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Plasma testosterone and the course of major depressive disorder in older men and women

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Conflict of interest: All authors declare there are no potential conflicts of interest to disclose

Abstract

Objective: To investigate associations between testosterone levels and major depressive disorder (MDD) in older men and women.

Design: Cross-sectional, two-year prospective analyses within the Netherlands Study on Depression in Older persons (NESDO) cohort study.

Setting: Multi-site naturalistic setting.

Participants: The 469 participants comprised 350 patients with MDD and 119 non-depressed participants in the comparison group; mean age 70.5 ± 7.3 years, 166 (35.4%) were men.

Measurements: MDD was assessed by the Composite International Diagnostic Interview (CIDI). Baseline plasma total testosterone and sex hormone binding globulin (SHBG) were assessed to calculate free testosterone. The Inventory of Depressive Symptomatology (IDS) was assessed every 6 months.

Results: Whereas SHBG levels did not differ between the depressed/non-depressed groups ($F(1,149)=0.075$, $P=0.78$), men with MDD had lower mean total and free testosterone levels than the comparison group in the multivariate adjusted analyses ($F(1,150)=7.249$, $P=0.008$, Cohen's $d=0.51$; and $F(1,149)=8.548$, $P=0.004$ Cohen's $d=0.55$). This could be ascribed to lower testosterone in men with 'pure' MDD and not in men with MDD and comorbid anxiety. Nine men (5.4%) had a total testosterone level < 8 nmol/L of whom 8 suffered from MDD. In women, hormone levels showed no significant difference between the groups. In men (using all 5 measurement points during follow-up) baseline free testosterone was inversely associated with depression severity in the adjusted analyses ($\beta = -0.15$; $t(151) = -2.15$; $P=0.03$).

Conclusions: Testosterone levels were lower in men with MDD compared to healthy men

after adjustment for confounders, such as body mass index. No significant associations were found in women.

Key words: testosterone; major depressive disorder; older persons; sex-hormone binding globulin; anxiety disorders

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Introduction

Testosterone is a sex steroid hormone that is primarily responsible for androgenizing effects, such as the development of sexual characteristics. In men, testosterone is mainly produced by the Leydig cells of the testes and in women by the ovaries and adrenal glands. Women have about 10 to 20-fold lower levels of total and free testosterone than men. Of the total blood testosterone, approximately 98% is bound to protein, with 50-60% being tightly bound to sex hormone-binding globulin (SHBG) and 40-50% loosely bound to albumin. In men, total and free testosterone levels gradually decline with age by as much as 40% between the ages of 40 and 70 years.(1, 2) In aging older men, very low levels of testosterone are frequently reported. When a low testosterone level (i.e., total testosterone levels < 8 nmol/L and free testosterone levels < 160 pmol/L) is accompanied by symptoms such as loss of libido and erectile dysfunction this is called late-onset hypogonadism.(3) In women, testosterone levels may also decline during aging.(4)

Androgen and estrogen receptors are located throughout the human body, including the limbic systems in the brain.(5, 6) Therefore, it is not surprising that not only health problems like impotence, osteoporosis and muscle weakness are associated with androgen deficiency in old age, but also fatigue, sleep disturbances, irritability(7), and a depressed mood.(8-10) It is suggested that the age-related decline in testosterone levels plays a role in the occurrence of major and minor depression in older men.(11) Depression in late life often has an unfavorable prognosis, such as higher relapse rates, somatic comorbidity and a higher risk of mortality.(12) In Europe, a mean level of 12.3% of older women and 8.6% of older men suffer from depression, as assessed using a computerized semi-structured clinical interview.(13) Besides this increased likelihood of suffering from major depressive disorder (MDD) in women, women also experience on average a higher severity of symptoms and

more subjective distress than men,(14) suggesting a role for the protective effect of androgens.

Previous cross-sectional and longitudinal studies on testosterone and depressive symptoms in older men used self-report instruments like the Geriatric Depression Scale (GDS),(15, 16) the Beck Depression Inventory (BDI),(8) the Center for Epidemiologic Studies Depression scale (CES-D) (17) or the Patient Health Questionnaire (PHQ), as well as electronic clinical records, (18-20) to assess depressive symptoms.

Three prospective cohort studies found no relationship with new depressive symptoms during 3-year follow-up,(15) or a relationship with only free but not total testosterone(17) or, in contrast, with total but not free testosterone.(20) Two other longitudinal studies in adult men (age 45 and ≥ 50 years, respectively) found that low total and free testosterone were associated with a higher incidence of clinical depression, as ascertained from clinical records.(18, 19) However, most cross-sectional and prospective studies lacked information on the clinical diagnoses of MDD as assessed through standardized clinical interviews for DSM-IV Axis I disorders, such as the Composite International Diagnostic Interview (CIDI).(8, 15-21) Such interviews place additional requirements on the training of assessors and are more time consuming and costly; however, self-reported outcomes and outcomes derived from trained assessors are not equivalent.(22) Studies focusing on associations with MDD, as assessed with standardized clinical interviews, are scarce - especially in women.(23, 24)

The Netherlands Study on Depression in Older persons (NESDO) is a longitudinal cohort study with 510 participants aged ≥ 60 years, that was established to investigate determinants of depressive disorders in older people.(25) The present study aimed to investigate the association between levels of plasma total and free testosterone, and the presence and course of MDD in older men and women. Our hypothesis was that lower free testosterone levels would be associated with MDD in older men and possibly also in women,

and that low testosterone levels would be associated with a poorer depression course over the 2-year follow-up period.

MDD is often accompanied by high levels of anxiety symptoms and anxiety diagnoses. Anxiety disorders and social anxiety symptoms were associated with lower salivary testosterone levels in adult women.(26) However, it remains unclear whether anxiety symptoms based on symptom severity scales (i.e., a dimensional approach), or as MDD with a concurrent anxiety diagnosis (i.e., syndromal approach)(27), affect the possible inverse association between testosterone and MDD in older men and women.

Method

Study participants

Data were used from the NESDO, a multi-site naturalistic prospective cohort study. Details on this study and recruitment of participants are published.⁽²⁵⁾ In summary, from 2007-2010, 510 depressed and non-depressed older persons were recruited in five regions in the Netherlands. Older persons suffering from late-life depression were recruited from mental healthcare institutes and via general practitioners (GPs) in order to include depressed persons in different stages of severity and development. Excluded from the study were older persons with insufficient Dutch language skills, a psychotic disorder, a bipolar disorder, or dementia. For the comparison group, non-depressed persons with no lifetime depression or other serious psychiatric disease were recruited via their GPs. Thereby, the baseline NESDO sample (n=510; age range 60-93 years) consisted of 378 depressed (according to the DSM-IV-R criteria) and 132 non-depressed participants.

Excluded from the study were 23 participants with incomplete data on sex hormone levels and 18 participants with minor depression or dysthymia without MDD, resulting in 469 (92%) participants for the present analysis. These participants were categorized into two groups according to their 6-month diagnosis of psychopathology: 119 non-depressed participants in the comparison group and 350 patients currently suffering from MDD. The latter group was further divided into 213 patients currently suffering from 'pure' MDD and 137 patients currently suffering from both MDD and comorbid anxiety disorder, i.e., general anxiety disorder (n=34), panic disorder (n=25), agoraphobia (n=35), and social phobia (n=64).

All participants gave written informed consent. The study protocol of NESDO has

been approved by the Ethical Review Board of the VU University Medical Center and, subsequently, by the local ethical review boards.

Measurements

The assessment included written questionnaires, several interviews, and a medical examination.⁽²⁵⁾ At baseline and at 2-year follow-up, the past six months diagnoses of MDD and comorbid anxiety disorders according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) criteria were assessed with the CIDI (WHO version 2.1), a structured clinical interview conducted by trained research assistants. The severity of depressive symptoms was assessed every six months with the Inventory of Depressive Symptoms-Self Report (IDS-SR).⁽²⁸⁾ For 28 symptoms, severity and frequency were rated on a scale from 0-3; total scores ranged from 0-84 with higher scores indicating more severe depression. The severity of anxiety was measured every 6 months using the self-report Beck Anxiety Index (BAI).⁽²⁹⁾ At each of the five subsequent measurement points, data on the IDS and BAI were available for 462, 418, 374, 371 and 369 participants, respectively.

The following sociodemographic and psychopathology characteristics were determined to be potential confounders: age, level of education, physical activity, alcohol use, smoking behavior, body mass index (BMI), number of prevalent chronic illnesses, and the use of androgen-affecting medication.^(3, 8) Of all 469 participants, 19 were using corticosteroid (systemic and inhaled corticosteroid therapy which may reduce testosterone levels), 6 (women) were using estrogen supplementation, 2 were using ketoconazole (which may block testosterone synthesis), and 2 were using spironolactone (which has anti-androgen effects). No participants were using androgens or anti-androgenic treatment (e.g., for prostate cancer).

The International Physical Activities Questionnaire (IPAQ) was used to measure

physical activity, which was measured in terms of time spent (minutes or hours, number of days/week) on different activities. Participants were then categorized into one of three groups with low, moderate or high activity level.(30)

The number of chronic diseases was determined using a questionnaire previously used in the Longitudinal Aging Study Amsterdam. Self-reports on selected chronic diseases proved to be fairly accurate.(31) Participants were asked whether they had previously, or currently, suffered from any of the following chronic diseases; cardiac diseases, peripheral atherosclerosis, stroke, diabetes mellitus, COPD, arthritis, cancer, or any other disease.

The use of alcohol was assessed using the Alcohol Use Disorders Identification Test (AUDIT) (32) and smoking behavior was evaluated with standard questions (e.g. number of years of smoking). Level of education was defined by the years of attained education. BMI was calculated from measured height/weight, and current medication use was determined by registering the medication that participants brought in using the WHO Anatomical Therapeutic Chemical (ATC) classification. Antidepressant medication was categorized into three dichotomous variables: tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRIs), and other antidepressants.

Testosterone

Fasting blood samples were collected in the morning during baseline measurements (between 8:10 and 10:45 h) with the average time of sampling at 9:04 (SD 0:23) h. From each participant 47.5 mL blood (in heparin, EDTA and serum tubes) was drawn. EDTA plasma was stored at -80°C and was thawed for the first time to determine total testosterone and SHBG concentrations (Endocrine Laboratory of the VU University Medical Center, Amsterdam, NL).

Testosterone was measured using a second-generation testosterone assay without

extraction (Architect i2000 analyzer, Abbott Diagnostics, Abbott Park, IL, USA) which, in men, showed a strong correlation with our ID-LC-MS/MS testosterone assay.(33) In women, correlation with the ID-LC-MS/MS testosterone assay was weaker: $r=0.903$.(34) In women, single testosterone values may be less reliable because of their lower mean levels, the day-to-day variation in testosterone levels, and the 20% upward bias (regression line: $y = 1.095x + 0.104$ nmol/L) that was found against the ID-LC-MS/MS.(34) This second-generation testosterone assay does not suffer from cross-reactivity by other steroid hormones (as does the first-generation assays) and is not influenced by SHBG concentrations.(35) Intra-assay variation at 0.2, 2 and 20 nmol/L was 8%, 4% and 4%, respectively, and inter-assay variation at the same concentrations was 10%, 6% and 6%, respectively. The lower limit of quantification was 0.45 nmol/L. Of the 303 women, in 22 (7.3%) the total testosterone level was ≤ 0.44 nmol/L and for these women we imputed the value of 0.44 nmol/L.

SHBG was measured using an automated immunometric assay (Architect i2000 analyzer, Abbott Diagnostics). Intra-assay and inter-assay variation was 4-6% over the whole concentration range. The lower limit of quantitation was 2 nmol/L.

Free testosterone, i.e. the amount of testosterone not bound to either SHBG or albumin (assumed to be 43 g/L), was calculated using the Vermeulen method (36) and also with the formula of Sartorius et al..(37)

Statistical analysis

Sociodemographic and psychopathology characteristics were analyzed according to the psychopathology groups using descriptive statistics. For categorical variables the number (percentages), and for continuous variables the mean with standard deviation (SD), are presented. As men have serum total and free testosterone levels that are 10-20 times higher than those found in women, and possible associations with depression may differ between

men and women, all further analyses were conducted stratified separately for men and women. *T*-tests and analyses of covariance (ANCOVA) were used to assess differences in androgen and SHBG levels between participants with a MDD and for those with a comorbid anxiety disorder, compared with the comparison group. The adjusted models took into account: age, level of education level, BMI, physical activity, smoking status, alcohol use, number of chronic diseases and androgen-affecting medication (i.e., oral or topical corticosteroids, ketoconazole or spironolactone; $n=24$). In case of significant differences, Cohen's d was calculated as an effect size measure (i.e., difference between the means divided by the pooled SD). As total and free testosterone levels were positively skewed in men ($n=166$; Skewness=0.669; SE=0.188; and Skewness=0.484; SE=0.189; respectively) and women ($n=303$; Skewness = 0.991; SE 0.140 and Skewness=1.374; SE=0.140; respectively), group comparisons were repeated using non-parametric analyses by independent-samples Mann-Whitney U test in unadjusted analyses (Table 2), and using naturally $\log_e$ transformed total and free testosterone levels in adjusted analyses. Using linear regression analyses, associations between total and free testosterone as independent variables, and IDS and BAI as dependent variables, at baseline were explored. Associations with psychotropic medication were also analyzed (i.e., SSRIs, TCAs, other antidepressants, antiepileptics, and antipsychotics).

Two approaches were used for the longitudinal analyses. First, using multilevel analysis (i.e., linear mixed models), associations between total and free testosterone in relation to IDS and BAI scores over the 2-year follow-up were analyzed. A compound symmetry covariance model was used. Participants were measured up to five times; thus, the two-level structure consisted of the observations (i.e., lower level) and the participant (i.e., higher level). Testosterone levels and time were added as fixed factors. The 'Testosterone' $\times$ 'time' interaction was added to the model to test whether low total and free testosterone

levels were associated with a poorer course of IDS and BAI scores over time. Next, non-linear associations were analyzed in multilevel analyses by total testosterone squared and free testosterone squared to the appropriate fully adjusted models. Subsequently, all previously mentioned baseline covariates were added to the model as fixed factors. The figures present the estimated marginal means (with standard error; SE). Second, binary logistic regression analysis was used to analyze the associations between baseline total and free testosterone and non-remission for depression and anxiety disorders. Findings are based on 120 men and 230 women with MDD at baseline, with the 6-months diagnosis of MDD or anxiety disorders at 2-year follow-up as the independent variables.

All tests were two-tailed with $P < 0.05$ denoting statistical significance. Analyses were performed with SPSS version 22.0 (IBM Corp., NY, USA).

Results

The study sample ($n=469$) consisted of 350 depressed and 119 non-depressed older persons; the mean (SD) age was 70.5 ± 7.3 (range 60-93) years and 166 were men (35.4%). Of the 350 depressed participants, 213 suffered from pure MDD and 137 from both MDD and anxiety disorder (Table 1). Participants with MDD were more likely to be abstinent of alcohol than the comparison group. Participants in both psychopathology groups were less physically active and more often smokers than in the comparison group. As expected, mean scores on the IDS and BAI were lower in the comparison group than in the group with psychopathology.

Cross-sectional associations

Table 2 shows the total and free testosterone levels in the MDD group and in the comparison group, separately for men and women. In both men and women, the numbers of chronic somatic diseases were unrelated to serum total and free testosterone. In the unadjusted analyses, men with MDD showed no significant difference in mean total and free testosterone levels as compared to the comparison group (Table 2). However, in the adjusted analyses, men with MDD had lower mean total and free testosterone levels than the comparison group ($F(1, 150)=7.249$, $P=0.008$, Cohen's $d=0.51$; and $F(1, 149)=8.548$, $P=0.004$ Cohen's $d=0.55$) while SHBG levels did not differ between the groups ($F(1,149)=0.075$, $P=0.78$). Adjusted analyses using log-transformed total and free testosterone levels yielded similar findings (data not shown).

Adjusted differences for total and free testosterone between MDD patients and the comparison group were largely due to differences between those MDD patients without anxiety disorders (Figure 1). With regard to total and free testosterone, men with pure MDD

had lower mean levels than the comparison group (Cohen's $d=0.66$ and 0.66 , respectively; Figure 1). Men with MDD and comorbid anxiety showed no significant differences in total and free testosterone levels as compared to the comparison group (Cohen's $d=0.21$ and 0.33 , respectively; Figure 1). Of the 166 male participants, 9 (5.4%) had a total testosterone level < 8 nmol/L of whom 8 (89%) suffered from MDD. In women, in the adjusted analyses those with MDD tended to have a lower mean total testosterone (but not a free testosterone) level than the comparison group (Table 2).

Baseline free testosterone was inversely associated with baseline IDS in men ($\beta = -0.17$; $t(149) = -2.22$; $P=0.03$) but not in women ($\beta = -0.07$; $t(267) = -1.22$; $P=0.23$), independent of covariates (i.e., age, level of education level, BMI, physical activity, smoking status, alcohol use, number of chronic disease, and androgen-affecting medication). No significant associations were found with severity of anxiety (i.e., BAI) in men or in women.

Prospective associations

Multilevel regression models were used to analyze the association between baseline testosterone and IDS and BAI scores over time. In adjusted analyses of multilevel regression models (Table 3 and Figure 2), higher baseline free testosterone was associated with lower IDS scores over time in men ($\beta = -0.15$; $t(151) = -2.15$; $P=0.03$) but not in women ($\beta = -0.09$; $t(269) = -1.63$; $P=0.10$). No significant associations were found for the severity of anxiety symptoms (i.e., BAI score). In multivariate models, beta coefficients became stronger in men; this was largely explained by the covariate BMI, which was inversely associated with total testosterone in men ($\beta = -0.297$; $t(164) = -3.98$; $P<0.001$), but not in women ($\beta = 0.058$; $t(300) = -1.01$; $P=0.32$).

When testing for nonlinear associations, we added the quadratic terms for (free)

testosterone to the models. It was found that the quadratic terms were not significant, except for total testosterone in women ($\beta = -0.117$; $t(266) = -2.27$; $P=0.02$; Figure 3); this suggests that, in women, testosterone levels ≥ 2.0 nmol/L are associated with a lower mean depression severity score. However, Figure 3A also suggests a threshold effect for total testosterone level in men of ≤ 10 nmol/L. When men were dichotomized according to a baseline total testosterone level of < 8 nmol/L and ≥ 8 nmol/L, the 9 men with low total testosterone had more depressive symptoms than the 157 remaining men (mean IDS score 27.5; SE 4.0 vs. 19.4; SE 0.9; $P<0.001$). Moreover, when we removed the 9 men with total testosterone < 8 nmol/L from the multilevel regression analysis, the adjusted inverse association became weaker by 36%. The beta coefficient was -0.121 ($t(150) = -1.707$; $P=0.09$) and became -0.077 ($t(141,7) = -0.961$; $P=0.34$). The inverse association with free testosterone also became slightly weaker by 18% ($\beta = -0.150$; $t(151) = -2.148$; $P=0.03$, became $\beta = -0.123$; $t(142.8) = -1.598$; $P=0.11$).

In all multilevel regression models the interaction terms of total and free testosterone over time did not add significantly to the models; thus, testosterone did not influence the differences in the course of IDS or BAI scores over the 2-year study period. Similar findings were found in the subgroup of 350 depressed older persons at baseline (data not shown). In these 350 older persons suffering from MDD at baseline, at follow-up 108 had a diagnosis of pure MDD, and 56 had a diagnosis of MDD and a comorbid anxiety disorder.

Of the 91 men with MDD at baseline and CIDI assessment at follow-up, 55 (45.8%) had remitted from MDD at 2-year follow-up. Also, of the 16 men who suffered from any type of anxiety disorder at follow-up, 13 also had an anxiety disorder at baseline (13/16; 81.3%). Of the 175 women with MDD at baseline and CIDI assessment at follow-up, 103 (44.8%) had remitted from MDD at 2-year follow-up. Of the 40 women who suffered from any type of anxiety disorder at follow-up, 25 also had an anxiety disorder at baseline (25/40; 62.5%).

Binary logistic regression analysis showed no association between free testosterone and non-remission for depression of anxiety disorders (Supplementary Table 1). Specifically, in the adjusted analyses, baseline free testosterone was not associated with MDD at 2-year follow-up either in men (odds ratio [OR] 1.00; 95% CI: 0.95-1.05; Wald [1] = 0.04; P=0.85) or in women (OR 1.03; 95% CI: 0.98-1.08; Wald [1] = 0.96; P=0.33).

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Discussion

In this study, low levels of total and free testosterone were associated with the presence of MDD in older men compared to the comparison group in the adjusted analyses but not in the crude analyses. Because the significant differences were mostly found in the adjusted analyses (and mainly for BMI), these results should be interpreted with caution. Additional analysis revealed that this difference was mainly due to lower total and free testosterone levels in men with MDD only, and not in those with a comorbid anxiety disorder. It is noteworthy that the majority of men with total testosterone <8 nmol/L also had MDD (i.e., 8 of 9 men). The baseline inverse association between free testosterone and severity of depressive symptoms persisted over the 2-year follow-up in men in the adjusted analyses only. Thus, although men with lower testosterone appeared to have more severe depression over time, no associations were found between testosterone and the rate of change in the IDS or BAI scores. In women, although lower total testosterone levels were weakly associated with the presence of MDD, this association was not found for free testosterone.

Our findings are largely in line with other studies suggesting an inverse association between depressive symptoms and free (or bioavailable) testosterone levels.(8, 15-21, 23) However, ours is among the first with data on total and free testosterone levels in older men and women with DSM-IV diagnoses of pure MDD, and MDD combined with comorbid anxiety disorders. Most previous studies were performed in population samples and lacked formally diagnosed MDD, because self-report instruments or medical records were used to measure depression or depressive symptoms.(8, 15-21, 23) Some studies showed an association between testosterone and depression only for the lowest testosterone levels, thereby suggesting a threshold effect.(17, 38) Our findings are in line with this idea that men with low testosterone are at increased risk to suffer from MDD and exhibit more depressive

symptoms during follow-up. The inverse association found in men between free testosterone levels and IDS scores suggests that lower free testosterone levels were associated with more severe depression. This is similar to findings in middle-aged men where this inverse association was found for both the BDI and the Hamilton Depression Rating Scale score.(21)

We also contribute to findings on testosterone in older patients with MDD and comorbid anxiety, by showing that their testosterone levels did not differ from our comparison group. Symptoms of anxiety have been associated with lower total and free testosterone levels in middle-aged and older men.(39) However, it is difficult to compare these latter findings with our results, as participants who report anxiety symptoms in self-report scales differ from patients with formally diagnosed (comorbid) anxiety disorders.(27)

Studies on the association between total/free testosterone and depression in older women are scarcer than in men and show conflicting results. The few studies that included women often lacked formally ascertained DSM-IV diagnoses.(23, 24, 40) Lower salivary testosterone levels are associated with both depression and (social) anxiety in younger and middle-aged women,(26, 40) while our results show that total testosterone levels were lower only in older women with MDD without comorbid anxiety.

Low testosterone may be both a result (due to the disease process, its behavioral result and psychotropic medication) and a causal factor in the etiology of depression. The underlying mechanisms of how testosterone affects the risk of depression are only partly understood.(5, 41) Testosterone crosses the blood-brain barrier where it affects neurogenesis, neural plasticity, and the neurotransmitters gamma-aminobutyric acid, dopamine and serotonin. It has both organizational effects (i.e., permanent masculinization during critical periods of development) and activational effects (i.e. more acute and transient effects throughout life). The brain expresses sex steroid receptors,(5, 6) and both androgen- and estrogen-dependent mechanisms are involved. Testosterone primarily acts through its 5 α -

reduced product dihydrotestosterone and the aromatase-derived estradiol in corticolimbic neural circuits, such as the dorsolateral prefrontal cortex, the amygdala, and the hippocampus.(5, 6, 41) In our sample of older men, conversion by adipose tissue aromatase of testosterone into estradiol may also explain the inverse association between BMI and total testosterone.

Strengths of this study include: i) assessment of the diagnosis based on the DSM-IV-R, ii) use of the validated CIDI, iii) use of many potential confounders, and iv) the inclusion of older women. The present study also has several limitations. First, a single blood sample was measured per participant, whereas serial blood samples would have been preferable given the intra-individual variability. Moreover, a direct testosterone immunoassay was used without extraction; this is a significant limitation, especially in women who have low levels of total testosterone. This resulted in 7.3% of the values being left-censored in women, and these values were imputed with the value of the lower limit of detection, which may have introduced bias. Second, since there were no follow-up testosterone levels, we could not establish whether men with chronic low testosterone had persistently more severe depression than men with chronic higher testosterone levels. Third, because participants in NESDO were almost entirely Caucasian, our findings need to be replicated in participants from other ethnic origins. Fourth, luteinizing hormone levels were not measured; this acts on the interstitial Leydig cells of the testes, stimulating them to produce testosterone. Fifth, although the Vermeulen formula is frequently used, calculation of free testosterone is reported to deviate (over/underestimation) from direct measurements (e.g. isotope dilution equilibrium dialysis, or centrifugal ultrafiltration).(42) When we used the more accurate formula of Sartorius et al.,(37) estimates of free testosterone were about 22% lower in men, suggesting an upward bias of the results derived from the Vermeulen formula. However, after using both these formulae, the significant differences found between the groups were similar. Nevertheless,

calculations using various formulae, as well as the clinical value of derived free testosterone calculations, remains debatable and depends mainly on SHBG levels. The idea that free testosterone is more biologically active than that bound to SHBG and albumin has been criticized.⁽⁴³⁾ Therefore, the assessment of total testosterone should be considered the most useful measure in epidemiological and clinical research in psychiatry. Finally, the multiple stratified analyses without correcting for multiple testing may have increased the chance of a type I error.

Of the earlier prospective studies on testosterone in adult men (18-20) and older men,^(15, 17) most found that low testosterone predicted the onset of depressive symptoms or incident clinical diagnoses of depression. However, the findings were inconsistent: for example, one study found no association,⁽¹⁵⁾ two studies found associations only for total testosterone,^(18, 20) one study found associations only for free testosterone,⁽¹⁷⁾ and another study based its findings on combined free and total testosterone.⁽¹⁹⁾ It should be noted that these latter studies included men free of depressive symptoms or depression at baseline, whereas in our study most of the older men and women (n=350) suffered from MDD at baseline.

In conclusion, the present study shows that total and free testosterone levels were lower in men with MDD than in healthy participants, especially when there was no comorbid anxiety disorder. Depressive symptoms were most pronounced in men with total testosterone < 8 nmol/L. Moreover, low free testosterone in men was related to the course of depression (i.e., a course with chronically higher depression severity). However, there were no associations between testosterone and the rate of change in IDS or BAI scores. This latter finding corroborates the absence of a clinical benefit from testosterone supplementation vs. placebo in patients with MDD and with low testosterone levels.^(5, 44, 45)

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Figure legends

Figure 1.

Bar graphs showing the mean levels of (A) total, (B) and free testosterone levels in the following groups of men: i) the comparison group, ii) patients with MDD without a comorbid anxiety disorder (i.e., ‘pure MDD’), and iii) patients with MDD and a comorbid anxiety disorder. Error bars represent standard errors. Assessed by analyses of covariance (ANCOVA), adjusted for age, level of education, body mass index, physical activity, smoking status, alcohol use, number of chronic diseases and androgen-affecting medication. Test statistics: [A] $F(1, 113) = 10.732$ and $F(1, 69) = 0.023$ for total testosterone; [B] $F(1, 112) = 12.492$ and $F(1, 69) = 0.724$ for free testosterone.

Figure 2.

Time course of depressive symptoms (i.e., Inventory of Depressive Symptomatology [IDS] scores) at baseline and at 0.5, 1, 1.5 and 2-year follow-up according to tertiles of free testosterone (FT) levels at baseline. These analyses were done separately for men [A and C] and women [B and D]. The estimated marginal means (with error bars representing standard errors) are presented. To facilitate presentation of data, FT was categorized into tertiles and calculated separately for men and women. All data were adjusted for age, level of education level, BMI, physical activity, smoking status, alcohol use, number of chronic disease, and androgen-affecting medication using multilevel analyses (i.e., MIXED analysis in SPSS) with continuous total testosterone (TT) and free testosterone (FT) data (for test statistics t-values, see Table 3). The appropriate interaction terms for $TT \times \text{time}$ and $FT \times \text{time}$ were not significant.

Figure 3.

Adjusted mean values of depressive symptoms (i.e., Inventory of Depressive Symptomatology [IDS] scores) using all data from (up to) 5 time points according to binned total (TT) and free testosterone (FT) levels at baseline. These analyses were done separately for men [A and C] and women [B and D]. The size of the boxes matches the number of participants in that particular bin. Standardized beta coefficients and p-values are based on the continuous values in order not to lose statistical power; binned values of TT and FT were used only to facilitate presentation of data. All data were adjusted for age, level of education level, BMI, physical activity, smoking status, alcohol use, number of chronic disease, and androgen-affecting medication using multilevel analyses (i.e., MIXED analysis in SPSS) with continuous total testosterone (TT) and free testosterone (FT) data, and their quadratic terms (TT^2 and FT^2 , respectively). Test statistics: [A] $t(149) = -1.736$ and $t(146) = 0.951$; [B] $t(268) = -1.745$ and $t(266) = -2.270$; [C] $t(149) = -2.206$ and $t(147) = 0.840$; and [D] $t(267) = -1.596$ and $t(267) = -0.524$.

Table 1. Sociodemographic and clinical characteristics of participants of NESDO according to gender

	Total	Males	Females
	n	(n=166)	(n=303)
Sociodemographic characteristics:			
Age (years): mean $\pm$ SD	469	69.8 $\pm$ 7.4	70.9 $\pm$ 7.3
Level of education (years): mean $\pm$ SD	469	11.8 $\pm$ 3.8	10.5 $\pm$ 3.3
BMI (kg/m ²): mean $\pm$ SD	468	26.5 $\pm$ 3.7	26.4 $\pm$ 4.5
Physical Activity Categorical Score: n (%)	456		
• Low		44 (26.7)	79 (27.1)
• Moderate		74 (44.8)	106 (36.4)
• High		47 (28.5)	106 (36.4)
Smoking status: n (%)	466		
• Never		23 (13.9)	116 (38.7)
• Former		112 (67.5)	114 (38)
• Current		31 (18.7)	70 (23.3)
Alcohol use: n (%)	462		
• No use (<1 unit per month)		42 (25.6)	108 (36.2)
• ≤ 3 units per week		65 (39.6)	110 (36.9)
• > 3 units per week		57 (34.8)	80 (26.8)
Psychosomatic characteristics:			
Major depressive disorder: n (%)	469	120 (72.3)	230 (75.9)
Number of chronic diseases: mean $\pm$ SD	468	1.6 $\pm$ 1.2	2.0 $\pm$ 1.4
IDS: mean $\pm$ SD	462	22.8 $\pm$ 14.4	25.4 $\pm$ 15.8
Beck Anxiety Index: mean $\pm$ SD	440	12.1 $\pm$ 10.1	14.8 $\pm$ 12.3
Medication use: n (%)			
• SSRI	468	27 (16.3)	65 (21.5)
• TCA	468	22 (13.3)	59 (19.5)
• Other antidepressant	468	43 (25.9)	57 (18.9)
• Antiepileptic	469	10 (6)	14 (4.6)
• Antipsychotic	469	17 (10.2)	34 (11.2)
• Androgen-affecting medication	469	10 (6.0)	19 (6.3)

NESDO, Netherlands Study on Depression in Older persons; SD, standard deviation; BMI, body mass index; IDS, Inventory of Depressive Symptomatology (self-rated); SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant. Other antidepressants comprised monoamine oxidase inhibitors, serotonin-norepinephrine reuptake inhibitors, and mirtazapine.

Table 2. Baseline total and free testosterone levels according to the presence of a major depressive disorder (MDD), for men and women separately

	Comparison group	MDD	Test statistic	P-value
Men (n=166):	(n=46)	(n=120)		
Total testosterone (nmol/L)				
• Crude (median, IQR)	16.4 (11.5; 21.4)	16.2 (12.4; 19.9)	$z = -0.485$	0.63
• Crude (mean, SE)	17.5 (1.1)	16.4 (0.5)	$F(1,164)=0.969$	0.33
• Adjusted* (mean, SE)	19.1 (1.0)	15.8 (0.6)	$F(1,150)=7.249$	0.008
SHBG (nmol/L)				
• Crude (median, IQR)	38.7 (26.8; 49.0)	41.6 (31.4; 51.8)	$z = 1.524$	0.13
• Crude (mean, SE)	40.6 (2.9)	43.2 (1.5)	$F(1,163)=0.765$	0.38
• Adjusted* (mean, SE)	43.1 (2.6)	42.2 (1.5)	$F(1,149)=0.075$	0.78
Free testosterone (pmol/L), Vermeulen formula				
• Crude (median, IQR)	314 (257; 386)	298 (225; 353)	$z = -1.392$	0.16
• Crude (mean, SE)	322.4 (15.8)	294.1 (9.3)	$F(1,163)=2.521$	0.11
• Adjusted* (mean, SE)	345.6 (16.3)	287.2 (9.4)	$F(1,149)=8.548$	0.004
Free testosterone (pmol/L), Sartorius formula				
• Crude (median, IQR)	238 (195; 292)	237 (189; 268)	$z = -0.938$	0.35
• Crude (mean, SE)	245.0 (10.8)	229.6 (6.3)	$F(1,163)=1.625$	0.20
• Adjusted* (mean, SE)	264.1 (11.0)	223.5 (6.3)	$F(1,149)=9.077$	0.003
Women (n=303):	(n=73)	(n=230)		
Total testosterone (nmol/L)				
• Crude (median, IQR)	0.94 (0.74; 1.32)	0.86 (0.64; 1.14)	$z = -2.121$	0.03
• Crude (mean, SE)	1.05 (0.05)	0.93 (0.02)	$F(1,301)=5.770$	0.02
• Adjusted* (mean, SE)	1.04 (0.05)	0.92 (0.03)	$F(1,269)=3.837$	0.051
SHBG (nmol/L)				
• Crude (median, IQR)	47.1 (38.3; 58.5)	48.8 (34.6; 70.0)	$z = 0.981$	0.33
• Crude (mean, SE)	49.5 (2.4)	54.2 (1.8)	$F(1,300)=1.757$	0.19
• Adjusted* (mean, SE)	52.2 (3.2)	52.7 (1.7)	$F(1,268)=0.019$	0.89
Free testosterone (pmol/L), Vermeulen formula				
• Crude (median, IQR)	13.1 (9.7; 20.4)	11.9 (8.3; 17.5)	$z = -2.100$	0.04
• Crude (mean, SE)	15.6 (1.0)	13.4 (0.5)	$F(1,300)=4.765$	0.03
• Adjusted* (mean, SE)	15.0 (0.9)	13.6 (0.5)	$F(1,268)=1.600$	0.21
Free testosterone (pmol/L), Sartorius formula				
• Crude (median, IQR)	13.6 (10.4; 19.1)	12.1 (9.0; 16.6)	$z = -1.943$	0.052
• Crude (mean, SE)	15.3 (0.8)	13.4 (0.4)	$F(1,300)=5.277$	0.02
• Adjusted* (mean, SE)	14.9 (0.8)	13.4 (0.4)	$F(1,268)=.2486$	0.12

SHBG, sex hormone-binding globulin; IQR, interquartile range.

Analyses by independent-samples Mann-Whitney U test, or analysis of (co)variance.

* Data are means (with standard errors [SE]) by analysis of covariance (ANCOVA), adjusted for age, level of education, body mass index, physical activity, smoking status, alcohol use, number of chronic diseases and androgen-affecting medication.

1 **Table 3.** Longitudinal associations between total/free testosterone levels, and depressive and anxiety symptom severity, during the 2-year follow-up for men
 2 and women separately

	Crude model			Adjusted model		
	Beta	t-value	P-value	Beta	t-value	P-value
Men (n=164):						
Inventory of Depressive Symptomatology (IDS):						
total testosterone	-0.04	t(161)= -0.591	0.56	-0.12	t(150)= -1.707	0.09
total testosterone × time	0.00	t(548)= -0.226	0.82	0.00	t(545)= -0.173	0.86
free testosterone	-0.11	t(163)= -1.590	0.11	-0.15	t(151)= -2.148	0.03
free testosterone × time	0.00	t(549)= -0.019	0.99	0.00	t(546)= 0.045	0.96
Beck Anxiety Index (BAI):						
total testosterone	0.01	t(160)= 0.186	0.85	-0.05	t(146)= -0.780	0.44
total testosterone × time	0.00	t(546)= 0.163	0.87	0.00	t(540)= 0.063	0.95
free testosterone	-0.05	t(162)= -0.745	0.46	-0.09	t(147)= -1.355	0.18
free testosterone × time	0.02	t(548)= 0.694	0.49	0.01	t(541)= 0.548	0.58
Women (n=298):						
Inventory of Depressive Symptomatology (IDS):						
total testosterone	-0.13	t(300)= -2.502	0.01	-0.09	t(270)= -1.806	0.07
total testosterone × time	0.02	t(999)= 1.005	0.32	0.02	t(942)= 1.392	0.16
free testosterone	-0.12	t(298)= -2.366	0.02	-0.09	t(269)= -1.631	0.10
free testosterone × time	-0.01	t(996)= -0.381	0.70	0.00	t(938)= -0.279	0.78
Beck Anxiety Index (BAI):						
total testosterone	-0.10	t(294)= -1.786	0.08	-0.06	t(262)= -1.229	0.22
total testosterone × time	0.02	t(985)= 1.081	0.28	0.02	t(926)= 1.196	0.23
free testosterone	-0.09	t(292)= -1.744	0.08	-0.09	t(260)= -1.744	0.08
free testosterone × time	0.00	t(981)= -0.181	0.86	0.00	t(922)= -0.181	0.86

- 1 Data are beta coefficients assessed by linear mixed models (i.e., multilevel regression analysis), adjusted for age, level of education level, body mass index, physical activity,
- 2 smoking status, alcohol use, number of chronic disease, and androgen-affecting medication.
- 3 NB: 2 men and 5 women did not complete the IDS or BAI at any measurement point and were not included in the analysis.
- 4

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Figure 1.

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Figure 2.

Figure 3.