Dynamics of serum testosterone and biological aging in men: insights from Chinese, American, and British populations

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Summary

Background Biological age (BA) is considered a better predictor of aging than chronological age (CA). BA acceleration was defined as the disparity between BA and CA. However, there has been inconclusive evidence on whether BA acceleration might be reversed by increased total testosterone (TT). The goal of this cross-sectional study was to investigate the relationship between TT and BA acceleration.

Methods Klemera and Doubal method biological age (KDM-BA) was employed as an indicator of BA in this study. This study involved participants of three ancestries from five cohorts, including the National Health and Nutrition Examination Survey (NHANES, recruited between 1 January 2011 and 31 December 2016), West China Health and Aging Trend (WCHAT, recruited between 1 January 2018 and 31 December 2018), West China Natural Population Cohort Study (WCNPCS, recruited between 1 January 2019 and 31 December 2020), UK Biobank (UKB, recruited between 3 March 2016 and 1 October 2020), and the West China Hospital robot-assisted radical prostatectomy (RARP) cohort (recruited between 1 January 2017 and 31 December 2023). Association between serum TT and BA acceleration were assessed by multivariable linear regression in NHANES, WCHAT, and WCNPCS. Change-to-change analysis was performed to evaluate the association between the dynamic of serum TT and BA acceleration in the UKB. 38 prostate cancer patients underwent adjuvant androgen deprivation therapy (ADT) were included to exploring whether ADT deteriorates BA acceleration.

Findings A total of 6976, 2080, 2133, and 6058 participants from NHANES, WCHAT, WCNPCS, and UKB, respectively, were included in the study. Higher serum TT was consistently associated with attenuated BA acceleration across NHANES, WCHAT, and WCNPCS. In UKB, Box–Cox with negatives allowed (BCN)-transformed relative TT change was significantly associated with decreased BCN-transformed BA acceleration change (β: −0.047, 95% CI: −0.057

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to –0.038). The results were remained robust in the stratification analysis, restricted cubic spline regression (RCS), and sensitivity analysis. The pre-and-post ADT paired test further indicated the deprivation of TT accelerated biological aging of prostate cancer patients.

Interpretation This study reveals that higher serum TT was associated with reduced biological aging. Men with increased serum TT on repeat testing demonstrated reduced BA acceleration. Comparison of men with prostate cancer revealed advanced BA acceleration after receiving ADT. This study suggests TT may be a reasonable indicator for biological aging.

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Keywords: Biological aging; Testosterone levels; Testosterone deficiency; NHANES; UK biobank

Research in context

Evidence before this study

We searched PubMed for studies published from database inception to April 8, 2024, using the combination of terms (biological age) AND (((testosterone) OR (TT)) OR (TD)), without language restrictions. Individuals experiencing biological age acceleration may not immediately exhibit aging-related phenotypes but have a higher risk of developing aging-related diseases and mortality. However, there is a significant lack of multi-ethnic and multi-center data to explore the impact of testosterone (TT) on biological age acceleration, as well as on the association of longitudinal changes in testosterone levels on aging in real-world settings, suggesting a significant knowledge gap regarding the association of testosterone levels, dynamic changes in testosterone, and androgen deprivation therapy in prostate cancer patients on biological age acceleration.

Added value of this study

Utilizing data from five cohorts (NHANES, WCHAT, WCNPCS, UKB, and West China Hospital RARP cohort) across three countries, we found that higher or increasing serum TT was consistently associated with decreased BA acceleration across population from different countries. Our research establish a clear link between testosterone levels and biological aging, providing robust, multi-source evidence that supports testosterone's anti-aging properties and reveal the potential risks of androgen deprivation therapy (ADT) in accelerating biological aging for prostate cancer patients. These findings underscore the importance of considering the broader implications of testosterone management in clinical settings, particularly in aging-related interventions.

Implications of all the available evidence

This study, along with existing evidence suggests a need to monitoring the aging related diseases when administrating ADT in prostate cancer patients. Integrating TT assessment and management into aging-related healthcare guidelines could enhance the effectiveness of interventions designed to promote healthy aging and reduce the risk of age-related diseases. Future research should focus on exploring the long-term effects of TT modulation on aging processes through large-scale, longitudinal studies and clinical trials.

Introduction

Aging is a complex process involving various physiological systems and timescales, resulting in the gradual maturation and eventual decline of living organisms.[1] Research suggests that Klemera and Doubal method biological age (KDM-BA) may better predict an individual's "true life expectancy" than chronological age, as it accounts for genetic and nutritional factors in addition to the passage of time.[2] Previous studies have shown that acceleration in KDM-BA is linked to disability, functional impairment, and increased mortality risk.[3]

Serum total testosterone (TT) levels decrease with age due to reduced Leydig cell activity, however, its potential role in mitigating biological aging remains unclear. Previous research has suggested that TT may help preventing various aging-related conditions, including osteoporosis, metabolic syndrome, diabetes, and cardiovascular disease.[4–7] Our previous research along with that of Zhang et al. identified association between TT and aging hallmarks, including klotho and inflammation level.[8,9] Nevertheless, the understanding of the relationship between TT and biological aging, as evaluated through composite biomarkers that integrate clinical indicators from various organs and systems, is still limited.

Therefore, we aimed to explore the association between TT level and TT dynamic in BA acceleration based on data from five distinct cohorts: the National Health

and Nutrition Examination Survey (NHANES), West China Health and Aging Trend (WCHAT), West China Natural Population Cohort Study (WCNPCS), UK Biobank (UKB), and the West China Hospital RARP cohort, using a well performed BA estimated by KDM algorithm with composite clinical biomarkers (Figure S1).

Methods

Study populations

The present study comprised three sets of data and analysis (Figure S1): 3 large databases with single TT measurement (NHANES, WCHAT, and WCNPCS), 1 database with repeated TT measurement (UKB), and prostate cancer patients with BA measurement before and after receiving ADT (West China Hospital RARP cohort).

Specifically, we assessed the association between the natural occurring TT level and BA acceleration in NHANES, WCHAT, and WCNPCS. In UKB, we further investigate the association of individual TT dynamic and BA acceleration. Data from West China Hospital RARP cohort was utilized to assess the effect of androgen deprivation therapy (ADT) on BA acceleration (Figure S1). The basic information regarding these cohorts is described in the Supplementary Materials.

For NHANES, WCNPCS, and WCHAT, we applied same inclusion and exclusion criteria. Specifically, we excluded individuals with missing TT measurements and those lacking biomarker required for the construction of BA. To minimize the impact of outliers on regression estimates, we also excluded outliers in TT measurements and BA acceleration. For the UKB, we included only those individuals who had no missing data for TT and biomarkers used to construct BA at baseline and during the first repeated assessment visit. The flowchart of participants enrollment in these datasets were illustrated in Fig. 1.

For the West China Hospital RARP cohort, we included patients who received ADT after RARP. The BA measured preoperatively was used as the BA before ADT. We collected information on the first set of biomarkers required for BA construction measured during the outpatient visit after ADT initiation and constructed the post-ADT BA. Patients for whom BA could not be determined both before and after ADT were excluded. Due to the significant reduction in TT levels observed in the majority of patients receiving ADT within one month,[10–12] we included only those patients who had received ADT for more than one month prior to the measurement of post-ADT BA. Additionally, to minimize bias introduced by time factors, we restricted the inclusion to individuals whose two BA measurements were taken within a two-year interval. Ultimately, a total of 38 patients were included in the study. To control for biases introduced by RARP, we used propensity score matching (PSM) to match, patients who received RARP alone and had BA measured both pre- and postoperatively with those who received adjuvant ADT after RARP and had similar characteristics. Then BA acceleration pre- and post RARP was compared.

Measurement of biological aging markers

KDM-BA, derived from a series of blood-chemistry associated biomarkers, has gained widespread acceptance as a tool for evaluating an individual's BA.[2] The aging-related biomarkers to construct the KDM-BA was selected based on the Levine's research and the available data from these four cohorts.[13] Overall, ten available clinical biomarkers, including systolic blood pressure, albumin, alkaline phosphatase (ALP), blood urea nitrogen, creatinine, glucose, total cholesterol, lymphocyte percentage, white blood cell counts, and mean cell volume. Additionally, ALP was excluded as a biomarker in the KDM-BA calculation for the WCHAT cohort, since it was not measured in this specific cohort.

KDM-BA acceleration was defined as the disparity between an individual's KDM-BA and CA.[3] A positive value of BA acceleration indicates that the BA is greater than the chronological age, suggesting that the individual is in a state of biological aging. Moreover, the greater the positive value, the more severe the aging relative to the chronological age. Conversely, a negative value of BA acceleration indicates that the BA is less than the chronological age, suggesting that the individual is biologically younger. Furthermore, the more negative the value, the younger the individual is relative to their chronological age. The KDM-BA construction was performed using the R package BioAge (https://github.com/dayoonkwon/BioAge).

As depicted in Figure S2, a strong correlation was observed between Levine's KDM-BA and our modified KDM-BA (Spearman correlation coefficient = 0.9768), even after excluding ALP from the algorithms, the correlation remained robust (Spearman correlation coefficient = 0.9749). The spearman correlation coefficient with 95% CI were computed through nonparametric bootstrapping with 1000 replicates using RVAideMemoire R package (https://CRAN.R-project.org/package=RVAideMemoire). The CI boundaries were derived from the 2.5th and 97.5th percentiles of the bootstrap distribution. The correlation between chronological age, KDM-BA and KDM-BA acceleration in four study population were illustrated in Figure S3. Table S1 and Table S2 demonstrates that KDM-BA acceleration constructed in the present study related to functional test performance, subjective health ratings, and mortality risk. Therefore, our findings indicate that our KDM biological aging measures are valid and comparable to those in Levine's original version.

Measurement of TT

The methods of TT measurements are listed in Table S3. The serum samples were prepared and stored at –80 °C. In NHANES, TT was tested by using liquid

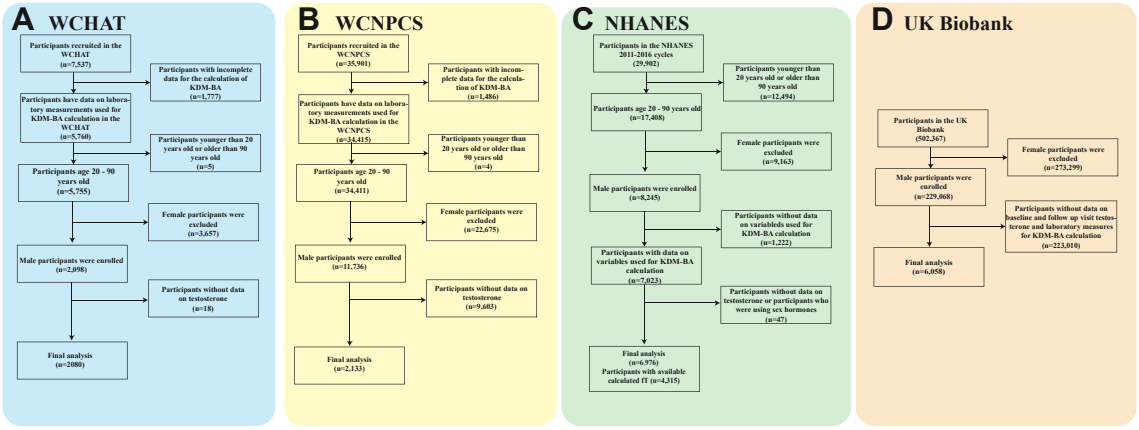

Fig. 1: Flow chart of the study design and participants selection in four cohorts. A: WCHAT; B: NHANES; C: WCNPCS; D: UKB.

chromatography and tandem mass spectrometry,[14,15] whereas for WCNPCS, WCHAT, and UKB,[16,17] it was tested by chemiluminescence immunoassay. In UKB, TT levels were measured repeatedly at the initial assessment visit (2006–2010) and the first repeat assessment visit (2012–2013). Moreover, all blood samples were collected in China in the morning and throughout the day in the UK and U.S. Free testosterone (fT) was calculated according to Vermeulen.[18]

Measurements of covariates

Potential covariates were identified by previous studies and the directed acyclic graph (DAG) was constructed in Figure S4.[14,19,20] The minimal sufficient set of covariates were selected based on DAG and backdoor criteria, including age, educational level, body mass index (BMI), smoking status (current/former/never), alcohol intake (yes/no), venipuncture time, marital status (married or separated/divorced/widowed or never), and physical activity. Of note, race was categorized as white, black, Asian, and other in the NHANES and was as white, Asian or Asian British, Black or Black British, Chinese, other ethnic group, and mixed ethnicity in the UKB. Venipuncture time was defined as morning/afternoon/evening in the NHANES database.[14] For WCHAT, nationality was categorized as Han, Zang, or Qiang, and others. The definition of physical activity in WCNPCS was categorized as 0–2 times per week, 3–5 times per week, and more than 6 times per week, whereas those in NHANES was collected according to physical activity questionnaire (mild/moderate/vigorous). In WCHAT, physical activity was evaluated by using the China Leisure Time Physical Activity Questionnaire (CLTPAQ). The metabolic equivalents of task (MET) were calculated and categorized as tertiles. The lowest, middle, and highest tertiles were defined as mild, moderate, and vigorous, respectively. Chronic diseases were also considered as covariate if available, including diabetes, cardiovascular disease, and hypertension.

Statistical analyses

Assessment of linear regression assumption and sample size estimation

Continuous variables were expressed as mean and standard deviation (SD), and category variables were expressed number and proportion (%). We used the performance R package to create diagnostic plots for testing the underlying assumptions of the main linear regression models in this study, including the normality and homogeneity of variance of residuals, as well as the linearity of quantitative predictors. We found no significant deviation from these assumptions (Figure S5). Sample size estimations were performed by Multiple Regression using Effect Size module in the PASS software version 21.0.3. According to Cohen, an effect size (f^2) of 0.02, 0.15, and 0.35 were defined as small, medium, and large effect size.[21] In our calculation, a sample size of 1108, 162, and 78 achieves 90% power to detect an effect size (f^2) of 0.02, 0.15, 0.35 attributable to 12 independent variables using an F-Test with a significance level (alpha) of 0.05 (Table S4). The variables tested are adjusted for an additional 1 independent variable(s). The calculations assume an unconditional (random X's) model. In our study, the cohort with the smallest sample size is WCHAT (n = 2080) (Fig. 1), and the sample size required to detect a small effect size (f = 0.02) is 1108. Therefore, the sample sizes of our four datasets all meet the requirement to detect an effect size of 0.02.

Multiple imputations for missing data

The combination and proportion of missing data were illustrated in Figure S6. Missing value of covariates was imputed using multiple imputation by chained equations (MICE) whereby we generated and analyzed 5 imputed datasets, the estimates were combined by Rubin rule.[22] Variables with missing values were imputed using other predictors within each dataset,

except for the variable itself.[23–25] Predictors for multiple imputation across datasets were shown in Table S5. As suggested by Buuren et al., the imputation method depends on the imputed variable. For binary variables, logistic regression model was used; for unordered categorical variables, multinomial logit model was used; for ordered categorical variables, ordered logit model was used.[22]

Analysis conducted in NHANES, WCNPCNS, and WCHAT

Multivariable linear regression model was employed to evaluate the association between TT level/dynamic of TT and BA acceleration/change in BA acceleration. To ensure the regression model comply with the underlying assumptions, TT and BA acceleration were transformed by Box–Cox with negatives allowed (BCN).[26–30] Specifically, the maximum-likelihood estimation was used to optimize the tuning power parameter, λ, such that the distribution of the transformed data has the largest similarity to normality. When zero or negative values occur in the data, a second parameter, γ, was also estimated to reduce transformation bias and added to make all values positive before the data can be handled by the Box–Cox transformation. The transformation was performed using Companion to Applied Regression (car) R package (https://cran.r-project.org/package=car). BCN-transformed TT was categorized into four quartiles for further analysis, and linear trend test was conducted. For NHANES, the first quartile (Q1) of BCN-transformed TT range from 1.569 to 2.180, the second quartile (Q2) range from 2.180 to 2.336, the third quartile (Q3) range from 2.336 to 2.495, the fourth quartile range from 2.495 to 3.468. For WCNPCS, Q1 range from 3.327 to 6.061, Q2 range from 6.061 to 6.918, Q3 range from 6.918 to 7.818, Q4 range from 7.818 to 11.391. For WCHAT, Q1 range from 1.670 to 3.369, Q2 range from 3.369 to 3.787, Q3 range from 3.787 to 4.192, Q4 range from 4.192 to 3.085. In the linear regression model for the association between TT level and BA acceleration, the independent variable of interest is BCN-transformed TT level, the response variable is BCN-transformed BA acceleration, the adjusted covariates are age, race, education level, marital status, BMI, physical activity, smoking status, alcohol intake, and venipuncture time, diabetes, hypertension, and cardiovascular disease (when applicable). Stratified analyses were performed to assess heterogeneity among different subgroups, the interaction effect was tested by incorporating an interaction term into the linear regression model and evaluated by Wald test.[31–33] Details of survival analysis was presented in Supplementary Materials.

Analysis conducted in UKB

In the UKB, TT levels and the biomarkers required for the construction of BA were measured twice during the initial assessment visit and the first repeat assessment visit, which allowed us to investigate the longitudinal dynamic changes in testosterone levels and their association with changes in BA acceleration by change-to-change analysis. Change-to-change analysis is a self-controlled method that utilizes within-individual data, shown to produce reliable results comparable to randomized controlled trials by minimizing unobserved time-invariant confounding factors.[34] To reduce the impact of measurement variability and baseline TT levels, we took advantage of the relative change in TT to reflect the dynamic changes in TT. The relative change in TT was calculated as the difference between the TT levels measured at the first repeat assessment visit and those measured at the initial assessment visit, divided by the TT levels measured at the initial assessment visit. The change in BA acceleration was defined as the difference between the BA acceleration calculated at the first repeat assessment visit and that calculated at the initial assessment visit. To ensure the regression model comply with the underlying assumptions, relative TT change and change in BA acceleration were also transformed using BCN. Regarding the linear regression model for the association between dynamic of TT and change in BA acceleration, the independent variable of interest is BCN-transformed relative TT change, and the response variable is BCN-transformed BA acceleration change, adjusted covariates included age, race, education level, BMI, physical activity, smoking status, alcohol intake, diabetes, cardiovascular disease, and BA acceleration measured in the initial assessment visit to avoid collider bias.[35] BCN-transformed relative TT change was categorized into four quartiles for further analysis, and linear trend test was conducted. In UKB, Q1 of BCN-transformed relative TT change range from −5.970 to −2.931, Q2 range from −2.931 to −2.258, Q3 range from −2.258 to −1.574, Q4 range from −1.574 to 2.140. Stratified analysis and test of interaction effect were also performed in UKB. Restricted cubic splines (RCS) were conducted to examine the robustness of the results. According to Harrell,[36] using four knots provides adequate model fit and represents a good compromise between flexibility and the loss of degrees of freedom due to overfitting in small samples. Therefore, we selected four knots positioned at the 5th, 35th, 65th, and 95th percentiles of relative TT change. Similar to previous studies, the positions of these knots were predetermined based on the distribution of the relative change in TT levels to ensure that each interval contains a sufficient number of observations for estimating the cubic polynomial.[37,38]

Analysis conducted in West China hospital RARP cohort

A paired t-test was employed to compare KDM-BA acceleration before and after ADT administration in prostate cancer patients. The assumption of normality for the differences in the paired t-test was assessed using the Shapiro–Wilk test, and we found no significant

deviation from the assumption (Table S6). PSM was performed using "MatchIt" R package, using nearest neighbor matching on a 1:1 basis, with age, diabetes, and hypertension as the matching variables.

Sensitivity analysis

To assess the robustness of the findings, we conducted several sensitivity analyses. First, we repeated the analysis using complete cases sample (n = 6515 for NHANES, n = 1179 for WCNPCS, n = 1585 for WCHAT, n = 5255 for UKB). Second, since fT was the bioactive form of testosterone, we assessed the relationship of the calculated fT with BA acceleration in NHANES. Last, we calculated the E-value to assess the potential impact of unmeasured confounders.[39] Similar to previous studies,[40–42] E-values for beta estimates and 95% CI were calculated using EValue R package[43] (https://cran.r-project.org/package=EValue). The formula for calculating E-value was adopted from VanderWeele et al.[39] For continuous outcomes, the E-value was approximated by incorporating the risk ratio (RR) approximation, RR ≈ exp (0.91 × d), into the E-value formula, where d denotes the standardized effect size. The corresponding approximate confidence interval was determined using the formula [exp (0.91 × d – 1.78 × sd), exp (0.91 × d + 1.78 × sd)], with sd representing the standard error associated with d. The E-value for continuous outcomes rely on the following approximations and assumption. First, an approximate conversion between standardized effect sizes and OR assumes that a binary variable in fact based on an underlying continuous variable with a particular cut-off. The approximate odd ratio (OR) R ≈ exp (1.81 × d) and an approximate confidence interval by [exp (1.81 × d – 3.55 × sd), exp (1.81 × d – 3.55 × sd)]. The approximation was considered accurate when the data follow a normal or logistic model and when the binary outcome does not have very low or very high probability. Second, the approximate RR can be obtained from the OR using a square root conversion RR ≈ sqrt (OR).[39]

All analyses were performed with R software, Free statistic software[44] and Empower software (http://www.empowerstats.com). Two-tailed P values < 0.05 were considered statistically significant.

Ethics

The NHANES was approved by the National Center for Health Statistics Research Ethics Review Board. The WCHAT received approval from the Ethics Committee of West China Hospital (No. 2017-445). The WCNPCS was approved by the Ethics Committee of West China Hospital/Sichuan University (No. 2020-145, and 2021-1142). The UKB received ethical approval from the North-West Multicentre Research Ethics Committee (REC reference: 11/NW/03820). The West China hospital RARP cohort was approved by the Institutional Ethics Review Board of West China Hospital of Sichuan University (Approval No. 2017324). All study participants of NHANES, WCHAT, WCNPCS, and UKB gave written informed consent. Due to the retrospective design of the study, the requirement for informed consent was waived by the Institutional Ethics Review Board of West China Hospital (Sichuan University, Chengdu, China).

Role of the funding sources

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Population characteristics of NHANES, WCHAT, WCNPCS, and UKB

The population characteristics of the four cohorts, including UKB, NHANES, WCNCPS, and WCHAT, are presented in Table 1. The analysis included 6979, 2080, 2133, and 6058 participants from the NHANES, WCHAT, WCNPCS, and UKB cohorts, respectively. The mean (±SD) BA values were 46.8 ± 17.6, 58.6 ± 12.1, 55.4 ± 10.7, and 57.1 ± 7.7 for the NHANES, WCHAT, WCNPCS, and UKB cohorts, respectively. And the mean (±SD) TT levels (nmol/L) in the NHANES, WCHAT, WCNPCS, and UKB were 14.4 ± 6.6, 16.0 ± 6.1, 17.8 ± 7.0, and 12.2 ± 3.6, respectively. And mean (±SD) for BA acceleration values were –2.2 ± 5.5 for NHANES, –5.0 ± 10.2 for WCHAT, –5.4 ± 6.6 for WCNPCS, and –0.6 ± 2.1 for UKB. Of note, for all included UKB participants, the mean (±SD) of change in TT was 0.12 (3.0) nmol/L, and time interval between the first and second measurement were 51.9 (10.7) months (Table S7).

Higher serum TT was associated with decreased BA acceleration

The relationship between serum TT levels and BA acceleration was initially investigated in three different surveys conducted in China and America. As outlined in Table 2, BCN-transformed TT was negatively associated with BCN-transformed BA acceleration (β: –0.078, 95% CI: –0.099 to –0.057) in NHANES. For WCHAT, each unit increase in BCN-transformed TT corresponded to 0.015-years decrease in BCN-transformed BA acceleration (β: –0.015, 95% CI: –0.023 to –0.006). In WCNPCS, per unit increase in BCN-transformed TT associated with 0.010-year decrease of BCN-transformed BA acceleration (β: –0.010, 95% CI: –0.015 to –0.006). These results remained robust when serum TT levels were categorized into quartiles for analysis.

In stratified analysis, the directions and magnitudes of the associations were generally consistent across various subgroups in all three cohorts (Fig. 2). Of note, in NHANES, the negative association between TT and BA acceleration was more pronounced among

Characteristics of NHANES participants	NHANES (n = 6976)	Characteristics of WCHAT participants	WCHAT (n = 2080)	Characteristics of WCNPCS participants	WCNPCS (n = 2133)	Characteristics of UK Biobanks participants	UKB (n = 6058)
KDM BA, Mean ± SD	46.8 ± 17.6	KDM BA, Mean ± SD	58.6 ± 12.1	KDM BA, Mean ± SD	55.4 ± 10.7	Baseline KDM BA, Mean ± SD	57.1 ± 7.7
KDM BA acceleration (Years), Mean ± SD	−2.2 ± 5.5	KDM BA acceleration (Years), Mean ± SD	−5.0 ± 10.2	KDM BA acceleration (Years), Mean ± SD	−5.4 ± 6.6	Baseline KDM BA acceleration (Years), Mean ± SD	−0.6 ± 2.1
Testosterone (nmol/L), Mean ± SD	14.4 ± 6.6	Testosterone (ng/dL), Mean ± SD	16.0 ± 6.1	Testosterone (ng/dL), Mean ± SD	17.8 ± 7.0	Baseline Testosterone (ng/dL), Mean ± SD	12.2 ± 3.6
Age (years), Mean ± SD	49.0 ± 17.7	Age (years), Mean ± SD	63.6 ± 8.4	Age (years), Mean ± SD	60.7 ± 8.9	Age (years), Mean ± SD	57.7 ± 7.5
Race, n(%)		**Race, n(%)**		**Race was not collected in this cohort**		**Race, n(%)**	
Mexican American	964 (13.8)	Han	762 (36.6)	–	–	White	5923 (98.0)
Other Hispanic	694 (9.9)	Zang or Qiang	966 (46.4)	–	–	Mixed	14 (0.2)
Non-Hispanic White	2749 (39.4)	Other race	352 (16.9)	–	–	Asian or Asian British	41 (0.7)
Non-Hispanic Black	1472 (21.1)	–	–	–	–	Black or Black British	22 (0.4)
Other race	1097 (15.7)	–	–	–	–	Chinese	14 (0.2)
–		–	–	–	–	Other ethnic group	31 (0.5)
Education level, n (%)		**Education level, n (%)**		**Education level, n (%)**		**Education level, n (%)**	
Under high school	1627 (23.3)	Under high school	1682 (81.3)	Under high school	1420 (77.6)	College or above	2629 (43.6)
High school	1623 (23.3)	High school	267 (12.9)	High school	288 (15.7)	A levels or AS levels	718 (11.9)
Above High school	3726 (53.4)	Above High school	120 (5.8)	Above High school	121 (6.6)	Under A level	2088 (34.6)
-	–	–	–	–	–	None of the above	598 (9.9)
Martial status, n (%)		**Martial status, n (%)**		**Martial status, n (%)**		**Martial status was not collected in this cohort**	
Married	4485 (64.3)	Married	1886 (91.2)	Married	1995 (95.6)	–	–
Divorced/Separated/ Widowed	1055 (15.1)	Divorced/Separated/ Widowed	159 (7.7)	Divorced/Separated/ Widowed	73 (3.5)	–	–
Never married	1436 (20.6)	Never married	24 (1.2)	Never married	18 (0.9)	–	–
Physical activity, n (%)		**Physical activity, n (%)**		**Physical activity, n (%)**		**Physical activity, n (%)**	
Mild	3367 (48.3)	Mild	530 (33.3)	0-2 times per week	215 (14.9)	Mild	1064 (20.0)
Moderate	664 (9.5)	Moderate	530 (33.3)	3-5 times per week	123 (8.5)	Moderate	2067 (38.8)
Vigorous	2945 (42.2)	Vigorous	531 (33.4)	more than 6 times per week	1104 (76.6)	Vigorous	2190 (41.2)
Smoking status, n (%)		**Smoking status, n (%)**		**Smoking status, n (%)**		**Smoking status, n (%)**	
Never	3285 (47.1)	Never	1071 (52.2)	Never	837 (40.2)	Never	3232 (53.5)
Former	2034 (29.2)	Former	242 (11.8)	Former	291 (14.0)	Former	2337 (38.7)
Now	1651 (23.7)	Now	740 (36.0)	Now	956 (45.9)	Now	475 (7.9)
Alcohol intake, n (%)		**Alcohol intake, n (%)**		**Alcohol intake, n (%)**		**Alcohol intake, n (%)**	
No	1077 (16.5)	No	1270 (61.5)	No	976 (46.9)	No	259 (4.3)
Yes	5444 (83.5)	Yes	794 (38.5)	Yes	1104 (53.1)	Yes	5796 (95.7)
BMI category, n (%)		**BMI category, n (%)**		**BMI category, n (%)**		**BMI category, n (%)**	
Underweight or normal weight (<25 kg/m^2)	1977 (28.3)	Underweight or normal weight (<25 kg/m^2)	1095 (53.1)	Underweight or normal weight (<25 kg/m^2)	1218 (57.1)	Underweight or normal weight (<25 kg/m^2)	1750 (29.0)
Overweight (25~30 kg/m^2)	2673 (38.3)	Overweight (25~30 kg/m^2)	810 (39.3)	Overweight (25~30 kg/m^2)	817 (38.3)	Overweight (25~30 kg/m^2)	3028 (50.1)
Obese (>30 kg/m^2)	2326 (33.3)	Obese (>30 kg/m^2)	158 (7.7)	Obese (>30 kg/m^2)	98 (4.6)	Obese (>30 kg/m^2)	1263 (20.9)
Venipuncture time		**Venipuncture time**		**Venipuncture time**		**Venipuncture time was not collected in this cohort**	
Morning	3369 (48.3)	Morning	2080 (100%)	Morning	2133 (100%)	–	–
Afternoon	2550 (36.6)	Afternoon	–	Afternoon	–	–	–
Evening	1057 (15.2)	Evening	–	Evening	–	–	–
Diabetes, n (%)		**Diabetes, n (%)**		**Diabetes, n (%)**		**Diabetes, n (%)**	
No	5656 (81.1)	No	1904 (92.2)	No	1939 (92.8)	No	5736 (94.9)
Yes	1320 (18.9)	Yes	161 (7.8)	Yes	151 (7.2)	Yes	311 (5.1)
Hypertension, n (%)		**Hypertension, n (%)**		**Hypertension, n (%)**		**Hypertension was intergrated into cardiovascular disease in this cohort**	
No	3992 (57.2)	No	1520 (73.6)	No	1711 (81.9)	–	–
Yes	2984 (42.8)	Yes	545 (26.4)	Yes	379 (18.1)	–	–

(Table 1 continues on next page)

Cardiovascular disease, n (%)		Cardiovascular disease, n (%)		Cardiovascular disease was not collected in this cohort		Cardiovascular disease, n (%)	
No	6167 (88.4)	No	1999 (96.8)	–	–	No	4187 (69.2)
Yes	809 (11.6)	Yes	66 (3.2)	–	–	Yes	1863 (30.8)

–: not available.

Table 1: **Characteristics of study participants among NHANES, WCHAT, WCNPCS and UKB cohort.**

participants older than 60 years old (β: –0.109, 95% CI: –0.152 to –0.066) than participants younger (Age≤60 years) (β: –0.042, 95% CI: –0.065 to –0.019). The interaction effect of age in NHANES was significant (P = 0.031). In WCHAT, the association between TT and BA acceleration was significant only among current smokers (β: –0.026, 95% CI: –0.040 to –0.012), and the interaction effect of smoke was significant (P = 0.015). Besides, the association was significant only among participants without hypertension (β: –0.019, 95% CI: –0.028 to –0.009), and the interaction effect of hypertension was significant (0.038).

The longitudinal dynamic of serum TT and BA acceleration change

Since serum TT exhibit negative association on BA acceleration in cross-sectional settings, we sought to explore whether the dynamic of serum TT have the potential for reversing BA acceleration. As shown in Table 3, BCN-transformed relative TT change was significantly associated with decreased BCN-transformed BA acceleration change (β: –0.047, 95% CI: –0.057 to –0.038). Participants in the highest quartile of BCN-transformed relative TT change was significantly associated 0.120 years decreased in BCN-transformed BA accelration change (β: –0.120, 95% CI: –0.146 to –0.094). The RCS (Fig. 3A) and trend test (Table 3) further validated the linear trend of the association between relative TT dynamics and BA acceleration change. In the stratified analysis, the results were robust across all subgroups, showing same direction of the effect of serum TT dynamics on BA acceleration change (Fig. 3B). Of note, the association between BCN-transformed relative TT change and BCN-transformed BA acceleration change was more pronounced among participants with DM (DM participants: β: –0.102, 95% CI: –0.159 to –0.044; Non-DM participants: β: –0.044, 95% CI: –0.054 to –0.034), and the interaction effect of DM was significant (P = 0.0040). The RCS of the other four imputed datasets from the UKB were illustrated in Figure S7. The conclusions drawn from these datasets were consistent across all imputed datasets.

Prostate cancer patients underwent ADT experienced deteriorated BA acceleration

Among prostate cancer patients who administrated adjuvant ADT after RARP with the mean time of 13.10 months, we found that the BA acceleration was significantly increased by an average 1.804 years after administration of ADT (Fig. 3C). To eliminated bias caused by RARP, we matched 38 prostate cancer patients who only received RARP and compared their BA acceleration pre-and-post surgery. As demonstrated by Fig. 3D, RARP did not result in significant change in BA acceleration (P = 0.18).

Sensitivity analysis

The association of TT level and relative change in TT with BA acceleration or change in BA acceleration were repeatedly assessed in complete cases sample of NHANES, WCHAT, WCNPCS (Table S8), and UKB (Table S9). The results remain robust in complete cases analysis. Since fT was considered as the bioactive form of T, and substantial evidence suggests that free

Variable	NHANES[a]		WCHAT[b]		WCNPCS[c]	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
BCN-transformed Serum TT	−0.078 (−0.099 to −0.057)	<0.0001	−0.015 (−0.023 to −0.006)	0.00050	−0.010 (−0.015 to −0.006)	<0.0001
Quartiles						
Q1	0 (Ref)		0 (Ref)		0 (Ref)	
Q2	−0.023 (−0.036 to −0.010)	0.00047	−0.003 (−0.016 to 0.010)	0.65	−0.009 (−0.026 to 0.008)	0.30
Q3	−0.034 (−0.048 to −0.021)	<0.0001	−0.019 (−0.033 to −0.006)	0.0056	−0.029 (−0.047 to −0.012)	0.0010
Q4	−0.044 (−0.058 to −0.030)	<0.0001	−0.020 (−0.033 to −0.006)	0.0048	−0.042 (−0.060 to −0.024)	<0.0001
P for Trend.		<0.0001		0.00079		<0.0001

[a]For NHANES, adjusted for age, race, education level, marital status, BMI, physical activity, smoking status, alcohol intake, and venipuncture time, diabetes, hypertension, and cardiovascular disease. [b]For WCHAT, adjusted for age, race, education level, marital status, BMI, physical activity, smoking status, alcohol intake, diabetes, hypertension, and cardiovascular disease. Venipuncture time were not adjusted since all blood were collected in the morning. [c]For WCNPCS, adjusted for age, education level, marital status, BMI, physical activity, smoking status, alcohol intake, diabetes, and hypertension. Race, venipuncture time were not adjusted since race were not collected and blood were collected in the morning.

Table 2: **Association of serum TT and BA acceleration in the NHANES, WCHAT, and WCNPCS.**

testosterone severe as a more accurate marker of androgen status,[45] we also investigated the relationship between ft and BA acceleration in NHANES (Table S10). Similarly to what we found regarding the relationship between TT and BA acceleration, fT also demonstrated a negative association with BA acceleration. Each unit increase in BCN-transformed fT was associated with 0.171-years decrease in BCN-transformed BA acceleration (β: –0.171 95% CI: –0.292~ –0.049). The linear trend test (Table S10) and RCS (Figure S8) demonstrated nonlinear association between BCN-transformed TT and BCN-transformed BA acceleration. Lastly, the E-value for the estimated association between BCN-transformed TT level and BCN-transformed BA acceleration were 2.147, 1.497, and 1.322 in NHANES, WCHAT, and WCNPCS respectively (Table S11), which indicated that the observed estimates could be explained away by an unmeasured confounder that was associated with both the exposure and the outcome by a risk ratio of 2.147, 1.497 or 1.322-fold each, above and beyond the measured confounders, but weaker confounding could not do so. Similarly, the E-value for the estimated association between BCN-transformed relative TT change and BCN-transformed BA acceleration change in UKB were calculated, E-value for beta estimate was 1.496 (Table S12).

Discussion

In this study, we identified a negative association between serum TT levels and BA acceleration across NHANES, WCHAT, and WCNPCS, although the directions of these association were consistently negative, the estimation of TT on BA acceleration differ across these cohorts. The most pronounced estimate of BCN-transformed TT on BCN-transformed BA acceleration was observed in the analysis of NHANES (β: –0.078), while in WCHAT (β: –0.015) and WCNPCS (β: –0.010), the estimates were relatively closer. This discrepancy may be attributed to the distinct racial compositions, age distributions, and lifestyle factors of the populations included in each study. In the subgroup analysis and interaction effect test, we observed significant interaction effect of smoke and hypertension on the association between TT and BA acceleration in WCHAT. Besides, the interaction effect of age on TT and BA acceleration was also significant in NHANES. Moreover, the interaction effect of DM was also significant in UKB. These results might indicate that the increase of TT may benefit the elderly, smokers and people with diabetes more for delaying aging, but the anti-aging effect of TT may be weakened by hypertension. However, the interaction effect of both smoking and age was not significant across all cohorts, and the estimation of effect values may be influenced by small sample sizes in certain subgroups. Therefore, this result needs further confirmation in future large sample prospective studies.

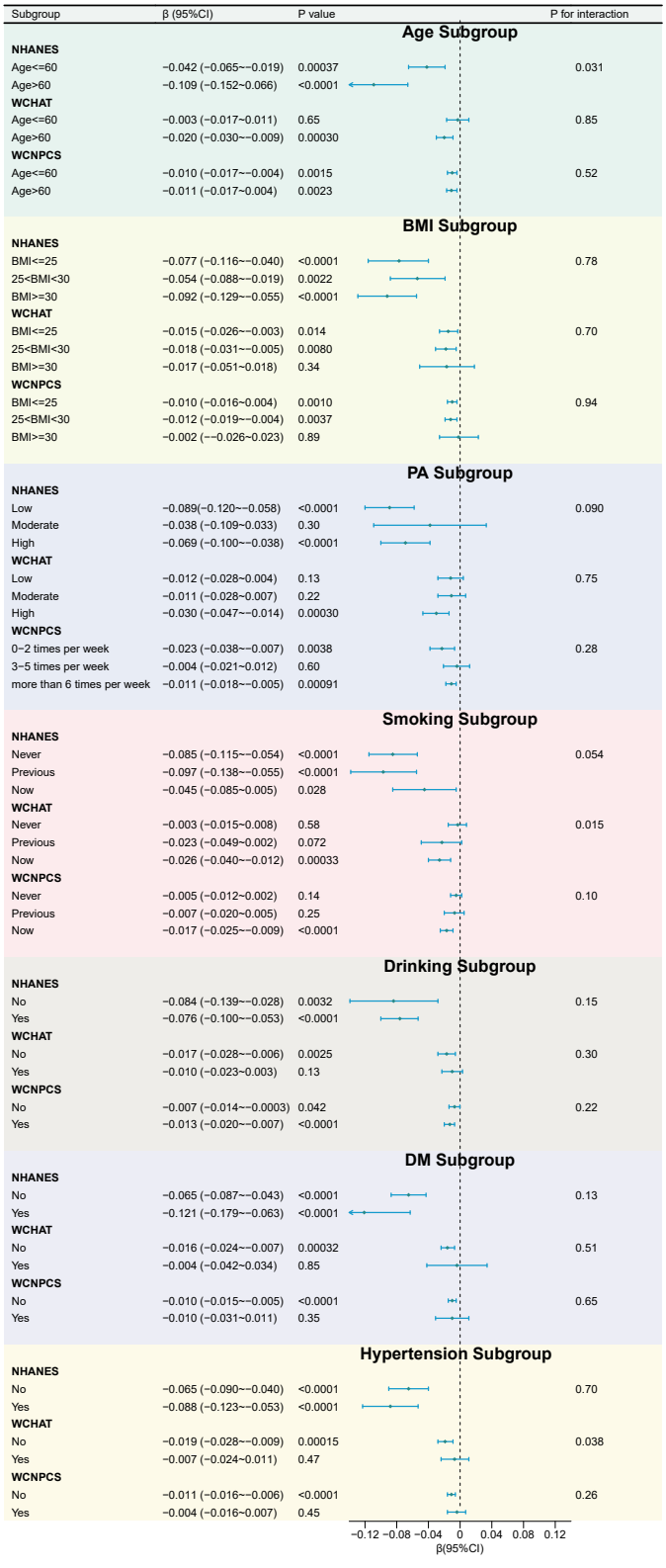

Fig. 2: Stratified analysis of BCN-transformed TT and BCN-transformed BA acceleration among NHANES IV, WCHAT, WCNPCS cohorts.

Transformed relative TT change	β (95% CI)	P value
Continuous	−0.047 (−0.057 to −0.038)	<0.0001
Quartile		
Q1	0 (Ref)	
Q2	−0.038 (−0.063 to −0.012)	0.0038
Q3	−0.068 (−0.093 to −0.042)	<0.0001
Q4	−0.120 (−0.146 to −0.094)	<0.0001
P for Trend		<0.0001

Adjusted for age, race, education, physical activity, drinking status, smoking status, BMI, diabetes, cardiovascular disease, and baseline KDM-BA acceleration.

Table 3: **The association of relative TT dynamics and change in BA acceleration.**

Our analysis mainly focused on the association of TT and BA acceleration, since TT the standard measure for research study. Of note, substantial evidence suggested that fT is the biologically active form of testosterone, although it accounts for only 2%–5% of total testosterone.[46,47] However, in populations with normal total testosterone, the decrease in free testosterone is still associated with symptoms of androgen deficiency.[48] In our analysis, we also observed nonlinear negative association between fT and BA acceleration (Table S10, Figure S8). However, due to the high cost and difficulty in clinical promotion of fT, as well as certain limitations in its calculation,[49] our research still focuses on the association between TT and biological aging, and has not delved into whether the reduction of fT accelerates biological aging in individuals with normal TT. High quality research is needed in the future to address this issue and clarify the importance of fT as a biological aging biomarker. Furthermore, a longitudinal change-to-change analysis showed that increase in relative TT change was negatively associated with BA acceleration change, providing valuable insight on considering TT management as an anti-aging approach. The promotion effect of ADT on biological aging was observed among patients receiving ADT after RARP. However, the interpretation of this result needs to be cautious, not only because of its small sample size, but also because although we used self-pairing design to control for confounding between individuals and limited the two BA measurements time interval to two years to minimize the impact of other confounding factors on BA during ADT treatment, bias may still exist. Future studies with higher level of evidences are required to validate the results.

Supporting our findings, previous studies have shown that decreased serum TT is associated with aging-related diseases, including cardiovascular disease and type 2 diabetes.[50] Prospective studies have also suggested that lower serum TT levels are linked to increased all-cause and cardiovascular mortality. As hallmarks of the aging process, inflammation and metabolic disorders have also been associated with low TT levels. Zhang et al. reported that TT deficiency was linked to higher levels of inflammation, and long-term TT therapy reduced inflammation in hypogonadal men.[9] Additionally, conditions such as metabolic syndrome, insulin resistance, hyperlipidemia, and hyperglycemia have been associated with low TT levels.[50,51]

Although the relationship between serum TT and biological age has been previously studied, most research has used indicators such as epigenetic age, telomere length, and the frailty index (FI) to measure

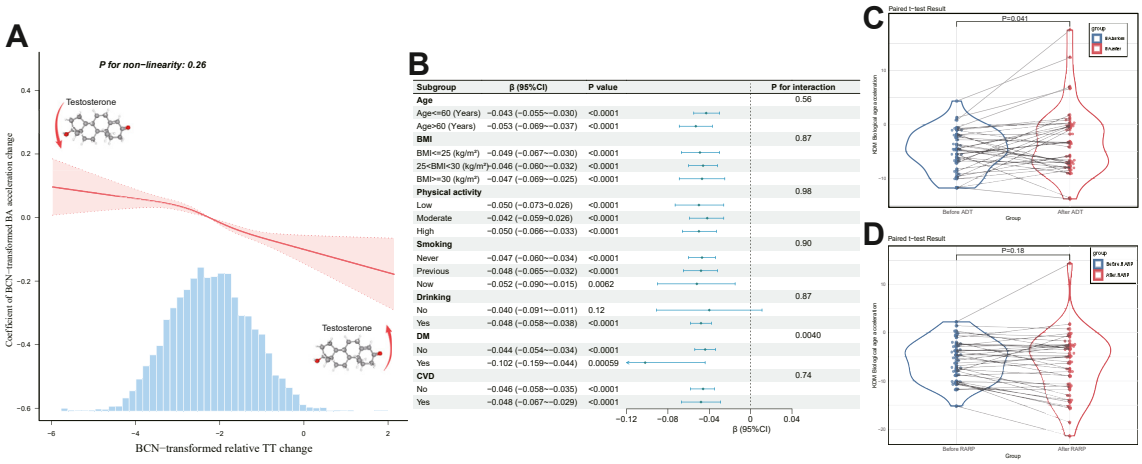

Fig. 3: The longitudinal dynamics of serum TT and BA acceleration: A. The RCS of BCN-transformed relative TT dynamics and BCN-transformed BA acceleration change. The shaded area refers to 95% confidence band. B. Stratified analysis of the association between BCN-transformed relative TT dynamics and BCN-transformed BA acceleration change. C. BA acceleration deteriorated after the administration of ADT in prostate cancer patients. D. BA acceleration did not exhibit significant change after RARP.

biological aging, leading to controversial conclusions. In mammalian models, castration in mice and sheep has been shown to extend lifespan and slow epigenetic aging in males.[52,53] Of note, in these studies, castration surgery was performed before puberty. However, there are distinct differences between prepubertal and postpubertal castration. Animals and men subjected to prepubertal castration exhibit a lack of sexual behavior and secondary sexual characteristics, whereas these features can still be observed in animals that undergo postpubertal castration.[54,55] Consequently, the pattern of TT declines in animals castrated before puberty may significantly differ from the gradual decline in male testosterone levels observed in epidemiological studies, which may explain the inconsistent results between animal studies and epidemiological studies. Additionally, some studies have found an inverse association between TT and telomere length, which contradicts the potential anti-aging effects of TT.[56] However, other research by Yeap, Coburn, and colleagues reported no association between TT and telomere length in men.[57,58] Regarding frailty, lifelong TT deficiency did not worsen frailty in aging male mice.[59] Conversely, two cohort studies conducted in Europe and Australia suggested that higher TT levels protect elderly men from worsening frailty.[60,61]

The inconsistencies between these studies and ours may be attributed to the different measures of biological age used. Previous research has indicated that various biological age metrics reflect distinct aspects of the aging process, as their correlations tend to be relatively weak.[62] Molecular metrics, such as epigenetic clocks, telomere length, and omics age, are designed to capture age-related modifications at the cellular and molecular levels. In contrast, the frailty index (FI) highlights significant age-related functional decline and health impairments, including symptoms, diseases, and disabilities. In our study, the composite biomarker BA assesses age-related changes in physiological integrity across multiple organs and systems, acknowledging that aging is a multidimensional process involving various bodily systems. Additionally, the rate of aging varies significantly among populations, with individuals of the same chronological age or disease status potentially exhibiting different levels of aging. By using BA acceleration as our outcome measure, our findings offer further evidence supporting the anti-aging potential of TT from a more comprehensive perspective.

In our change-to-change analysis, we found that increase in relative TT change significantly associated with increased BA acceleration change, further enhance the consistency of our finding. To the best of our knowledge, rare epidemiological studies have investigated the dynamics of TT and progression of biological aging.

To the best of our knowledge, this is the first study to explore the association between longitudinal TT dynamics and BA acceleration, which more comprehensively reflects the aging rate of multiple organs and systems. A notable strength of our study is the large sample size with diverse ethnic representation, and the consistency of our findings across different populations. Furthermore, our results were validated in a prostate cancer population, reinforcing the robustness of our conclusions.

However, we acknowledge several limitations in our study. Although our sample size was relatively large, the analyses in NHANES, WCHAT, and WCNPCS were conducted at baseline due to the lack of repeated assessments of serum TT and BA. Notably, repeated measurements of serum TT and BA are planned for future visits, and we intend to conduct a prospective study to evaluate the causal effects of TT on biological aging in these cohorts. Additionally, serum TT was measured at a single time point in the three cohorts, which may not fully represent typical concentrations due to intra-individual and diurnal variations; multiple measurements are recommended according to AUA guidelines.[14] Third, due to the intrinsic nature of an observational study, residual confounding from unmeasured confounders and residual confounding due to measurement error in confounders is still inevitable. Fourth, the inconsistency in TT measurement methods and between four large databases may also introduce bias into our study results. In NHANES, TT level was measured by Isotope dilution liquid chromatography and tandem mass spectrometry (ID/GC–MS), while in WCHAT, WCNPCS, and UKB, TT level was measured by chemiluminescent immunoassay (Table S3). As suggested by Taieb et al.,[63] the immunoassays underestimated TT concentrations in samples from men compared to ID/GC–MS, which might attenuated the protective effect of TT on BA acceleration. Fifth, although various methods have been adopted in UKB to minimize and mitigate the impact of potential errors (systematic bias and random errors) in biomarker measurements, in order to provide high-quality biomarker data, sample collection and processing, sample retrieval, and detection data monitoring methods have been used to minimize drift, bias, and measurement errors (https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=5636). In change-to-change analysis, changes in testosterone may still be caused by normal measurement variations. Lastly, in the study on ADT-treated prostate cancer patients, the retrospective design may introduce potential confounding factors, which could affect the reliability of the results. Moreover, the statistical power did not reach 0.8 (Table S13). However, this is the first study to investigate the dynamics of BA acceleration influenced by ADT treatment. We plan to include more participants in future studies to enhance the statistical power of the results, and a paired design was used to minimize bias.

In conclusion, our findings suggest that TT has the potential to reverse BA acceleration, providing significant insights into the possible anti-aging properties of TT. These results highlight TT as a promising target for

interventions aimed at slowing the aging process. We suggest that future research should explore the impact of testosterone therapy on the acceleration of biological aging in men with testosterone deficiency, both before and after treatment, employing the KDM-BA acceleration method as an approach.

Contributors

Shi Qiu, Lu Yang, Birong Dong, Shengfeng Wang, and Weichao Huang contributed to study design. Weichao Huang, Shi Qiu, Qiaorui Wen, and Shengfeng Wang have accessed and verified the data. Weichao Huang, Linghui Deng, and Qiaorui Wen contributed to formal analysis, and writing the original draft. Weichao Huang, Linghui Deng, Qiaorui Wen, Colucci Manuel, Robesti Daniele, Aurora Valdata, and Qiang Wei contributed to reviewing and editing the manuscript. Zilong Zhang, Chichen Zhang, Xianghong Zhou, and Yuming Jin contributed to figures. Qiaorui Wen, Xiaoli Zou, Dan Hu and Zhongyuan Jiang, Yu Zhan, Lei Chen, Shaoheng Luo, Zuoqiu Sophia, and Jirong Yue contributed to data collection and data curation. Linghui Deng contributed to methodology. All authors reviewed the manuscript drafts, critically revised the manuscript and approved the final manuscript.

Data sharing statement

UK Biobank data can be accessed by applying through their website at https://www.ukbiobank.ac.uk/. NHANES data is publicly available at https://www.cdc.gov/nchs/nhanes/index.htm. Data from WCHAT, WCNPCS, and West China Hospital RARP cohort is available upon reasonable request.

Declaration of interests

The authors declare not competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2025.103178.

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