/wk), daily energy intake (kcal, continuous), and serum SHBG concentration (nmol/L, continuous). Additionally, 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 hormones 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 IL6, hsCRP, and TNF-±-R2. In the second sensitivity analysis, we stratified the analyses by BMI with a cutoff point at 25 kg/m^2. To explore potential non-linear relation between sex hormone concentrations and TL, we used restricted cubic spline models. We

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conducted all statistical analyses using SAS (version 9.3; SAS institute, Cary, NC). All *P* values were two tailed, and false discovery rate (FDR) were adopted to control the effects of multi-testing.

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 old. On average, the Asian/Pacific Islanders had a lower BMI, 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 concentration of SHBG, and lower concentrations of inflammation markers (hsCRP, IL6, and TNF-±) 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*-trend=0.14 for free estradiol and P-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 continuous measure of estradiol were in the same direction as the pooled analysis. We did not observe significant interaction between estradiol concentration and race/ethnicity (*P*=0.48 for free estradiol and 0.63 for total estradiol).

Total and free testosterone concentrations were modestly associated with TL in the pooled analysis (*P*-trend=0.04 for free testosterone and *P*-trend=0.02 for total testosterone), where the mean TL appeared shorter in higher testosterone concentrations 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*-trend=0.003, 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 785 bp shorter TL (95% CI, 48

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to 1522 bp) than women in the lowest quartile (median 0.012 ng/dL). In Asian/Pacific Islanders, doubling of serum free testosterone concentration was associated with 202 bp shorter TL (95% CI, 51 to 353 bp). No associations between free testosterone concentration and TL were observed in black and Hispanic women. In sensitivity analyses, neither additional adjustment of 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*-trend=0.08). In cubic spline models (Figure 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 significant inverse association between total testosterone concentration and TL in Asian/Pacific Islander women (*P*-trend=0.008, FDR<0.05). In multivariable-adjusted models, Asian/Pacific Islander women with highest serum concentration of total testosterone (median, 22.4 ng/dL) had 600 bp shorter TL (95% CI, 45 to 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 203 bp shorter TL (95% CI, 50 to 355 bp). No association between total testosterone concentration and TL were observed in Black and Hispanic women. In the sensitivity analyses, neither additional adjustment of 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 not longer significant (*P*-trend=0.07).

DISCUSSION

Overall, we did not find significant associations of estradiol levels with leukocyte TL in these women. However, in Asian/Pacific Islander women, total and free testosterone 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, doubling of free or total testosterone concentration was associated with approximately 9.2 times of this average annual attrition. Among black and Hispanic women, however, no associations were observed between testosterone levels and TL. When we restricted our analyses to women who had never used HRT,

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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.[15,25-29] 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 levels.

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, [30,31] which has long been attributed to sex-differences in social or lifestyle factors (e.g. cigarette smoking, alcohol consumption, job stress, and medical services utilization). [32] Recent work has also identified altered serum lipid levels by sex steroid hormones levels as potential biological mechanisms responsible for the sex-difference in cardiovascular disease. [26,33] For women after the menopausal transition, not only does endogenous estrogen plummet, 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 hormone replacement therapy. [34] Although TL is a well-known indicator of biologic aging and senescence and has been associated with chronic diseases, [35-38] few studies have investigated the relationship between sex steroid hormones and TL.

In animal studies, estrogen deficiency has been associated with telomere shortening. [39,40] Estrogen was suggested to diminish oxidative stress, [41] which is fundamental to biologic aging and can accelerate telomere shortening and stimulate the transcription of the gene encoding telomerase. [42] In a human study examining the relationship between estradiol levels 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. [43] In the current study, we observed longer TL in women with higher concentrations of free or total estradiol, albeit these associated were not statistically significant at the conventional ±=0.05 level. One limitation of our study is that we could not accurately evaluate and control the lifetime exposure of sex hormones. However, we did assess

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the difference between age at menopause and age at menarche as a surrogate to adjust for potential confounding of lifetime exposure of estrogen.

Previous studies investigating the association between androgen levels and TL are scarce. One study in healthy elderly men in Belgium reported no statistically significant association between age-corrected testosterone concentrations and TL. [44] In this 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 levels have been associated with insulin resistance, metabolic syndrome, and cardiovascular disease in elderly women [45] and also associated with higher levels of cardiovascular risk factors in a multiethnic women population. [46] Interestingly, our data suggest that the Asian/Pacific Islander population generally had elevated markers typically associated with good health, including lower BMI, lower levels of inflammation, higher SHBG concentrations, and resultant lower free testosterone levels compared to black and Hispanic women.

It is interesting to note that Asian/Pacific Islander women had the lowest testosterone levels 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 which can also contribute to racial heterogeneity.

Another possible mechanism for the ethnic discrepancy is related to 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, [47] and fewer CAG repeats in the AR gene result in higher transcriptional activity and higher levels of serum androgens. [48] 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

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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 this study that need to be kept in mind when interpreting findings in the current study. First, this is cross-sectional 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 levels and TL are clearly warranted to further establish the causal relationship and investigate the role of sex hormones in affecting changes in TL among postmenopausal women. Second, although the analyses have controlled for confounding of the association to the extent possible by including potential predictors of sex hormone levels 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 current study did not include men and white women. As the current study shows racial heterogeneity of the association between sex hormone and TL, future studies with a broader population coverage are also warranted to investigate the association in other populations. Lastly, there were likely some measurement errors associated with both plasma levels of sex steroids and measures of LTL. Measurement errors, when they are not dependent, are likely to bias the parameter of interest toward the null. In this 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 the samples were blind duplicate samples. The overall intraplate coefficient of variation was 0.8%, and the interplate coefficient of variation of the telomere assays was 5.7%.[15]

In conclusion, serum concentration of estradiol was not significantly associated with leukocyte TL in the multiethnic postmenopausal women population. However, higher total and free testosterone concentrations appeared 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 level on biologic aging, although

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

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Disclosure

All authors declare no conflict of interest.

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

Table 1. Baseline characteristics of 1857 postmenopausal women by race/ethnicity.

	Race/ethnicity			P^a
	Black (n=1124)	Hispanic (n=444)	Asian/Pacific Islander (n=289)	
Age, mean (SD), y	60.9 (6.7)	60.2 (6.8)	63.6 (7.8)	<0.001
Smoking, %				<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, median (IQR), MET-h/wk	6.0 (0.8-15.0)	6.8 (1.2-15.2)	8.6 (3.0-18.4)	0.001
BMI, mean (SD), kg/m^2	30.9 (7.0)	28.9 (5.7)	24.9 (4.6)	<0.001
Lifetime estrogen exposure[b], mean (SD), year	33.9 (7.4)	35.2 (6.3)	35.9 (6.3)	<0.001
Age at menarche, mean (SD), y	12.6 (1.6)	12.6 (1.6)	12.7 (1.6)	0.545
Age at menopause, mean (SD), y	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, median (IQR)[c]				
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
IL6, pg/mL	2.19 (1.31-4.24)	2.08 (1.31-3.59)	1.29 (0.84-2.31)	<0.001
TNF-±, pg/mL	2290 (1880-2770)	2430 (1950-2890)	2190 (1820-2590)	<0.001

[a] *P* values were obtained from chi-square tests for categorical variables, from ANOVA for normal-distributed continuous variables, and from Kruskal-Wallis tests for non-normal-distributed continuous variables.

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

[c] SI conversion factors: estradiol (pg/mL) × 3.67 = (pmol/L); testosterone (ng/dL) × 0.0347 = (nmol/L).

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Table 2. Leukocyte telomere length in base pairs according to serum levels of estradiol.

Model	Quartiles of estradiol				*P*-trend	Continuous (per doubling)
	Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Free estradiol						
Pooled						
Median, pg/mL	0.07	0.20	0.34	0.61		
Age-adjusted	0 (Reference)	14 (-179 to 207)	-123 (-316 to 71)	20 (-177 to 216)	0.99	-23 (-72 to 27)
Multivariable	0 (Reference)	149 (-80 to 378)	109 (-130 to 347)	223 (-34 to 480)	0.14	21 (-46 to 89)
Blacks						
Median, pg/mL	0.09	0.22	0.36	0.62		
Age-adjusted	0 (Reference)	-64 (-306 to 179)	-139 (-382 to 104)	-27 (-273 to 219)	0.85	-35 (-101 to 30)
Multivariable	0 (Reference)	-4 (-290 to 282)	76 (-224 to 376)	97 (-224 to 417)	0.48	9 (-79 to 96)
Hispanics						
Median, pg/mL	0.06	0.20	0.34	0.62		
Age-adjusted	0 (Reference)	99 (-319 to 518)	34 (-389 to 458)	103 (-324 to 529)	0.72	31 (-73 to 135)
Multivariable	0 (Reference)	356 (-158 to 870)	223 (-303 to 749)	359 (-214 to 931)	0.36	79 (-72 to 230)
Asians/Pacific Islanders						
Median, pg/mL	0.04	0.14	0.28	0.56		
Age-adjusted	0 (Reference)	-232 (-719 to 254)	-307 (-791 to 178)	-385 (-874 to 103)	0.15	-74 (-185 to 37)
Multivariable	0 (Reference)	-212 (-803 to 380)	-110 (-712 to 491)	-167 (-832 to 498)	0.77	6 (-147 to 158)
Total estradiol						
Pooled						
Median, pg/mL	6.3	15.3	27.4	59.1		
Age-adjusted	0 (Reference)	-70 (-264 to 123)	-75 (-267 to 118)	-24 (-220 to 172)	0.99	-17 (-64 to 30)
Multivariable	0 (Reference)	-26 (-255 to 203)	134 (-112 to 381)	161 (-112 to 435)	0.19	20 (-46 to 87)
Blacks						
Median, pg/mL	7.5	16.3	27.5	58.6		
Age-adjusted	0 (Reference)	51 (-191 to 293)	-60 (-301 to 181)	-11 (-257 to 236)	0.78	-34 (-97 to 29)
Multivariable	0 (Reference)	114 (-171 to 400)	166 (-143 to 474)	212 (-123 to 547)	0.28	7 (-80 to 94)
Hispanics						
Median, pg/mL	6.3	14.3	28.9	67.6		
Age-adjusted	0 (Reference)	148 (-275 to 571)	48 (-374 to 469)	144 (-282 to 570)	0.66	28 (-69 to 125)
Multivariable	0 (Reference)	166 (-355 to 687)	-7 (-557 to 544)	320 (-299 to 938)	0.29	72 (-78 to 222)
Asians/Pacific Islanders						
Median, pg/mL	2.5	11.9	24.3	54.5		
Age-adjusted	0 (Reference)	-178 (-663 to 306)	-291 (-777 to 194)	-89 (-574 to 396)	0.88	-51 (-154 to 53)
Multivariable	0 (Reference)	-122 (-723 to 478)	29 (-614 to 671)	138 (-580 to 856)	0.61	11 (-140 to 163)

Numbers are adjusted difference of telomere length (base pairs) with the reference group (95% CI). * False discovery rate < 0.05.

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Multivariable: adjusted for age, race/ethnicity, HRT, years between menarche and menopause, case/control, BMI, physical exercise, total energy intake, smoking, alcohol consumption, SHBG, and testosterone.

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Table 3. Leukocyte telomere length in base pairs according to serum levels of testosterone.

Model	Quartiles of testosterone				*P*-trend	Continuous (per doubling)
	Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Free testosterone						
Pooled						
Median, ng/dL	0.015	0.053	0.124	0.304		
Age-adjusted	0 (Reference)	-49 (-242 to 143)	-138 (-331 to 54)	-199 (-391 to -7)	0.04	-39 (-78 to -1)
Multivariable	0 (Reference)	-37 (-260 to 185)	-158 (-395 to 79)	-269 (-547 to 8)	0.04	-56 (-114 to 1)
Blacks						
Median, ng/dL	0.017	0.063	0.142	0.329		
Age-adjusted	0 (Reference)	-187 (-428 to 54)	-240 (-481 to 1)	-88 (-329 to 153)	0.85	-19 (-68 to 29)
Multivariable	0 (Reference)	-138 (-417 to 141)	-179 (-479 to 121)	-14 (-359 to 331)	0.71	-17 (-90 to 55)
Hispanics						
Median, ng/dL	0.015	0.045	0.115	0.270		
Age-adjusted	0 (Reference)	241 (-177 to 660)	54 (-367 to 476)	14 (-406 to 434)	0.64	-23 (-110 to 64)
Multivariable	0 (Reference)	396 (-97 to 889)	182 (-334 to 698)	-2 (-597 to 594)	0.52	-19 (-148 to 110)
Asians/Pacific Islanders						
Median, ng/dL	0.012	0.034	0.095	0.241		
Age-adjusted	0 (Reference)	201 (-279 to 682)	-18 (-498 to 462)	-541 (-1027 to -55)	0.003*	-150 (-245 to -55)
Multivariable	0 (Reference)	415 (-159 to 989)	-180 (-789 to 430)	-785 (-1522 to -48)	0.003*	-202 (-353 to -51)
Total testosterone						
Pooled						
Median, ng/dL	1.9	7.6	15.3	29.9		
Age-adjusted	0 (Reference)	-41 (-233 to 151)	-220 (-411 to -28)	-198 (-391 to -6)	0.02	-54 (-101 to -7)
Multivariable	0 (Reference)	-17 (-238 to 204)	-233 (-457 to -9)	-182 (-420 to 56)	0.08	-57 (-115 to 1)
Blacks						
Median, ng/dL	1.9	8.5	16.6	32.1		
Age-adjusted	0 (Reference)	-123 (-365 to 119)	-277 (-518 to -37)	-49 (-290 to 193)	0.78	-30 (-89 to 29)
Multivariable	0 (Reference)	-148 (-426 to 129)	-209 (-492 to 73)	-25 (-325 to 274)	0.93	-18 (-91 to 55)
Hispanics						
Median, ng/dL	1.9	7.0	13.1	28.2		
Age-adjusted	0 (Reference)	193 (-228 to 615)	9 (-407 to 426)	-110 (-531 to 311)	0.35	-30 (-135 to 75)
Multivariable	0 (Reference)	324 (-170 to 817)	68 (-444 to 581)	-22 (-540 to 497)	0.55	-21 (-150 to 109)
Asians/Pacific Islanders						
Median, ng/dL	1.9	5.5	11.6	22.4		
Age-adjusted	0 (Reference)	5 (-476 to 486)	-283 (-763 to 197)	-681 (-1161 to -202)	0.001*	-206 (-323 to -88)
Multivariable	0 (Reference)	178 (-397 to 753)	-190 (-775 to 395)	-660 (-1274 to -45)	0.008*	-203 (-355 to -50)

Numbers are adjusted difference of telomere length (base pairs) with the reference group (95% CI). * False discovery rate < 0.05.

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Multivariable: adjusted for age, race/ethnicity, HRT, years between menarche and menopause, case/control, BMI, physical exercise, total energy intake, smoking, alcohol consumption, SHBG, and estradiol.

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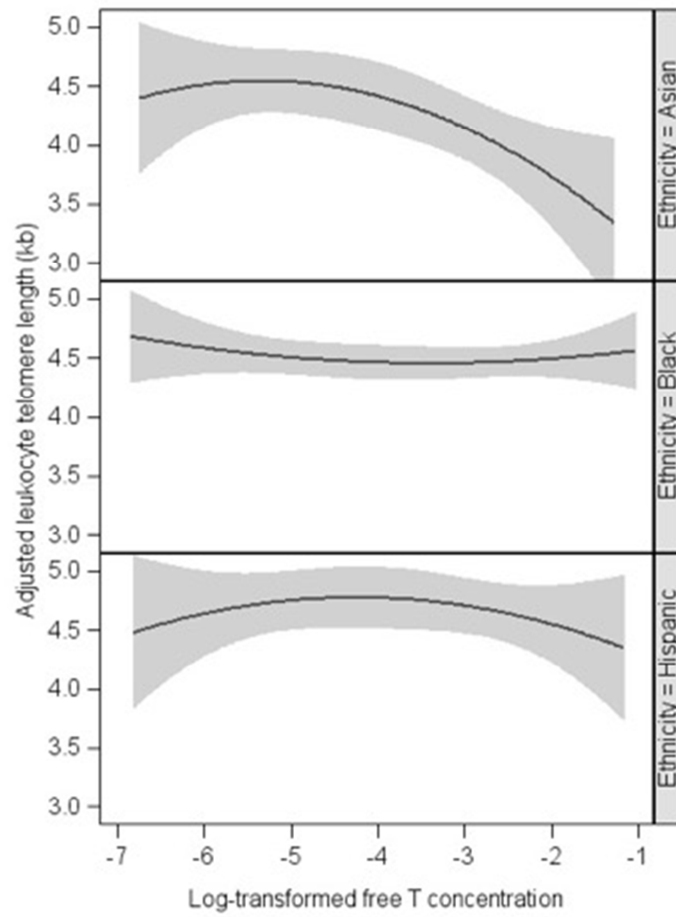

Figure 1. Cubic spline models of the association between free testosterone concentration and leukocyte telomere length by race/ethnicity

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