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Research Article

Testosterone Concentration and Incident Depression in Older Men: A Longitudinal Cohort Study

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Abstract

Background: Testosterone has been implicated in mood regulation, yet its role in the development and treatment of depression remains unclear. This study investigated the association between testosterone concentrations and the incidence of depression in older men.

Methods: We utilized data from 4 107 men aged 70 years and older who participated in the Aspirin in Reducing Events in the Elderly (ASPREE) and ASPREE-XT studies. Serum total testosterone concentrations were measured at baseline and year 3. Depressive symptoms were assessed annually using the CES-D-10 scale, with incident depression defined as a CES-D-10 score of ≥8. Cox proportional hazards regression models were used to estimate the hazard ratios (HR) for incident depression, adjusted for potential confounders.

Results: During a median follow-up of 8.4 years, 1 449 participants experienced an episode of depression. Baseline total testosterone concentrations were not significantly associated with the risk of incident depression, whether treated as continuous variables (HR 1.00, 95% CI 0.99–1.01) or when categorized into quintiles. Similarly, changes in testosterone concentrations from baseline to year 3 did not predict incident depression (aHR 1.03, 95% CI 0.99–1.08). A subgroup analysis focusing on men with biochemical evidence of hypogonadism also found no association with incident depression.

Conclusions: Our findings do not support an association between testosterone concentrations and the risk of developing depression in older men. These results suggest that testosterone is not an important factor in the pathogenesis of depression in this population. There may still be individual variability in response to testosterone changes and its potential impact on mood disorders.

Keywords: Depression, Depressive symptoms, Mental health, Mood disorders; Psychiatry; Testosterone

Late-life depression is a significant public health concern, associated with increased morbidity, cognitive decline, and reduced quality of life in older adults (1). Among the various biological factors, testosterone has been hypothesized to play a role in the development of depression in older men. This interest in testosterone's potential influence on mood has coincided with an increase in testosterone therapy prescriptions over the past two decades, with an average annual percent increase of 15.5% in the United States between 2007 and 2014. Although testosterone is primarily prescribed for hypogonadism, approximately 4% of total prescriptions are to treat depression (2).

Testosterone exerts its effects on the body and brain through a complex physiological pathway. It is secreted in response to luteinizing hormone (LH) stimulation, with concentrations peaking in the morning and declining throughout the day. In circulation, testosterone is predominantly protein-bound, with only a small fraction acting on androgen receptors within various tissues, including the brain (3). Testosterone concentrations decline from around the third decade and, from the age of 55, total testosterone concentrations decline by 0.8% per year, reflecting Leydig cell impairment and hypothalamic-pituitary insensitivity (4).

Testosterone has long been implicated in the regulation of mood and behavior. In 1948, just over a decade after the Nobel Prize was awarded for the isolation of testosterone and other sex hormones, testosterone was trialed as a treatment for major depressive disorder (5). Although early studies reported promise, 7 decades on, the role of testosterone in the pathogenesis and treatment of depression remains disputed. Although some studies suggest a link between low testosterone concentrations and depressive symptoms (6), others have failed to establish a consistent association (7), and a recent editorial concluded that the role of testosterone in the pathophysiology of depression in men remains steeped in controversy (8). Given the conflicting findings on the relationship between testosterone and depression, this study sought to clarify the relationship between testosterone concentrations

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and the incidence of depression in a cohort of relatively healthy older men.

Method

Design and Participants

This study utilized data from the ASPirin in Reducing Events in the Elderly (ASPREE) clinical trial and the ASPREE eXTension (ASPREE-XT) observational study. ASPREE was a randomized, double-blind, placebo-controlled clinical trial designed to determine if daily low-dose aspirin (100 mg) could prolong survival free from dementia and physical disability in healthy older adults. A total of 19 114 community-dwelling individuals aged 70 years and older (65 years and older for U.S. minorities) were recruited between 2010 and 2014 from Australia (n = 16 703) and the United States (n = 2,411) (9). Participants were free of overt cardiovascular disease, dementia, independence-limiting physical disability, conditions with a high risk of bleeding, anemia, conditions likely to cause death within 5 years, current use of antiplatelet or antithrombotic medication, current use of aspirin for secondary prevention, and uncontrolled hypertension.

Following the completion of the ASPREE trial, participants were invited to enroll in the ASPREE-XT study, an observational study designed to track long-term health outcomes, including dementia, cancer, and functional decline. Participants underwent annual health assessments, including standardized evaluations of physical and cognitive function, medical history, and medication use (10).

Testosterone Concentration Assessment

Blood samples were collected at baseline and year 3 from consenting Australian participants as part of the ASPREE Healthy Ageing Biobank sub-study, which aimed to support future research into biomarkers and genetic factors related to aging and associated diseases (11). The samples were processed and stored at −80 °C until analysis. Serum total testosterone was measured using a chemiluminescence microparticle immunoassay (Abbott Alinity ci, Abbott Diagnostics, Macquarie Park, NSW, Australia), with a reference range of 8.0–30.0 nmol/L. The assay's coefficient of variation (%CV) was 4% at 3.3 nmol/L, 3.8% at 14.4 nmol/L, and 3.4% at 29.2 nmol/L.

Depression assessment

Depressive symptoms were assessed using the short version of the Center for Epidemiological Studies Depression scale (CES-D-10). The CES-D-10 is a self-reported questionnaire that evaluates the frequency of depressive symptoms over the past week. This instrument has demonstrated strong performance in classifying depressive symptoms (12). Construct validity studies have shown that the CES-D-10 is a reliable and valid measure of depression in older populations (13).

Participants completed the CES-D-10 at baseline and annually during follow-up visits in the ASPREE and ASPREE-XT studies. The primary outcome of this study was the incidence of depression, defined as a CES-D-10 score of 8 or higher on annual follow-up assessment, a threshold supported by previous research in older adults (14). A depression endpoint was also recorded where there was evidence of depression being the primary reason for a hospital admission for > 24 hours.

Depression symptom trajectories were also modeled using linear and nonlinear latent class mixed models to characterize dynamic change and identify unobserved heterogeneity. Four distinct trajectories were identified, corresponding to low ("nondepressed"), consistently mild ("subthreshold depression"), consistently moderate ("persistent depression"), and initially low but increasing ("emerging depression") CES-D-10 depressive symptoms. Further information on the method is outlined elsewhere (15,16).

Covariate Assessment

Baseline covariate data were collected through a combination of anthropometric measurements, laboratory assessments, and participant-completed questionnaires. These included assessments of comorbidities, social and medical history, physical and cognitive function, and lifestyle factors. Annual in-person visits and bi-annual phone calls were conducted for retention purposes and to reassess participants and collect information on the occurrence of prespecified endpoints, randomized medication compliance or aspirin use, and other health-related data. All covariates came from the ASPREE and ASPREE-XT studies except for physical activity and resistance training measures, which came from ASPREE Longitudinal Study of Older Persons (ALSOP) sub-study, described elsewhere (17). These measures of physical activity were self-reported.

Exclusion Criteria

All U.S. participants were excluded from the analysis as testosterone blood samples were not available at baseline. Australian participants were excluded if they did not have a valid measure of testosterone or had depression at baseline. Baseline depression was defined by a baseline CES-D-10 score of 8 or higher or the use of any antidepressant medication. Participants with a history of prostate cancer or those who were prescribed androgen or antiandrogen therapies at baseline were also excluded due to their effects on testosterone concentrations.

Statistical Analysis

The relationship between baseline testosterone concentrations and the risk of incident depression was analyzed using Cox proportional hazards regression models. The hazard ratios (HR) and 95% confidence intervals (CI) for incident depression were estimated, with adjustments for potential confounders including age, educational status, living status, socioeconomic status, smoking status, alcohol consumption, physical activity, body mass index (BMI), diabetes mellitus, chronic kidney disease, and cognitive function, as measured by the total Modified Mini-Mental State Examination score. Confounding variables were selected based on their association with both testosterone concentrations and depression, excluding any instrumental variables.

Testosterone concentrations were initially treated as continuous variables. Subsequently, they were categorized into quintiles (Q), with Q3 serving as the reference category and Q1 representing the lowest testosterone concentrations. To investigate the association between changes in testosterone concentrations and incident depression, testosterone was analyzed as the change from baseline to year 3, presented both as a percentage and in nmol/L. A sensitivity analysis, using higher CES-D-10 cut-scores of 10 and 12, was undertaken to assess the robustness of the findings across different thresholds for depression diagnosis. The linearity of the relationship between testosterone concentrations and incident depression was examined using a three-knot restricted cubic spline

model, with the median testosterone value as the reference point. Statistical analyses were conducted using R version 4.2.0 (18).

A subgroup analysis was conducted to examine the association between hypogonadism and incident depression. Although late-onset hypogonadism is defined by the presence of at least three sexual symptoms associated with a total testosterone concentration of less than 11 nmol/L and a free testosterone concentration of less than 220 pmol/L (19), we utilized a total testosterone cut-score of 6.4 nmol/L, given the absence of data on sexual symptoms. This cut score was selected as it represented the 2.5th percentile reference range for testosterone concentrations in a previous study in older men (20). A symptom level cross-sectional analysis was conducted between testosterone concentrations and depressive symptoms at baseline using a network model. The network was estimated using the EBICglasso method, which applies a graphical lasso algorithm with extended Bayesian information criterion (EBIC) for optimal model selection. Spearman correlations were used to account for the nonparametric nature of the data. The analysis focused on 10 depressive symptoms from the CES-D scale and testosterone concentrations, constructing a sparse network where only the most meaningful associations were retained (21). Finally, a multinomial logistic regression was undertaken with participants grouped into nondepressed, subthreshold, persistent and emerging depressive trajectories.

Results

Study Population

Of the 16 703 Australian ASPREE participants eligible for clinical biochemistry analysis, 7 524 were male. After excluding those with depression at baseline, those receiving androgen and antiandrogen therapies, and those with a history of prostate cancer, 4 107 individuals with baseline measures of serum total testosterone concentrations were included in the analysis (Figure 1). The mean (SD) age of the study population was 74.9 years (4.2) and the median interquartile range (IQR) follow-up time was 8.4 years (2.2 years).

At baseline, 139 (3.4%) of participants were current smokers and 3 514 (85.6%) were current alcohol drinkers. There were 1 787 (43.5%) of participants with more than 12 years of education. The mean BMI was 27.9 kg/m^2 (Table 1).

Testosterone and Incident Depression

The association between testosterone and depression is shown in Table 2. In the unadjusted model, baseline testosterone concentrations did not have a statistically significant association with incident depression, both in its continuous form [HR (95% CI): 1.00 (0.99-1.01)] or when divided into quintiles. The results were similar in the fully adjusted model.

When analyzing how the percentage change in testosterone concentrations affects the incidence of depression, there was no statistically significant association observed when testosterone was treated as a continuous variable [aHR (95%CI): 1.03 (0.97-1.08)]. The results were similar when observing the change in testosterone concentrations in nmol/L. In its continuous form, the HR (95%CI) for the incidence of depression in the fully adjusted model was 1.00 (0.98-1.02), and results were again similar when observing testosterone concentrations in quintiles. Sensitivity analyses with higher CES-D-10 cut-scores of 10 and 12 yielded results consistent with the primary analysis, showing no significant differences from the reference group. The Kaplan-Meier curve comparing survival free of depression over the follow-up period for each quintile can be found in Figure 2. Restricted cubic spline illustrating the relationship between total testosterone and depression can be found in Figure 3.

In the subgroup analysis comparing men with testosterone concentrations below 6.4 nmol/L to those with testosterone concentrations of 6.4 nmol/L or higher, a total of 102 men were included in the low testosterone group. The analysis revealed an adjusted HR of 1.02 (95% CI: 0.71–1.46) for the association between low testosterone and incident depression (Supplementary Material). In the network model exploring the association between testosterone as a continuous variable and individual depressive symptoms, testosterone was weakly correlated with feelings of loneliness and fearfulness (items within the CES-D-10). However, testosterone had low centrality within the network, indicating that it had a limited influence on the broader structure of depressive symptoms (Supplementary Material). Multinomial logistic regression found testosterone level had no impact on membership of different depressive symptom trajectory groups (Supplementary Material).

Discussion

This study sought to test the hypothesis that lower testosterone concentrations are associated with an increased incidence of depression in older men. Our findings do not

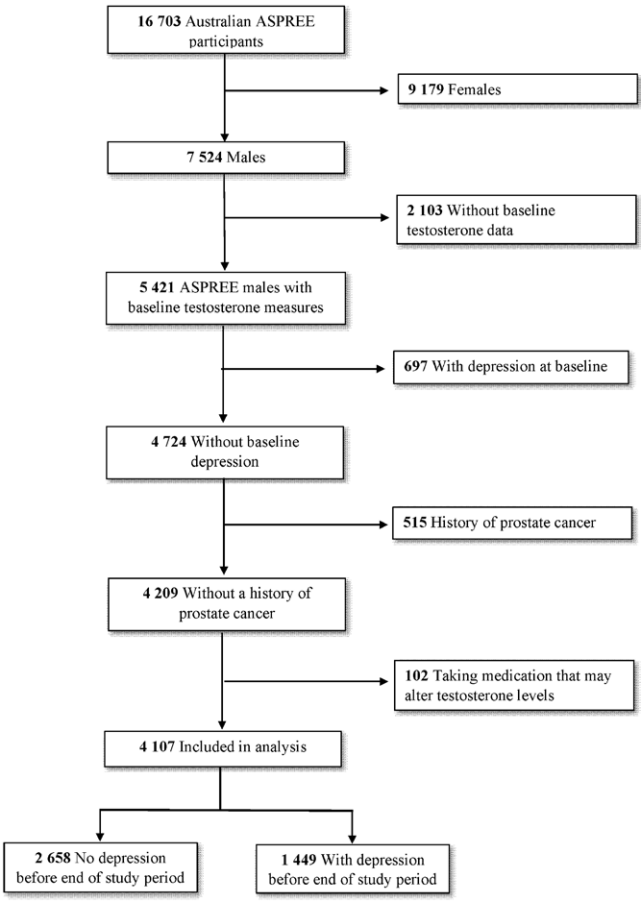

Figure 1. STROBE flow diagram.

Table 1. Baseline Characteristics of Participants

	No depression (*N* = 2 658)	Depression (*N* = 1 449)	Total (*N* = 4 107)	*p*-Value*
Age (mean [*SD*])	74.7 (4.12)	75.3 (4.19)	74.9 (4.16)	<.01
Smoking history, *n* (%)				<.01
Current	87 (3.3%)	52 (3.6%)	139 (3.4%)	
Former	1 319 (49.6%)	817 (56.4%)	2 136 (52.0%)	
Never	1252 (47.1%)	580 (40.0%)	1 832 (44.6%)	
Alcohol use, *n* (%)				.06
Current	2 264 (85.2%)	1 250 (86.3%)	3 514 (85.6%)	
Former	126 (4.7%)	82 (5.7%)	208 (5.1%)	
Never	268 (10.1%)	117 (8.1%)	385 (9.4%)	
IRSAD decile[1] (mean [*SD*])	6.29 (2.83)	6.21 (2.84)	6.26 (2.83)	.37
Living situation				.45
Living alone	484 (18.2%)	287 (19.8%)	771 (18.8%)	
Living with others	2 166 (81.5%)	1 158 (79.9%)	3 324 (80.9%)	
Education, *n* (%)				.06
≤12 years	1 473 (55.4%)	847 (58.5%)	2 320 (56.5%)	
>12 years	1 185 (44.6%)	602 (41.5%)	1 787 (43.5%)	
Physical activity[2α], *n* (%)				<.01
None	24 (0.9%)	9 (0.6%)	33 (0.8%)	
Light	451 (17.0%)	347 (23.9%)	798 (19.4%)	
Moderate	1 170 (44.0%)	632 (43.6%)	1 802 (43.9%)	
Vigorous	473 (17.8%)	220 (15.2%)	693 (16.9%)	
Resistance training[3α], *n* (%)				.83
Yes	630 (23.7%)	364 (25.1%)	994 (24.2%)	
BMI category[4], *n* (%)				.14
Underweight	2 (0.1%)	2 (0.1%)	4 (0.1%)	
Normal weight	598 (22.5%)	292 (20.2%)	890 (21.7%)	
Overweight	1 412 (53.1%)	764 (52.7%)	2 176 (53.0%)	
Obese	639 (24.0%)	387 (26.7%)	1 026 (25.0%)	
Diabetes[5], *n* (%)	297 (11.2%)	162 (11.2%)	459 (11.2%)	.99
Chronic kidney disease[6], *n* (%)	607 (22.8%)	338 (23.3%)	945 (23.0%)	.97
3MS score[7] (mean [SD])	93.0 (4.51)	92.7 (4.58)	92.9 (4.54)	.02
Testosterone level[8] (median [IQR])	16.6 (6.30)	16.5 (6.22)	16.6 (6.27)	.64

Notes: BMI = body mass index; IRSAD = Index of Relative Socio-economic Advantage and Disadvantage; IQR = interquartile range.
[1]Index of relative socioeconomic advantage and disadvantage score (1 most disadvantaged areas to 10 most advantaged areas).
[2]Physical activity was defined as the level of activity in a typical week.
[3]Resistance training was defined as "activities to increase muscle strength, such as lifting weights or resistance training" in a typical week.
[4]BMI categories: underweight (<18.5), normal weight (18.5–24.9), overweight (25.0–29.9), obese (≥ 30.0).
[5]Defined as self-report or the use of any drug use for the treatment of diabetes, including insulin, or a fasting blood glucose level of greater than or equal to 7 mmol/L.
[6]Defined an estimated glomerular filtration rate of less than 60 mL/min/1.73 m^2.
[7]3MS is the Modified Mini-Mental State Examination.
[8]To convert testosterone from nmol/L to ng/dL, multiply by 28.82.
[α]This variable may have been collected up to 2 years after baseline for some participants.
*t-tests were used for continuous variables and chi-squared test's for categorical variables.

support this hypothesis. Using both continuous and categorical measures of testosterone, we found no significant association between testosterone concentrations and the risk of developing depression. Moreover, our analysis of changes in testosterone over three years did not show any significant relationship with incident depression. These results were consistent when assessing the change in testosterone as a percentage change and change in nmol/L. Additionally, symptom-level network analysis revealed that testosterone was weakly associated with certain symptoms, such as loneliness and fearfulness, but did not play a central role in the broader depressive symptom network.

Our findings contrast with some previous epidemiological studies that have reported associations between low testosterone and depression. For instance, Kische et al. (22) observed a significant relationship between testosterone change and depression incidence over a four-year follow-up period. Similarly, Ford et al. (6) reported an increased risk of depression in men with low testosterone concentrations, defined as a concentration of 6.4 nmol/L, compared to men

Table 2. Multivariable analysis of testosterone and risk of incident depression

A						
	Whole Cohort (*N* = 4 107) Testosterone mean (*SD*) nmol/L: 16.5 (6.3)	**Testosterone Quintiles [median nmol/L (IQR)]**				
		Q1 *N* = 821 [9.7 (2.6)]	**Q2 *N* = 822 [13.1 (1.4)]**	**Q3 *N* = 822 [15.9 (1.3)]**	**Q4 *N* = 821 [19.0 (2.0)]**	**Q5 *N* = 821 [24.7 (5.1)]**
Model 1	1.00 (0.99–1.00)	1.02 (0.87–1.2)	0.98 (0.84–1.16)	1.00	1.01 (0.86–1.19)	0.98 (0.83–1.15)
Model 2	1.00 (0.99–1.00)	1.01 (0.86–1.19)	0.97 (0.83–1.15)	1.00	1 (0.85–1.18)	0.99 (0.84–1.16)
Model 3	1.00 (0.99–1.01)	0.98 (0.82–1.17)	1.01 (0.85–1.21)	1.00	1.05 (0.88–1.26)	1.04 (0.87-1.25)
Model 4	1.00 (0.99–1.01)	0.94 (0.78–1.14)	1.03 (0.86–1.24)	1.00	1.03 (0.86–1.24)	1.02 (0.85–1.23)
[a]B						
	Whole cohort (*N* = 3 105)	**Testosterone change in %**				
		>20% decrease *N* = 597	10%–20% decrease *N* = 458	Within 10% change *N* = 947	10%–20% increase *N* = 326	>20% increase *N* = 648
Model 1	1.02 (0.97–1.08)	1.13 (0.93–1.38)	1.06 (0.85–1.32)	1.00	1.09 (0.85–1.39)	0.94 (0.76–1.15)
Model 2	1.03 (0.98–1.07)	1.14 (0.94–1.39)	1.06 (0.86–1.32)	1.00	1.08 (0.85–1.39)	0.97 (0.79–1.2)
Model 3	1.03 (0.99–1.08)	1.14 (0.92–1.41)	1.05 (0.83–1.33)	1.00	1.09 (0.83–1.42)	0.96 (0.77–1.2)
Model 4	1.03 (0.99–1.08)	1.15 (0.92–1.43)	1.05 (0.82–1.34)	1.00	1.12 (0.85–1.48)	0.95 (0.75–1.2)
[b]C						
	Whole cohort (*N* = 3105)	**Testosterone change in whole numbers**				
		>5 nmol decrease *N* = 339	2–5 nmol decrease *N* = 605	Within 2 nmol change *N* = 1206	2–5 nmol increase *N* = 509	>5 nmol increase *N* = 317
Model 1	0.99 (0.98–1.01)	1.1 (0.88–1.38)	1.06 (0.88–1.29)	1.00	1.02 (0.83–1.25)	1.02 (0.8–1.31)
Model 2	1.00 (0.99–1.02)	1.12 (0.89–1.41)	1.07 (0.88–1.29)	1.00	1.04 (0.85–1.28)	1.06 (0.83–1.36)
Model 3	1.00 (0.99–1.02)	1.06 (0.83–1.37)	1.03 (0.84–1.27)	1.00	1.01 (0.8–1.27)	1.04 (0.79–1.37)
Model 4	1.00 (0.98–1.02)	1.07 (0.82–1.39)	1.05 (0.85–1.29)	1.00	1.02 (0.8–1.3)	1.04 (0.78–1.37)

Notes: A = Cox proportional hazards regression for testosterone as a continuous variable and in quintiles; B = Cox proportional hazards regression for testosterone as a continuous variable and in % change; C = Cox proportional hazards regression for testosterone as a continuous variable and in whole number change. Data shown as HR (95%CI).
Model 1: Unadjusted.
Model 2: Adjusted for age, smoking status, alcohol status, and education.
Model 3: Model 2 + living status, socioeconomic status, physical activity, and body mass index.
Model 4: Model 3 + diabetes, chronic kidney disease, and Modified Mini-Mental State Examination (3MS) score.
[a]Within 10% change includes 10% change.
[b]Within 2 nmol includes 2 nmol change.

with concentrations above this cutoff. Our study differs in its longer follow-up period and more comprehensive adjustment for potential confounders. Another key difference that may partly account for the discrepancy in findings is that Ford et al. utilized clinical diagnoses of depression rather than proxy measures based on a screening tool. With respect to individual depressive symptoms, whereas our cross-sectional network model suggests there are weak associations between testosterone and specific emotional symptoms, testosterone does not appear to play a central role in the overall network of depressive symptoms. This is consistent with existing symptom-level analyses of the relationship between testosterone and depression (23).

The relationship between testosterone and depressive symptoms is complex. There is consistent evidence that men with prostate cancer receiving androgen deprivation therapy (ADT) for prostate cancer have higher rates of depression than men with prostate cancer not receiving ADT (24,25), which suggests that a significant and rapid reduction in testosterone concentrations may induce depressive symptoms. This may reflect state-dependent low mood, where there is an association between low testosterone and depressive symptoms cross-sectionally, but low testosterone does not necessarily predict later depression (26). It may be the case that reduced testosterone is a risk marker for depression rather than a risk factor for depression. In other words, reduced testosterone may be a byproduct of the physiological effects of stress and illness (27), or a reflection of increased comorbidity, rather than a direct causal risk factor for depression itself.

The findings of this study should be evaluated in conjunction with recent research on the effects of testosterone replacement therapy on emotional wellbeing. Three recent double-blind randomized controlled trials of testosterone supplementation have reported mixed effects on mood (28). The Testosterone's Effects on Atherosclerosis Progression in Aging Men (TEAAM) study, which followed 308 men aged 60 years with low or low-normal testosterone concentrations up to 36 months, found no significant difference between testosterone and placebo groups at any time point on the emotional well-being items (29). The Testosterone Vitality Trial,

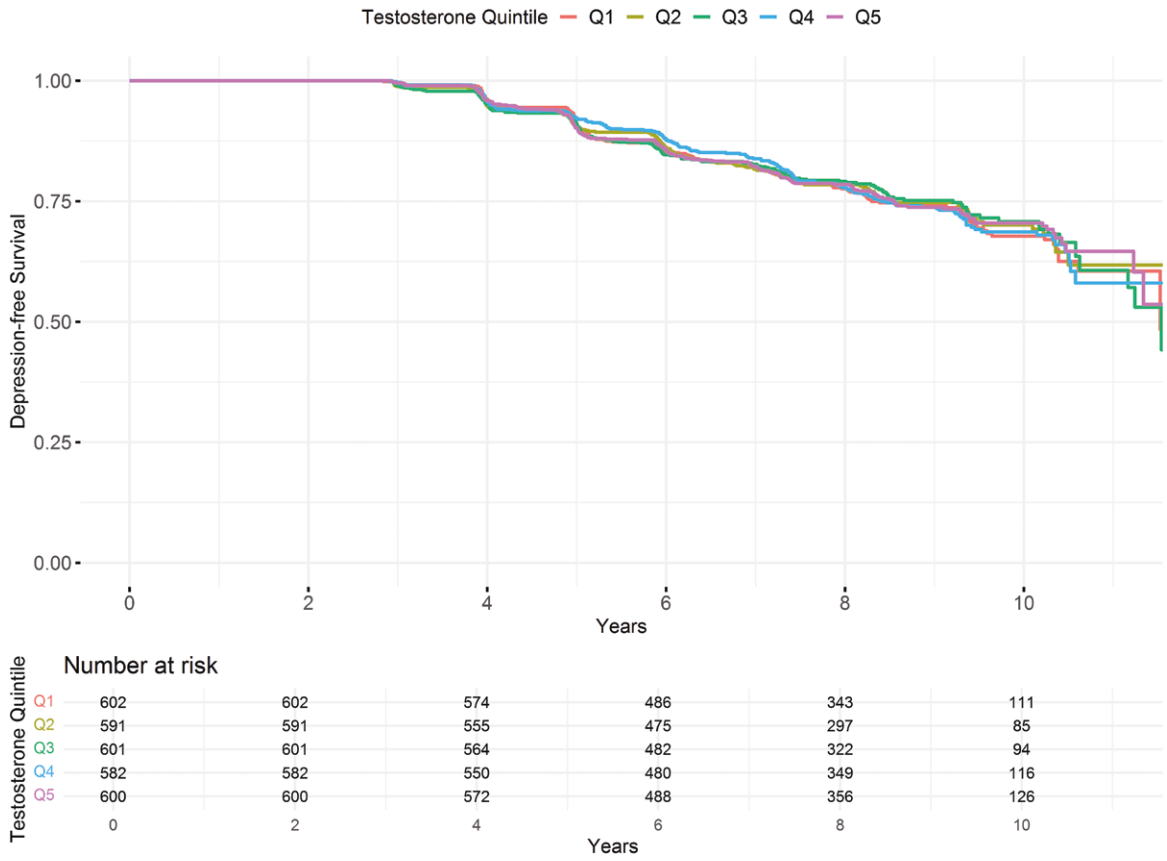

Figure 2. Kaplan–Meier curve of depression-free survival by testosterone quintile.

which included 464 men aged 65 years and older with PHQ-9 depression scores, found a difference of −0.72 (95% CI −1.20 to −0.23) favoring testosterone treatment over placebo. The PHQ-9 scale ranges from 0 to 27 so this represents a very small treatment effect (30). The Testosterone Replacement therapy for the Assessment of long-term Vascular Events and efficacy ResponSE in hypogonadal men (TRAVERSE) study, which assessed 5 204 men aged 45–80, found no difference between testosterone and placebo groups on the rate of low-grade persistent depressive disorder but found a difference of −1.3 (95% CI −2.3 to −0.2) at 24 months on the HIS-Q Mood scale, representing a small treatment effect (31). These findings suggest that while testosterone therapy may benefit specific individuals, its overall effect on mood and depressive symptoms in eugonadal older men is limited.

Our findings, alongside the recent trial evidence outlined above, have important implications for the clinical use of testosterone therapy in older men. Testosterone therapy has seen a significant rise in prescriptions, particularly in men without clear indications of hypogonadism (2). Given the lack of consistent evidence linking testosterone concentrations to depression, caution should be exercised when considering testosterone supplementation for mood disorders. Although testosterone therapy may be warranted for men with clinically confirmed hypogonadism, the clinical trial evidence does not support its widespread use as a treatment for depression in older men.

A key strength of our study is its large sample size, long follow-up period, and annual measurement of depression symptoms, allowing for robust estimation of the relationship between testosterone and depression. Additionally, as the cohort continued from a randomized controlled trial, there was greater uniformity in the enrolled participants and a reduced likelihood of residual confounding. Our analyses adjusted for a range of confounding variables, including cognitive function, physical activity, and socioeconomic status. This level of adjustment was not possible in many previous studies, including being unable to account for prostate cancer or testosterone deprivation or replacement (32).

There were several limitations. Foremost, the CES-D-10 score was used as a proxy for clinical depression (except where hospitalization for depression occurred), which may have resulted in some misclassification of depressive symptoms. The use of a CES-D score threshold of 8 to define depression was justified based on the association between low-grade persistent depressive disorder (31). However, this cut-score may have inadvertently led to misclassification of subclinical symptoms, or normal sadness (33), which could bias the results. However, sensitivity analyses with higher cut-scores found no association between testosterone concentrations and depression. A more general limitation of depression screening tools is that testosterone drives a range of dominance behaviors (34) and depression classification tools may not be adequate to delineate what reflects trait-dependent factors and what reflects state-dependent factors. The use of antidepressant treatment as a proxy of the presence of depression might have included some participants using antidepressants for other indications like anxiety.

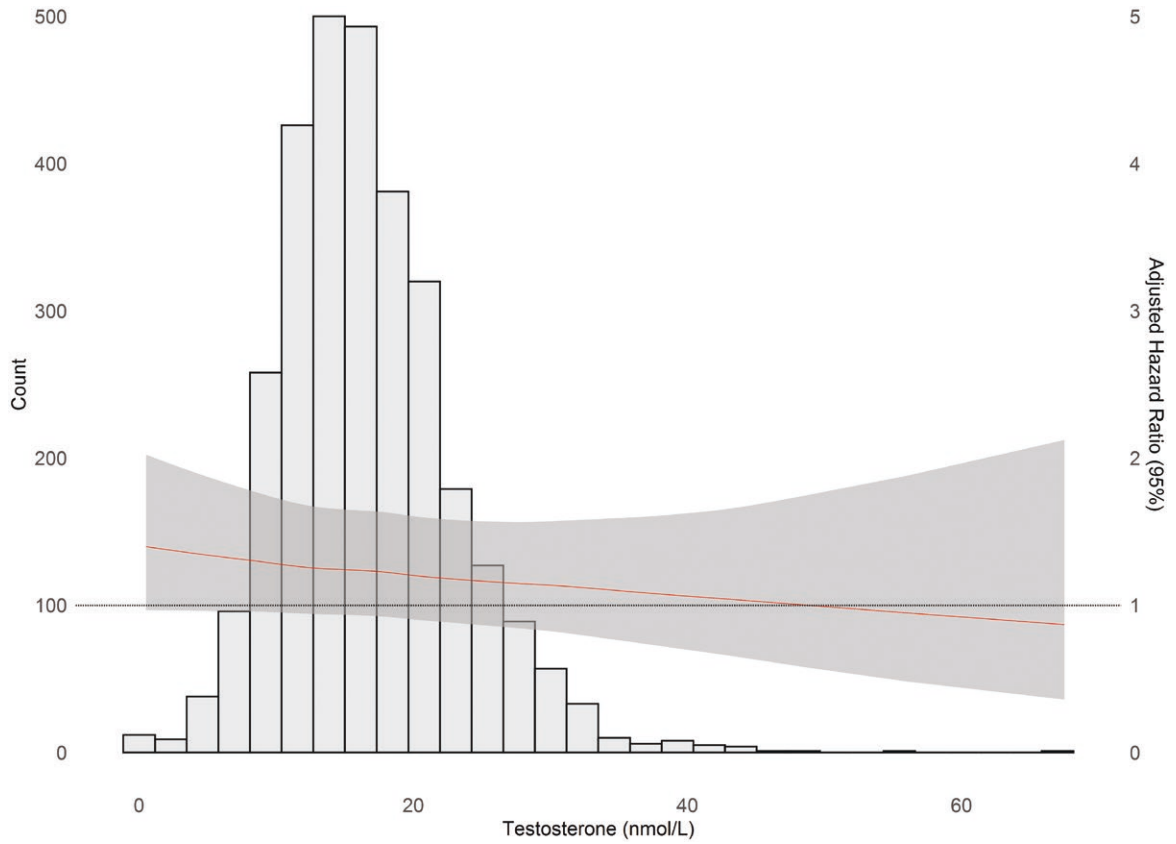

Figure 3: Restricted cubic spline curve of testosterone and adjusted hazard ratios for incident depression.

Regarding testosterone measurement, a limitation of our study is that we only measured testosterone concentrations and did not have sex-hormone binding globulin, dihydrotestosterone or free testosterone concentrations. It also did not include measures of androgen receptor polymorphisms (35), which may mediate individual variability in mood responses to testosterone. Finally, testosterone concentrations fluctuate throughout the day, and a single measurement may not accurately reflect an individual's average testosterone concentration (36). Blood samples were collected as nonfasting specimens, which may have contributed to lower testosterone concentrations and increased variability in measurements (37). However, given the large sample size, this variation can be considered random error and is unlikely to affect inference. Other limitations include the observational nature of the study precluding any determination of causality.

In conclusion, these findings suggest that testosterone is not an independent risk factor for the later development of depression in older men. Although hypogonadism may contribute to mild depressive symptoms (38), the role of testosterone in the etiology of major depression appears to be limited. Future studies should explore the potential for testosterone to affect specific depressive symptoms in real-time, using ecological momentary assessment, particularly symptoms related to social behavior, anxiety, and fearfulness.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None.

Data Availability

These data are not publicly available but they can be requested by research groups. Expressions of interest to analyze data from the ASPREE clinical trial and/or ASPREE-XT observational cohort, are co-ordinated through the ASPREE Access Management Site.

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