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Salivary testosterone: Associations with depression, anxiety disorders, and antidepressant use in a large cohort study

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ARTICLE INFO

Article history:

Received 7 September 2011

Received in revised form 21 November 2011

Accepted 22 November 2011

Keywords:

Salivary testosterone

Depression

Anxiety disorders

Antidepressants

Social phobia

Androgens

ABSTRACT

Objective: Low circulating levels of testosterone have been associated with major depression, but there is more limited evidence for differences in patients with anxiety disorders. The use of selective serotonin reuptake inhibitors (SSRIs) and other antidepressants is associated with sexual side effects, warranting testing for interactions with testosterone.

Methods: Data are from 722 male and 1380 female participants of The Netherlands Study of Depression and Anxiety (NESDA), who were recruited from the community, general practice care, and specialized mental health care. Depressive and anxiety diagnoses were assessed using the DSM-IV Composite International Diagnostic Interview. To smooth the episodic secretion, the four morning saliva samples per participant and the two evening samples were pooled before testosterone analysis.

Results: Morning median testosterone levels were 25.2 pg/ml in men and 16.2 pg/ml in women, with lower evening levels of 18.2 and 14.1 pg/ml, respectively. Significant determinants of testosterone levels were sex, age, time of the day, use of contraceptives, and smoking status. Female patients with a current (1-month) depressive disorder (effect size 0.29; $P = 0.002$), generalized anxiety disorder (0.25; $P = 0.01$), social phobia (0.30; $P < 0.001$), and agoraphobia without panic disorder (0.30; $P = 0.02$) had lower salivary testosterone levels than female controls. Higher testosterone levels were found in male and female participants using SSRIs than in non-users (effect size 0.26; $P < 0.001$).

Conclusion: Salivary testosterone levels are lower in female patients with a depressive disorder, generalized anxiety disorder, social phobia, and agoraphobia as compared to female controls. SSRIs may increase salivary testosterone in men and women.

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Introduction

There are important sex differences in prevalence proportions of psychiatric disorders, with women having a more than double the risk of depressive disorder, generalized anxiety disorder, social phobia, and panic disorders with or without agoraphobia [1–6]. Differences between the sexes have also been found for symptom profiles of depression, with an earlier age of onset, a higher symptom severity, more atypical depression, and a diminished libido in affected women compared to men [1,4,7]. Libido and other sexual functions may further worsen under the influence of antidepressant use [8,9]. Especially selective serotonin reuptake inhibitors (SSRI), generally preferred as the

first-line pharmacotherapy treatments for mild to moderate outpatient depression and anxiety disorders, are associated with sexual problems such as diminished libido and difficulties with sexual arousal and orgasm in at least 30% of patients [8–10].

Testosterone is an important circulating androgen. Its secretion is under the rapid pulsatile control of gonadotropin-releasing hormone (GnRH), which in turn stimulates the production of luteinizing hormone. Consequently, there are cyclic changes in serum testosterone levels (with a slight nocturnal rise), and therefore ideally at least several morning blood samples separated by 30-minute intervals are to be used to assess androgenic status in a meaningful way [11]. Salivary testosterone levels are strongly correlated with free serum testosterone levels in men [12,13], but less strongly in women, for whom there is less evidence [14,15].

Testosterone is involved in male secondary sexual characteristics, reproduction, and sexual function. The brain contains receptors for testosterone [16–19], and is capable of synthesizing and metabolizing

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testosterone to, for instance, estradiol [20–22]. In some studies, a low androgen status has been associated with symptoms of fatigue, irritability, dysphoria, declining vigor, low vitality, sexual dysfunction in men [23–26] and women [27], and an increased risk of depressive symptoms and depression in most [28–30] but not all studies [31,32]. Findings were mixed, however, as several other epidemiological studies did not show or showed non-linear relationships between testosterone levels and depressive symptoms [33]. Inconsistencies may partly be due to the link or interaction with the genetic cytosine–adenosine–guanine (CAG) repeat length polymorphism. Longer CAG repeats may diminish the androgen receptor transactivation capacity and has been linked to the vulnerability to depression and anxiety [34,35], though not consistently [36–38]. Evidence on the role of testosterone in women is scarce, but there is some evidence that low DHEA(S) is involved in depression in women [39,40]. Androgen deprivation therapy in men with prostate cancer induces many of these symptoms, which are reversible after cessation of therapy [41,42]. However, androgen withdrawal induced adverse mood symptoms only in a subgroup of 10% (3 out of 31) of healthy male volunteers [43]. Low testosterone levels have also been found in men with dysthymic disorder [44]. In a large community sample of almost 4000 elderly men, free testosterone concentration in the lowest quintile was associated with more depressive symptoms [29]. The evidence for a potential role of testosterone in anxiety disorders is much more limited. Low salivary testosterone levels were associated with depressive and anxiety symptoms in 106 adolescent boys but not in 107 girls [45] and weakly with anxiety symptoms in 3413 men [32], but other studies found no relationship between social anxiety and basal salivary testosterone levels in 20 adolescents [46] and 58 students [47]. Moreover, the urine testosterone level was comparable in 16 patients with panic disorder versus 13 controls [48]. Even so, testosterone has been associated with many behavioral aspects of dominance, competition, aggression, and sociality [49–56].

Data on serum testosterone level in anxiety disorders is scarce, whereas salivary testosterone levels have even less often been studied in small groups of patients with psychiatric disorders [57]. Also, there are few studies on the effects of antidepressants on the hypothalamic–pituitary–gonadal (HPG) axis [58]. We hypothesized that low salivary testosterone levels are associated with both depressive and anxiety disorders. Second, treatment of depressive and anxiety disorders with antidepressants decreases libido and this may be due to a reduction in testosterone levels. We measured salivary testosterone levels in the large Netherlands Study of Depression and Anxiety (NESDA). A number of 4 morning and 2 evening salivary samples were pooled before testosterone was quantified in order to minimize the effects of the pulsatile release of testosterone [11,15]. The use of a number of saliva samples had advantages over blood samples as they are more easily collected by participants themselves at several time points at their homes; it requires no pain- and stress-inducing venipunctures; and salivary testosterone reflects the free bio-available serum fraction.

Method

Participants took part in NESDA [59], a large longitudinal cohort study on the course of depressive and anxiety disorders. The NESDA sample consists of 2981 participants (mean age 41.9, range 18–65; 1002 men and 1979 women), of whom 807 persons were recruited through mental health care organizations, 564 persons through the community setting and the remaining 1610 through primary care. Exclusion criteria were a primary diagnosis of psychotic, obsessive–compulsive, bipolar or severe addiction disorder and not being fluent in Dutch. The baseline assessment was constituted by a medical exam, a face-to-face interview, saliva collection and several written questionnaires. Detailed objectives and methods of NESDA are described elsewhere [59]. The research protocol was approved by the ethical

committee of participating universities, and all of the respondents provided written informed consent.

There were 2329 participants with a lifetime diagnosis of depressive and/or anxiety disorder and 652 controls without a lifetime psychiatric diagnosis. Controls were defined as having no prior lifetime history of anxiety disorder (i.e., panic disorder, generalized anxiety disorder, or social phobia) or depressive disorder (i.e., major depressive disorder (MDD) or dysthymia).

Assessment of psychopathology and antidepressant use

Several instruments were used to assess psychopathology. The presence of a DSM-IV major depressive disorder or anxiety disorder (i.e., panic disorder, social phobia, generalized anxiety disorder, and agoraphobia) was assessed by the DSM-IV based Composite Interview Diagnostic Instrument (CIDI, WHO Version 2.1). The presence of psychiatric disorders within the past 12 months was used to categorize participants into those with no lifetime anxiety of depressive disorder ($n = 522$), those with a remitted (< 1 year) disorder ($n = 461$), with a recent (1-year) or current anxiety disorder ($n = 276$), those with a recent (1-year) or current depressive disorder ($n = 412$) and a recent or current comorbid disorder ($n = 471$). We also analyzed current psychiatric disorders within the preceding month for depressive disorder and anxiety disorders (i.e., social phobia, panic disorder and/or agoraphobia, and generalized anxiety disorder). The total score of the Inventory of Depressive Symptomatology Self Report (IDS-SR) was used to assess overall depression severity [60] and subdivided into five severity groups, i.e., low (total score 0–13), mild (14–25), moderate (26–38), severe (39–48), and very severe (49–84). Item 22 (i.e., interest in sex) from the IDS-SR was analyzed separately as an internal validation of the salivary testosterone assessment, that was rated on a four-point scale: 0 (i.e., usual interest), 1 (i.e., somewhat less interest or pleasure), 2 (i.e., little interest or pleasure), and 3 (i.e., no interest or pleasure). The total score of the Beck Anxiety Inventory was used to assess affective and somatic symptoms of anxiety [61] and subdivided into four severity groups, i.e., normal (total score 0–9), mild (10–18), moderate (19–29), and severe (30–63) [62]. For 15-item Fear Questionnaire cut-off scores of 19 for the 5-item agoraphobia AG subscale and 18 for 5-item social phobia (SO) subscale were used [63].

Medication use during the month prior to baseline interview was registered by observation of drug containers brought to the interview or self-report. Using the World Health Organization Anatomical Therapeutic Chemical classification [64], psychoactive medication was categorized into antidepressants (i.e., tricyclic antidepressants [TCA; N06AA], SSRIs [N06AB], and other antidepressants [N06AF, N06AG, N06AX]), and benzodiazepine use (N03AE, N05BA, N05CD, N05CF) was dichotomized.

Salivary testosterone measurement

At baseline, respondents were instructed to collect saliva samples at home on a regular (preferably working) day shortly after the interview. The median time between the interview and saliva sampling was 9 days (25th–75th percentile, 4–22 days). Instructions prohibited eating, smoking, drinking, or brushing teeth within 15 min. Saliva samples were obtained using Salivettes (Sarstedt AG and Co., Nümbrecht, Germany) at 6 time points: four morning samples (at awakening and at 30, 45, and 60 min later) and 2 evening samples (at 22:00 h and 23:00 h). Indirect evidence suggests that compliance of participants to the protocol was good, as the cortisol awakening response showed the characteristic curve within the first hour of awakening in the large majority of participants, with a steep decline in both evening samples [65]. Samples were stored in refrigerators and returned by regular mail. After receipt, Salivettes were centrifuged at 2000 g for 10 min, aliquoted, and stored at -80 °C. Samples had been thawed once for the

assessment of salivary cortisol, in which results have been published previously [65]. While storage at -80°C of salivary samples does not affect testosterone levels during at least two years, the extra freeze-thaw cycle may have contributed some error to testosterone measurement [14]. Salivary testosterone levels have been shown to be relatively stable over time, with a test–retest stability of 0.65 in men and 0.78 in women over two weeks [66].

To smooth the episodic secretion [11,15], 75 μl of each of the 4 samples collected in the morning (at wake-up and after 30, 45 and 60 min) were mixed to yield one morning sample, and 150 μl of each of the 2 evening samples collected at 22:00 and 23:00 h were mixed to yield also one evening sample. So for every participant, one mixed morning sample and one mixed evening sample were assayed. If one of the samples was missing, a corresponding volume of the other sample(s) was taken. The 11 subjects who did not provide any evening sample were excluded. For every other subject who provided saliva, both a morning and evening sample could be combined. Biochemical analysis of free testosterone in saliva was measured in duplo by the testosterone in saliva assay from Diagnostic Biochem Canada (EiAsy Testosterone Saliva, DBC: CAN-TE-300) using $2 \times 100 \mu\text{l}$ material. The sensitivity of the kit is 1.0 pg/ml and there are hardly cross-reactivities with other steroids. In every assay a standard control was used, with a mean of 26.9 pg/ml (SD 2.1) that was reproducible with a coefficient of variation (CV) of 7.8%. The intra assay precision was 7.1, 3.4, and 6.7% at the concentrations of resp. 14, 38 and 123 pg/ml ($n=10$). In our study about 120 kits were used, all of the same lot. The CV over all testosterone measurements was 10.2%.

To validate the use of Salivettes for testosterone measurement, we also compared saliva obtained through Salivettes with those obtained through Salicaps (a passive drooling device) in a repeated-measures design. We included 10 healthy volunteers (age 35 [SD 12] year; 5 females). Saliva was collected at 6 time-points with alternating Salicaps (IBL International GmbH, Hamburg, Germany) (3 times) and Salivettes (Sarstedt, Newton, NC, USA) (3 times) with 10 min in between and always starting with a Salicap. Participants were instructed to minimize physical exercise and not to eat, drink or smoke, 1 h before the start and during the sampling period. Moreover, to avoid cross-contamination, participants were instructed to rinse the mouth with pure water immediately after each sample (always 10 min before the next sampling). All samples were frozen and after a single freeze–thaw cycle centrifuged for 10 min at 5000 g to remove mucins and to extract the saliva from the Salivettes. The three Salivette samples were also combined to one sample (mixed sample), and the same was done for the three Salicap samples. Testosterone was measured in duplo in every sample as well as the mixed samples using the method described above. There was a significant higher testosterone level in saliva obtained by Salivettes than by Salicaps (as was shown previously) [14], but this difference was highly constant. Pearson's correlation coefficients between the individuals' mean values obtained with Salivettes and Salicaps were $r=0.87$ ($p<0.001$) for the calculated mean and $r=0.87$ ($p<0.001$) for the measured value from the mixed samples. Using ranked-order Spearman's correlation coefficients similar findings were obtained ($r=0.82$ and $r=0.82$, respectively).

Potential covariates

Sociodemographic factors included sex, age, and educational level (years of attained education). Age was categorized by decade. Body mass index (BMI) was calculated as weight divided by length squared, and categorized into 4 groups ($<20 \text{ kg/m}^2/20$ to $25 \text{ kg/m}^2/25$ to $30 \text{ kg/m}^2/\geq 30 \text{ kg/m}^2$). Smoking status was dichotomized into non-smoker or current smoker. Alcohol consumption was categorized into 3 groups (i.e., non-user/a number of units of alcohol of less than 2 per day/2 or more per day). Physical activity was assessed using the International Physical Activity Questionnaire (Craig et al., 2003) and

expressed per 1000 Metabolic Equivalent of Task (MET)/min a week, and subsequently categorized into 3 groups (i.e., $<3/3$ to $6/\geq 6$ of 1000 MET/min per week). A MET/min is defined as the metabolic equivalent of the number of calories consumed by a person (of 60 kg) per minute in an activity relative to the basal metabolic rate (www.ipaq.ki.se), and ≥ 3 indicates activities of at least moderate intensity. Respondents reported working status on the sampling day (i.e., whether (61.8%) or not (38.2%) the participant went to their job that day). Sampling data was used to categorize weekday versus weekend day and season, which was categorized into dark months (i.e., October to February) and months with more daylight (i.e., March through September).

For subgroup analyses in women, information on the use of oral contraceptives and on menstrual phase was obtained. Duration of the menstrual cycle and the number of days since the last menstruation were used to yield the phase of the menstrual cycle at the time of saliva sampling for women reporting a menstrual cycle between 28 and 32 days (i.e., 0–3 days of cycle was regarded as the early follicular phase, 4–13 days as the late follicular phase, and 14–32 days as the luteal phase). Remaining women were categorized as either using oral contraceptives or being post-menopausal.

Prevalent cardiovascular diseases (i.e., coronary disease, cardiac arrhythmia, angina pectoris, heart failure, and myocardial infarction) were ascertained using an algorithm based on self-report data and medication use.

Statistical analysis

The distributions of morning and evening salivary testosterone levels were strongly positively skewed and therefore naturally log-transformed values were used for analyses. Back-transformed geometric mean values are presented in tables and figure. Pearson's correlation coefficients were used to analyze the relationship between morning and evening salivary testosterone levels. Univariate analysis of variance was performed to evaluate the effects of sociodemographic factors, health indicators, and sampling factors on salivary testosterone levels, for morning and evening testosterone levels separately.

Because the morning and evening samples were highly intercorrelated, showing highly comparable associations with psychiatric characteristics, analyses reported in the paper were conducted over individual means of the z-scores of morning and evening samples. The effects of psychiatric characteristics and psychotropic medication on testosterone levels were analyzed in unadjusted and adjusted analyses of (co)variance (adjusted for sex, age, menstrual status, years of education, smoking status, prevalent cardiovascular disease, and northern European ancestry), in men and women separately. When analyzing the associations with psychopathology measures, we also adjusted for SSRI use, because of its strong association with salivary testosterone levels.

Two-tailed P values of less than 0.05 were considered to indicate statistical significance. For significant findings, effect sizes were calculated with Cohen's d. All of the analyses were conducted using SPSS version 17.0 statistical software (SPSS Inc, Chicago, Illinois).

Results

Participants

The 722 men were on average 45.0 (range 18 to 64) years old and the 1380 women 42.7 (range 18 to 65) (Table 1). The mean BMI was 26.2 kg/m^2 for men and 25.3 for women, and around 17% of men and women were obese (i.e., $\text{BMI} \geq 30 \text{ kg/m}^2$). Men were more often current smokers and consumed more often ≥ 2 units of alcohol per day than women. The prevalence of cardiovascular disease was approximately double in men versus women. There was a large range of salivary testosterone levels, that importantly overlapped in men and women. Nevertheless, men had higher median salivary testosterone levels than women both in the morning and evening samples (Table 1). Testosterone levels showed rank-order stability within each gender between morning and evening samples, with Spearman's rank correlation coefficients of 0.62 in men and also 0.62 in women.

Table 1
Characteristics in 2102 participants, according to sex.

Variables	Men (N = 722)	Women (N = 1380)
Age – year	44.9 ± 12.6	42.8 ± 13.2
Education – year	12.2 ± 3.3	12.4 ± 3.3
Northern European ancestry (no., %)	692 (95.8%)	1313 (95.1%)
Body-mass index – kg/m ²		
Mean	26.2 ± 4.4	25.3 ± 5.1
≥ 30 – no. (%)	127 (17.6%)	226 (16.4%)
Current smoker (no., %)	272 (37.7%)	426 (30.9%)
Alcohol consumption ≥ 2 units/d (no., %)	181 (25.1%)	166 (12.0%)
Physical activity – 1000 MET-min/week (median, IQR)	2.83 (1.37–4.88)	3.08 (1.53–4.95)
Prevalent cardiovascular disease (no., %)	70 (9.8%)	63 (4.6%)
Salivary testosterone level – pg/ml		
Morning (geometric mean, 95% CI)	25.7 (24.5–27.1)	17.7 (17.0–18.5)
Evening (geometric mean, 95% CI)	19.4 (18.4–20.5)	14.9 (14.1–15.6)

Determinants of salivary testosterone

The associations between age, menstrual status in women, health indicators (BMI, smoking, alcohol intake, and physical activity), and sampling factors (working status and season with more daylight) on salivary testosterone levels were analyzed as potential correlates may be considered as covariates in epidemiological association studies. In men and women, age was strongly and inversely associated with both morning and evening salivary testosterone levels (all $P < 0.001$). The lowest levels were found in men and women over age 50. Current smoking was associated with high salivary testosterone levels, especially for evening levels and more strongly so in women than in men. Although the menstrual status showed an association with salivary testosterone, this did not persist after adjustment for covariates (e.g., age, smoking status, and SSRI use; $P = 0.20$), but in post-hoc comparisons women using oral contraceptives (that increases SHBG which binds testosterone) had lower testosterone levels than premenopausal women not using oral contraceptives ($P = 0.03$). No univariate associations were found with other potential determinants.

Salivary testosterone, psychopathology and antidepressant use

We combined the morning and evening testosterone levels using the mean of z-scores, because morning and evening levels were intercorrelated and associations with measures of psychopathology were highly consistent for morning and evening testosterone levels in both men and women (data not shown). Tables 2 and 3 show the associations with measures of psychopathology and medication use separately in men and women, respectively.

In adjusted models in men, there were no associations with measures of psychopathology (Table 2). Those 106 men using SSRIs had higher testosterone levels than 558 men not using antidepressants (effect size 0.207; $P = 0.05$). When repeating our analysis in men with recent (1 year) or current psychopathology the mean difference did not importantly change (92 versus 245 men; effect size 0.192).

In adjusted models in women, there was a group difference when taking into account the 12-month diagnostic status in adjusted analyses, indicating that women with any psychiatric disorder had on average a lower testosterone level. When zooming in on the 1-month diagnostic categories, the female patients with a current depressive disorder ($P = 0.002$), current generalized anxiety disorder ($P = 0.01$), current social phobia ($P < 0.001$), current agoraphobia (without panic disorder; $P = 0.02$) had lower testosterone levels than the reference group of female participants having no lifetime history of depressive or anxiety disorder. The effect sizes were 0.287, 0.251, 0.299 and 0.304 for the depressive disorder, generalized anxiety disorder social phobia, and agoraphobia, respectively, indicating modest strengths. Again, more markedly increased testosterone levels were found in 224 women using SSRIs than 1052 non-users of antidepressants (effect size: 0.273; $P < 0.001$), but not in women using TCAs or other antidepressants. When repeating our analysis in women with recent (1 year) or current psychopathology the mean difference did increase slightly (184 versus 475 women; effect size 0.335). In women, the severity of anxiety symptoms ($P = 0.001$), agoraphobia (effect size 0.175; $P = 0.04$), and social phobia (effect size 0.294; $P < 0.001$) was related to lower testosterone levels. There was a linear trend for decreasing testosterone levels with increased social phobia scores (Fig. 1). The comparison between the groups with severe anxiety versus no anxiety yielded an effect size of 0.254. Although, there was also a difference between categories of severity of depressive symptoms ($P = 0.03$), this was not a linear association.

Because associations between SSRI use and salivary testosterone levels were comparable in men and women (combined effect size 0.258), we further explored the effects of antidepressants in all subjects combined. In adjusted analyses, SSRI users but not users of TCAs or other antidepressants showed higher salivary testosterone levels than non-users of antidepressants (Fig. 1). In post-hoc tests, we found that differences were statistically significant for fluvoxamine and paroxetine, although differences for sertraline, fluoxetine and citalopram showed a similar tendency that did not reach significance. The mean testosterone difference between SSRI users and non-users of antidepressants was of similar

strength in subjects with remitted disorders and current disorders (0.234 versus 0.268, respectively).

Discussion

We found that depressive disorder, generalized anxiety disorder, social phobia, and agoraphobia were associated with lower salivary testosterone levels in women, but not in men. Remarkably, SSRI use was associated with elevated testosterone levels in both men and women. While morning testosterone levels were higher than evening testosterone levels, we found that within-person correlation indicated rank-order stability over the day. Our study therefore provides support for the usefulness, validity and reliability of the noninvasive method of testosterone measurement in saliva. It also points to the importance of taking confounding variables into account, especially of sex, age, menstrual cycle, time of the day, and smoking status. The associations between smoking and physical activity with higher testosterone levels [67] and between oral contraceptive use and lower testosterone levels [66,68] are in line with previous findings.

Testosterone levels were lower in female patients with current depression versus female controls. Previous studies in women are relatively scarce [27], in part because blood levels of free testosterone in women were rather unreliable [69], until recently. Previous studies in men [23–26,28–30] found lower testosterone levels in men with depressive symptoms compared to male controls, which we could not confirm. Some previous epidemiological studies found no relationship between low testosterone and depressive symptoms [31,32]. However, androgen activity is not only the effect of bioavailable androgens but also the responsiveness of the androgen receptor in target cells. The androgen receptor gene (located on the X chromosome) contains a polymorphic CAG repeat sequence affecting androgen sensitivity. Previous studies yielded inconsistent results; CAG repeat length and depressive symptoms were positively associated [35], unrelated [36], or associated only in subgroups of black men [37] or patient groups [38]. In another study, low testosterone levels were associated with depressive symptoms in men with short CAG repeat lengths only [34]. As we did not take into account the CAG repeat length polymorphism, this may explain our null-finding in depressed men. Trials with testosterone administration showed inconsistent evidence for antidepressant properties, more so in hypogonadal than in eugonadal men [33]. These studies were done in participants who did [70–72] or did not [73] suffer from a depressive disorder. In supraphysiologic dosages, testosterone may induce euphoria and other symptoms of hypomania [74,75].

Yet, we included twice as many women than men in our study. Our findings of low testosterone levels in social anxiety disorder, generalized anxiety disorder, and agoraphobia are not in line with the previous smaller studies that found no relationship between social anxiety and basal salivary testosterone levels [46,47]. Yet, among the psychiatric disorders under study, particularly those that were characterized by social withdrawal (i.e., depression, social anxiety and agoraphobia) and high social anxiety scores were associated with low testosterone levels in women. No association with panic disorder was found. These findings fit previous notions that reduced testosterone was particularly evident for those primates and humans who behaved socially submissive and showed increased levels of social fear behavior, such as social withdrawal [49–52,54–56]. This could suggest that low testosterone is rather a consequence than a cause of the affective symptoms.

Nevertheless, there are putative biological mechanisms that point to a causal role of low testosterone. First, affective symptoms are importantly mediated by the ‘fear network’ involving the amygdala, hippocampus, and prefrontal cortex, while exogenous and endogenous testosterone significantly modulated local activity and interregional connectivity in the prefrontal cortex and the amygdala during social affective behavior [53,76]. Neurons in the amygdala [16,19], hippocampus [17,18] and

Table 2

Forest plot for mean standard scores of salivary testosterone levels according to psychiatric characteristics in 722 male subjects.

Variables	Unadjusted			Adjusted*		
	No.	Mean (SE)	P	Mean(SE)	P	
6-month diagnosis of psychopathology						
Never	200	-0.133 (SE0.053)	0.03	-0.098 (SE0.058)	0.14	
Remitted disorder	132	-0.022 (SE0.071)		0.013 (SE0.068)		
Anxiety disorder	93	0.123 (SE0.086)		0.104 (SE0.082)		
Depressive disorder	139	0.118 (SE0.070)		0.095 (SE0.067)		
Comorbid disorder	158	0.006 (SE0.061)		-0.038 (SE0.065)		
1-month diagnosis versus never-diagnosis of psychopathology						
Never	200	-0.133 (SE0.053)		-0.103 (SE0.057)		
Depressive disorder	98	0.043 (SE0.087)	0.07	-0.030 (SE0.087)	0.51	
Generalized anxiety disorder	103	0.008 (SE0.072)	0.12	-0.005 (SE0.083)	0.25	
Social phobia	135	-0.006 (SE0.068)	0.14	-0.066 (SE0.071)	0.76	
Panic disorder with agoraphobia	71	0.095 (SE0.085)	0.03	0.034 (SE0.103)	0.24	
Panic disorder without agoraphobia	40	0.098 (SE0.123)	0.08	0.119 (SE0.136)	0.09	
Agoraphobia	37	-0.225 (SE0.136)	0.50	-0.176 (SE0.140)	0.86	
Sexual interest**						
Usual interest	426	-0.043 (SE0.036)	0.20	-0.042 (SE0.039)	0.18	
Somewhat less interest or pleasure	210	0.097 (SE0.060)		0.097 (SE0.055)		
Little interest or pleasure	56	-0.028 (SE0.097)		-0.040 (SE0.106)		
No interest or pleasure	18	-0.080 (SE0.247)		-0.115 (SE0.191)		
Antidepressant use						
No use	558	-0.017 (SE0.034)	0.04	-0.008 (SE0.033)	0.04	
Tricyclic antidepressant	12	-0.178 (SE0.145)		-0.193 (SE0.225)		
Selective serotonin reuptake inhibitor	106	0.177 (SE0.080)		0.156 (SE0.077)		
Other antidepressant	46	-0.171 (SE0.101)		-0.215 (SE0.116)		
Benzodiazepine use						
No use	624	-0.004 (SE0.032)	0.79	-0.002 (SE0.032)	0.97	
Present use	98	0.019 (SE0.077)		0.002 (SE0.081)		
Inventory of depressive symptomatology						
None	282	-0.016 (SE0.047)	0.21	0.013 (SE0.048)	0.44	
Mild	187	-0.069 (SE0.060)		-0.067 (SE0.058)		
Moderate	161	0.124 (SE0.063)		0.084 (SE0.063)		
Severe	65	-0.058 (SE0.091)		-0.075 (SE0.098)		
Verysevere	23	0.049 (SE0.173)		-0.022 (SE0.175)		
Beck's anxiety inventory						
None	400	-0.030 (SE0.039)	0.42	-0.014 (SE0.040)	0.50	
Mild	161	-0.023 (SE0.067)		-0.044 (SE0.063)		
Moderate	116	0.107 (SE0.075)		0.095 (SE0.074)		
Severe	41	0.025 (SE0.118)		-0.034 (SE0.127)		
Fear questionnaire						
No agoraphobia	652	-0.017 (SE0.031)	0.13	-0.017 (SE0.031)	0.18	
Agoraphobia (≥ 19 on AG subscale)	66	0.137 (SE0.106)		0.123 (SE0.099)		
No social phobia	555	-0.032 (SE0.033)	0.06	-0.022 (SE0.033)	0.27	
Social phobia (≥ 18 on SO subscale)	158	0.100 (SE0.066)		0.058 (SE0.064)		

The size of each square is proportional to the number of participants; horizontal lines indicate standard errors. Standard scores were calculated as the mean of z-scores of the morning and evening testosterone levels, within each sex. P values are for the difference between the groups by analysis of variance.

*Adjusted for age, years of education, smoking status, prevalent cardiovascular disease, and northern European ancestry (as well as SSRI use [2 categories] when appropriate).

**Sexual interest was assessed with item 22 of the Inventory of Depressive Symptomatology Self Report (IDS-SR).

neocortex [19] indeed specifically express androgen receptors. Second, there may be an imbalance between testosterone and cortisol [56,77]. Androgens may downregulate the hypothalamo-pituitary-adrenal (HPA) axis in a direct way via androgen receptors which are expressed in corticotropin-releasing factor (CRF) producing neurons in the hypothalamus and which suppressed the promoter region of the CRF gene [17,18]. Elevated plasma cortisol levels and hyperactivity of CRF

neurons in the paraventricular nucleus (PVN) of the hypothalamus are consistent neurobiological findings in depressive and anxiety disorders [17,65,78,79], and the expression of androgen receptors is low in post-mortem brain tissue from patients with depression [17]. Therefore, our findings fit with the idea that low bioavailable testosterone increases the risk to several psychiatric disorders, that social anxiety and submission induces a lowering of androgens, or both.

Table 3

Forest plot for mean standard scores of salivary testosterone levels according to psychiatric characteristics in 1380 female subjects.

Variables	Unadjusted			Adjusted*	
	No.	Mean (SE)	P	Mean (SE)	P
6-month diagnosis of psychopathology					
Never	307	0.084 (SE 0.051)	0.23	0.175 (SE 0.054)	0.009
Remitted disorder	320	-0.086 (SE 0.045)		-0.015 (SE 0.052)	
Anxiety disorder	177	-0.027 (SE 0.079)		-0.082 (SE 0.070)	
Depressive disorder	267	-0.016 (SE 0.063)		-0.041 (SE 0.056)	
Comorbid disorder	309	0.035 (SE 0.057)		-0.068 (SE 0.054)	
1-month diagnosis versus never-diagnosis of psychopathology					
Never	307	0.084 (SE 0.051)		0.139 (SE 0.053)	
Depressive disorder	181	-0.056 (SE 0.073)	0.11	-0.151 (SE 0.072)	0.002
Generalized anxiety disorder	145	-0.038 (SE 0.081)	0.19	-0.131 (SE 0.082)	0.01
Social phobia	252	-0.091 (SE 0.061)	0.03	-0.161 (SE 0.061)	< 0.001
Panic disorder with agoraphobia	156	0.177 (SE 0.084)	0.32	0.013 (SE 0.083)	0.15
Panic disorder without agoraphobia	51	0.032 (SE 0.119)	0.69	0.003 (SE 0.130)	0.55
Agoraphobia	73	-0.221 (SE 0.135)	0.01	-0.220 (SE 0.116)	0.02
Sexual interest**					
Usual interest	690	0.025 (SE 0.035)	0.09	0.030 (SE 0.036)	0.06
Somewhat less interest or pleasure	298	0.070 (SE 0.058)		0.072 (SE 0.053)	
Little interest or pleasure	251	-0.117 (SE 0.061)		-0.100 (SE 0.059)	
No interest or pleasure	111	-0.072 (SE 0.089)		-0.131 (SE 0.089)	
Antidepressant use					
No use	1052	-0.044 (SE 0.029)	0.001	-0.040 (SE 0.028)	0.002
Tricyclic antidepressant	36	-0.008 (SE 0.174)		0.061 (SE 0.154)	
Selective serotonin reuptake inhibitor	224	0.242 (SE 0.063)		0.215 (SE 0.062)	
Other antidepressant	68	-0.098 (SE 0.097)		-0.116 (SE 0.112)	
Benzodiazepine use					
No use	1176	0.009 (SE 0.028)	0.40	0.013 (SE 0.027)	0.29
Present use	204	-0.051 (SE 0.065)		-0.063 (SE 0.066)	
Inventory of depressive symptomatology					
None	475	0.025 (SE 0.041)	0.50	0.094 (SE 0.043)	0.03
Mild	423	0.013 (SE 0.049)		0.017 (SE 0.045)	
Moderate	326	-0.064 (SE 0.053)		-0.121 (SE 0.051)	
Severe	112	-0.018 (SE 0.098)		-0.083 (SE 0.089)	
Very severe	39	0.181 (SE 0.142)		0.021 (SE 0.149)	
Beck's Anxiety Inventory					
None	689	0.041 (SE 0.037)	0.10	0.101 (SE 0.036)	0.001
Mild	394	-0.100 (SE 0.046)		-0.121 (SE 0.046)	
Moderate	218	0.031 (SE 0.065)		-0.034 (SE 0.063)	
Severe	76	0.067 (SE 0.107)		-0.142 (SE 0.109)	
Fear Questionnaire					
No agoraphobia	1216	0.002 (SE 0.027)	0.90	0.022 (SE 0.026)	0.04
Agoraphobia (≥19 on AG subscale)	161	-0.008 (SE 0.076)		-0.145 (SE 0.075)	
No social phobia	1016	0.049 (SE 0.030)	0.002	0.074 (SE 0.029)	< 0.001
Social phobia (≥18 on SO subscale)	359	-0.134 (SE 0.048)		-0.200 (SE 0.049)	

The size of each square is proportional to the number of participants; horizontal lines indicate standard errors.

Standard scores were calculated as the mean of z-scores of the morning and evening testosterone levels, within each sex.

P values are for the difference between the groups by analysis of variance.

*Adjusted for age, years of education, smoking status, prevalent cardiovascular disease, and northern European ancestry (as well as SSRI use [2 categories] when appropriate).

**Sexual interest was assessed with item 22 of the Inventory of Depressive Symptomatology Self Report (IDS-SR).

The use of SSRIs is associated with sexual problems, such as lowered libido and difficulties with sexual arousal or orgasm [8–10]. These side effects normally continue for as long as the individual is taking the medication and may partly be explained by increased prolactin levels in some cases [80], that subsequently may also induce gynecomastia and galactorrhoea. As androgens are pivotal for sexual function, we were interested in the relationship between antidepressant use and testosterone. Remarkably, we found that SSRI users had

higher salivary testosterone levels versus participants who did not use antidepressants, which is hard to reconcile with their sexual side effects, although SSRIs may have induced androgen antagonism or the higher testosterone levels may be associated with treatment-induced increases in prosocial behavior. The mechanisms of these side effects are likely complex, involving not only endocrine (e.g., prolactin and sex hormonal) but also directly effects on the central and peripheral nervous systems involved in sexual function. Besides systemic effect on

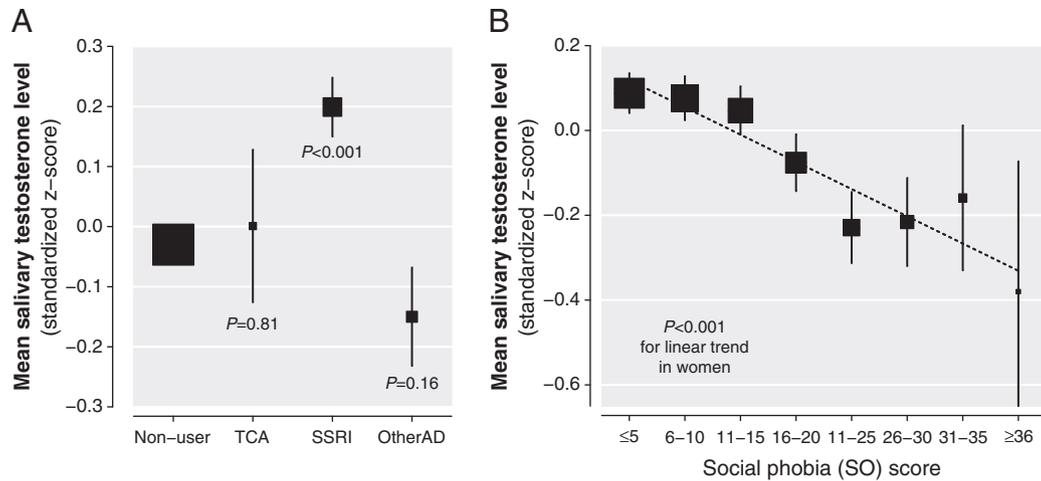


Fig. 1. The mean standard scores (with error bars representing standard errors) for morning and evening salivary testosterone levels, according (Box A) to the kind of antidepressant used in men and women combined, and (Box B) social phobia (SO) subscale score (part of the Fear Questionnaire) in women only. The black reference line shows the mean score in non-users of antidepressants. The size of each square is proportional to the number of participants. Vertical lines indicate standard errors. Scores are adjusted for sex (when appropriate), age, menstrual status, years of education, smoking status, prevalent cardiovascular disease, and northern European ancestry. In Box B, testosterone values were additionally adjusted for SSRI use. P values are (Box A) by analysis of covariance (compared to the non-user reference group), or (Box B) for linear trend in analysis of covariance. AD denotes antidepressant; SSRI denotes selective serotonin reuptake inhibitors; TCA denotes tricyclic antidepressants.

the circulating free testosterone level, SSRIs (and current smoking) may have had a local effect on salivary glands, as antidepressants reduce the salivary flow through muscarinic acetylcholine receptor occupancy, inducing the common complaint of a dry mouth. But effects such as a dry mouth are more commonly observed during TCA use, which in our study was not associated with higher testosterone levels [81].

Our study had some limitations. First, Salivettes were used for the assessment of salivary testosterone, where a passive drooling device would have been preferable. Since we observed a systematic elevation, all Salivette samples need to be collected and handled in the same way in order to yield reliable statistical results as done in the present study. As the difference in results was non-random (i.e., higher levels of testosterone using Salivettes as compared to passive drool saliva samples but with a strong correlation), the internal validity of this study was high. Second, our analyses were cross-sectional and therefore cannot indicate the causal direction. Third, we needed to rely on saliva sampling at the homes of the participants, but as we pooled either four or two saliva samples, compliance and timing with the sampling instructions seemed less of an issue. Fourth, most participants had "(either remitted or current)" anxiety or depressive disorders, and therefore the control group without any lifetime psychiatric diagnosis was relatively small. Nevertheless, noncompliance with instructions could have resulted in some measurement error. The CV of testosterone was around 10%, which error may have biased our findings somewhat toward the null hypothesis. All saliva samples were visually inspected for potential discoloration but not tested biochemically for potential blood contamination [14]. The strengths of our study included the large sample size, the 'gold standard' use of multiple samples that were combined [11], and the inclusion of patients with several anxiety disorders. We also confirmed findings of low testosterone levels found in participants who were female, using oral contraceptives, and were older, and high testosterone levels found in participants who smoke, which support the validity of salivary testosterone as a measure of androgenic activity.

We conclude that saliva samples are useful for non-invasive screening purposes. Salivary testosterone levels were significantly lower in female patients with depression, generalized anxiety disorder, social anxiety disorder, and agoraphobia. These findings stress the role of testosterone in social affective symptomatology in women. Future studies should explore whether testosterone treatment or augmentation is beneficial and safe not only in male but also in female patients with low androgenic status.

Conflict of interest

None.

Acknowledgments

The infrastructure for the Netherlands Study of Depression and Anxiety (NESDA) (<http://www.nesda.nl>) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw grant 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, IQ Healthcare, Netherlands Institute for Health Services Research [NIVEL], and Netherlands Institute of Mental Health and Addiction [Trimbos]). Funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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