

# Salivary Testosterone Is Consistently and Positively Associated with Extraversion: Results from The Netherlands Study of Depression and Anxiety

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## Key Words

Testosterone · Extraversion · Personality

## Abstract

**Background:** Testosterone has been postulated as a ‘social’ hormone, but the relationship between testosterone and personality traits linked with socially oriented behaviors such as extraversion remains unclear. The objective of our study was to investigate the association between baseline salivary testosterone levels and the Big Five personality traits. **Methods:** We studied the relationship between salivary testosterone (morning and evening) and NEO-FFI (Five-Factor Inventory) personality traits in 1,611 participants with lifetime or current depression and/or anxiety and 482 participants without depression/anxiety of the Netherlands Study of Depression and Anxiety (NESDA). **Results:** The personality domain of extraversion was independently associated with higher salivary testosterone, both in healthy subjects ( $\beta = 0.094$ ;  $p = 0.04$ ) and in subjects with lifetime or current depression and/or anxiety ( $\beta = 0.092$ ;  $p < 0.001$ ). In multivariable adjusted analyses, extraversion remained the only personality trait that was positively associated with sal-

ivary testosterone ( $\beta = 0.079$ ;  $p = 0.006$ ). **Conclusion:** We conclude that salivary testosterone is consistently and positively related to extraversion, supporting the notion of a hormonal basis of this personality trait, which may be linked to the tendency to strive for and maintain social status.

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## Introduction

Testosterone is a major circulating sex hormone both in men and in women [1]. There is increasing evidence that testosterone, in addition to physiological effects, enhances socially oriented behaviors [2–4]. As testosterone levels have high stability over time [5] with a substantial genetic component [6], testosterone may be regarded as a psychologically relevant biological trait variable [7]. The aim of the present study was to test the relationship between testosterone and personality traits linked with socially oriented behaviors such as extraversion.

The key social role of testosterone has been defined as the search for and maintenance of social status [2, 3]. Direct support to this notion has been lent by a study in male

baboons, showing a linear positive relationship between social dominance ranking and fecal testosterone levels [8]. A recent study in humans has shown a similar relationship between social status ranking and testosterone: in female athletes before-competition salivary levels of testosterone were positively related to social status assessed among their teammates [9].

Several human studies have indicated that testosterone is specifically associated with measures of dominance that are implicit – outside of conscious awareness [10, 11]. This has been shown both in social experiments and in studies using assessments of implicit power motivation [11]. For example, the administration of testosterone has been shown to alleviate gaze aversion [12] and avoidance [13] in eye tracking and social approach-avoidance tasks, respectively. Implicit power motivation, indexed by picture interpretations that are subsequently coded for power imagery [11], was also positively correlated with baseline testosterone [11]. As opposed to implicit power motivation, in several studies explicit, self-reported power motivation was not associated with baseline testosterone [11, 14], although 2 studies did demonstrate a positive association between self-reported dominance and testosterone [5, 15]. A recent study by Slatcher et al. [10] suggests a more complex situation, as self-reported dominance was found to moderate the effect of salivary testosterone levels on dominance behaviors in an experimental condition. The authors conclude that this finding is in line with other studies demonstrating interactions between explicit personality constructs, so-called personality traits and implicit personality constructs or implicit motives [10]. For example, Brunstein and Maier [16] demonstrated a strong association between implicit achievement motivation and performance on a laboratory task in students high in explicit achievement motivation but not in students low in explicit achievement motivation [16].

Of the explicit personality constructs, measured by means of validated self-report measures, the most extensively researched model is the Big Five framework of personality consisting of 5 dimensions: neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness [17]. It has been postulated that the Big Five trait of extraversion can be subdivided into 2 traits: the first trait, termed social closeness, agreeableness or affiliative extraversion, reflects an enjoyment of close interpersonal bonds and the other trait, termed social dominance, assertiveness, activity, or agentic extraversion, reflects an enjoyment of leadership roles and assertiveness [18]. There appear to be striking parallels between im-

PLICIT power motives, testosterone and the personality dimension of extraversion – in particular agentic extraversion.

The relationship between testosterone and the Big Five personality traits was tested in a study by Sellers et al. [5]. In this study in 72 psychology students no association was observed between salivary testosterone and either of the Big Five personality traits, with the exception of a negative correlation between testosterone and conscientiousness in women [5]. We are not aware of other studies on the association between testosterone and the Big Five dimensions, but there are a number of studies using other personality measures. Olweus et al. [19] found no associations between plasma testosterone levels and personality traits tested using the Eysenck Personality Questionnaire (EPQ) in 58 adolescent boys. King et al. [20] studied the relationship between serum testosterone and EPQ both in abstinent alcoholics and normal controls and observed a significant positive correlation between testosterone and extraversion in controls but not in alcoholics. Coccaro et al. [21] tested associations between testosterone in cerebrospinal fluid and the EPQ in male subjects with personality disorder and demonstrated a positive relationship for venturesomeness but not for other personality traits including neuroticism and extraversion. Finally, Daitzman and Zuckerman [22] studied the relationship between plasma androgens and the Sensation Seeking Scale (SSS), a scale related to the EPQ, and found a positive association between androgens and the SSS disinhibition subscale.

Limitations of the studies on the association between testosterone and personality mentioned above are the small numbers of participants. In addition, previous findings have not been adjusted for diagnoses of depression or anxiety disorders. This seems to be highly relevant, as there is evidence of reduced testosterone levels in depression [23–26] and social anxiety [24], even though the exact relationship between low testosterone levels and major depression has yet to be proven [27]. Depression has also been shown to be associated with reduced extraversion [28]. Therefore, in the present study we investigated the association between baseline salivary testosterone levels and the Big Five personality traits in a large cohort study, including 482 participants without and 1,611 participants with lifetime or current depression or anxiety. We hypothesized a positive association between salivary testosterone and extraversion – in particular agentic extraversion. We also tested the association between testosterone and the other Big Five traits to test the specificity of this relationship.

## Methods

### Sample

Study participants took part in the Netherlands Study of Depression and Anxiety (NESDA) [29], a large longitudinal cohort study on the course of depression and anxiety disorders. The NESDA sample consists of 2,981 participants. A detailed description of the NESDA study design, its rationale, methods and recruitment strategy, can be found elsewhere [29]. The research protocol was approved by the medical ethics committees of participating universities, and all of the respondents provided written informed consent.

There were 2,329 participants with a lifetime diagnosis of depressive and/or anxiety disorder and 652 controls without a lifetime psychiatric diagnosis. We selected all participants for whom NEO-FFI (Five-Factor Inventory) data as well as morning and evening salivary testosterone levels were available. Following these criteria, 1,611 participants with a lifetime diagnosis of depressive and/or anxiety disorder and 482 controls without a lifetime psychiatric diagnosis were included. The reasons for exclusion were as follows: no salivary sample available ( $n = 838$ ), no NEO-FFI data available ( $n = 10$ ), pregnancy ( $n = 10$ ), use of corticosteroids ( $n = 21$ ), and lacking information on the use of oral contraceptives ( $n = 9$ ). The 888 excluded subjects (29.8%) did not differ from the 2,093 included subjects for gender ( $p = 0.22$ ) but were on average younger (mean  $\pm$  standard deviation,  $SD = 37.9 \pm 12.3$  vs.  $43.6 \pm 13.0$  years;  $p < 0.001$ ) and had a lower mean level of education ( $11.8 \pm 3.3$  vs.  $12.3 \pm 3.3$  years;  $p < 0.001$ ). The excluded subjects also had a higher level of neuroticism ( $p < 0.001$ ) and lower levels of agreeableness and conscientiousness (both  $p < 0.001$ ) but did not differ in the level of extraversion ( $p = 0.24$ ) and openness ( $p = 0.80$ ) from the included participants.

### Measurements

#### Personality

Personality was operationalized using the NEO-FFI personality questionnaire, a 60-item questionnaire measuring 5 domains: neuroticism, extraversion, openness to experience, conscientiousness, and agreeableness. Items (e.g. 'I often feel inferior to others') are answered on a 5-point Likert scale, ranging from 'strongly disagree' to 'strongly agree' [30]. Each domain consists of 12 items, with scores ranging from 12 to 60 per domain. In our participants scores ranged from 12 to 60 for neuroticism, 14 to 57 for extraversion, 16 to 57 for openness to experience, 19 to 59 for conscientiousness, and 25 to 59 for agreeableness. Internal consistency values ranged from 0.74 to 0.89 [31]. In our participants Cronbach's  $\alpha$  for neuroticism, extraversion, openness, agreeableness, and conscientiousness were 0.90, 0.84, 0.69, 0.71, and 0.80, respectively. In addition to the 5 personality domains, the NEO-FFI also reliably provides 13 item cluster subcomponents [31]. For the personality trait of extraversion there are 3 item cluster subcomponents: sociability (referring to affiliative extraversion), activity (referring to agentic extraversion) and positive affect (as an additional subcomponent) [31].

#### Salivary Testosterone Measurement

At baseline, respondents were instructed to collect saliva samples at home on a regular (preferably working) day shortly after the interview, of whom 2,068 returned saliva samples that could be used in the current analyses [32]. The median time between the

interview and saliva sampling was 9 days (25–75th percentile, 4–22 days). Instructions prohibited eating, smoking, drinking, or brushing teeth within 15 min before saliva collection. Saliva samples were obtained using Salivettes (Sarstedt AG and Co., Nümbrecht, Germany) at 6 time points: 4 morning samples (at awakening and at 30, 45 and 60 min later) and 2 evening samples (at 22:00 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 [32]. Samples were stored at home in refrigerators and returned by regular mail. After receipt, the Salivettes were centrifuged at 2,000 g for 10 min, aliquoted and stored at  $-80^{\circ}\text{C}$ . The samples were thawed once more for the assessment of salivary cortisol, the results of which have been published elsewhere [33]. To smooth the episodic secretion, 75  $\mu\text{l}$  of each of the 4 samples collected in the morning were mixed to yield 1 morning sample, and 150  $\mu\text{l}$  of each of the 2 evening samples were mixed to yield 1 evening sample. So for every participant, 1 mixed morning sample and 1 mixed evening sample were assayed. If one of the samples was missing a corresponding volume of the other sample(s) was taken. Biochemical analysis of free testosterone in saliva was measured in duplicate by the testosterone in saliva assay from Diagnostic Biochem Canada (EiAsy Testosterone Saliva, DBC: CAN-TE-300) using  $2 \times 100 \mu\text{l}$  of material. The sensitivity of the kit is 1.0 pg/ml and there is hardly any cross-reactivity with other steroids. In every assay the same standard control was used, with a mean of  $26.9 \pm 2.1$  pg/ml that was reproducible with a coefficient of variation of 7.8%. The intra-assay precision values were 7.1, 3.4 and 6.7% at concentrations of 14, 38 and 123 pg/ml, respectively ( $n = 10$ ). The mean coefficient of variation of the duplicate measurements of all testosterone determinations was 10.2%. As previously described [24], in order to validate the use of Salivettes for testosterone measurement we compared saliva obtained with Salivettes with saliva obtained with Salicaps (a passive drooling device) in 10 healthy volunteers in a repeated-measures design; 3 Salivette samples were combined to 1 sample (mixed sample). Similarly, 3 Salicaps samples were combined to 1 mixed sample. Testosterone was measured in duplicate in every sample as well as the mixed samples. There was a significantly higher testosterone level in saliva obtained with Salivettes than with Salicaps, but this was highly consistent. 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. Similar findings were obtained using Spearman's rank-order correlation coefficients ( $r = 0.82$  and  $r = 0.82$ , respectively).

#### Potential Covariates

Age, sex and years of education were considered as basal covariates. Based on earlier research, adjustments were made for smoking [33], obesity [34], SSRI use [24], and menstrual status [35]. Participants reported their time of awakening on the sampling day. Average sleep duration in the previous 4 weeks was assessed using the Insomnia Rating Scale [36] and was dichotomized as more or less than 6 h per night. Gender and menstrual status was subdivided into the following 4 categories: being male, naturally cycling female, female using oral contraceptives and postmenopausal female. In the statistical analyses, 3 dummy variables were used for male versus female, use of contraceptives and postmenopausal ver-

**Table 1.** Characteristics in 2,093 participants, according to the presence of psychopathology (depression and/or anxiety)

Variables	Subjects free of lifetime psychopathology (n = 482)	Subjects with lifetime or current psychopathology (n = 1,611)	p value
Age, years	43.1±14.5	43.7±12.5	0.43
Sex and menstrual status			
Naturally cycling women	97 (20.1)	399 (24.8)	0.04
Women using oral contraceptives	84 (17.4)	262 (16.3)	0.55
Postmenopausal women	111 (23.0)	422 (26.2)	0.16
Men	190 (39.4)	528 (32.8)	0.04
Education, years	12.8±3.2	12.2±3.2	<0.001
Body mass index, kg/m <sup>2</sup>			
Mean	25.1±4.5	25.7±5.0	0.01
≥30	66 (13.7)	286 (17.8)	0.04
Current smoker	112 (23.2)	579 (35.9)	<0.001
Physical activity (1,000 MET min/week)	3.33 (1.59–4.89)	2.95 (1.44–4.93)	0.18
6-month diagnosis of psychopathology			
Remitted disorder	–	453 (28.1)	–
Anxiety disorder	–	268 (16.6)	–
Depressive disorder	–	403 (25.0)	–
Comorbid disorder	–	462 (28.7)	–
User of SSRIs	2 (0.4)	333 (20.8)	<0.001
Five-Factor Personality Scales of the NEO-FFI			
Neuroticism	27.0±7.6	38.5±8.1	<0.001
Extraversion	41.8±6.4	35.3±7.0	<0.001
Openness	37.9±5.6	38.4±6.0	0.14
Agreeableness	45.4±4.9	43.7±5.2	<0.001
Conscientiousness	45.0±5.5	41.2±6.3	<0.001
Salivary testosterone level, pg/ml			
Morning	19.8 (18.6–21.1)	20.2 (19.4–21.0)	0.60
Evening	16.9 (15.7–18.2)	16.1 (15.4–16.8)	0.26

Values are means ± SD or n (%). Physical activity is presented as median with interquartile range in parenthesis (because of the skewed distributions of physical activity and salivary testosterone). Salivary testosterone levels are presented as geometric means with 95% confidence intervals in parentheses. p values by t tests for independent samples or  $\chi^2$  tests, when appropriate.

sus nonpostmenopausal. As described in our previous publication [24], we used the item of sexual interest from the Inventory of Depressive Symptomatology Self Report (IDS-SR), dichotomized into 'usual or somewhat less interest or pleasure' versus 'little or no interest or pleasure', as a measure of sexual desire.

#### Statistical Analyses

Data were analyzed using the Statistical Package of the Social Sciences (IBM SPSS Statistics, version 20.0; SPSS Inc., Chicago, Ill., USA). Stratified analyses were performed for the group of patients with current or lifetime depression/anxiety and the control group without lifetime psychopathology. The distributions of morning and evening salivary testosterone levels were strongly positively skewed and therefore naturally log<sub>e</sub>-transformed values were used for analyses. Standardized z values (measurement minus mean divided by the SD) were calculated within the groups of men and of women. In addition to individual analyses with morning and eve-

ning values, we also averaged morning and evening z values to yield a 'pooled testosterone level'. The association between salivary testosterone z values and NEO-FFI personality domains and subscales were analyzed using multivariate regression analyses, while adjusting for the covariates. Two-sided p values of less than 0.05 were considered to indicate statistical significance.

## Results

### Participants

Participants free of lifetime depression and anxiety were on average 43.1 years old (SD = 14.5 years) and participants with lifetime or current depression/anxiety 43.7 years (SD = 12.5 years; table 1). In the group with lifetime

**Table 2.** Associations between salivary testosterone levels and NEO-FFI personality traits in 2,093 participants

Variables	Morning testosterone level		Evening testosterone level		Pooled testosterone level	
	unadjusted	adjusted <sup>1</sup>	unadjusted	adjusted <sup>1</sup>	unadjusted	adjusted <sup>1</sup>
<i>Neuroticism (n = 2,093)</i>	0.009 (p = 0.68)	<i>-0.085 (p &lt; 0.001)</i>	-0.013 (p = 0.57)	<i>-0.056 (p = 0.02)</i>	-0.002 (p = 0.93)	<i>-0.078 (p = 0.001)</i>
No lifetime psychopathology (n = 482)	0.054 (p = 0.24)	-0.019 (p = 0.68)	0.028 (p = 0.54)	0.033 (p = 0.48)	0.046 (p = 0.32)	0.007 (p = 0.88)
Lifetime or current psychopathology (n = 1,611)	-0.010 (p = 0.68)	<i>-0.096 (p = 0.001)</i>	-0.013 (p = 0.61)	<i>-0.072 (p = 0.01)</i>	-0.013 (p = 0.61)	<i>-0.093 (p = 0.001)</i>
<i>Extraversion (n = 2,093)</i>	<i>0.056 (p = 0.01)</i>	<i>0.101 (p &lt; 0.001)</i>	<i>0.086 (p &lt; 0.001)</i>	<i>0.075 (p = 0.001)</i>	<i>0.079 (p &lt; 0.001)</i>	<i>0.098 (p &lt; 0.001)</i>
No lifetime psychopathology (n = 482)	0.082 (p = 0.07)	<i>0.111 (p = 0.02)</i>	<i>0.144 (p = 0.004)</i>	0.057 (p = 0.22)	<i>0.127 (p = 0.005)</i>	<i>0.094 (p = 0.04)</i>
Lifetime or current psychopathology (n = 1,611)	<i>0.061 (p = 0.01)</i>	<i>0.091 (p = 0.001)</i>	<i>0.071 (p = 0.004)</i>	<i>0.076 (p = 0.004)</i>	<i>0.073 (p = 0.003)</i>	<i>0.092 (p &lt; 0.001)</i>
<i>Openness (n = 2,093)</i>	-0.006 (p = 0.77)	-0.013 (p = 0.57)	-0.013 (p = 0.56)	-0.003 (p = 0.91)	-0.011 (p = 0.63)	-0.009 (p = 0.70)
No lifetime psychopathology (n = 482)	0.020 (p = 0.66)	0.029 (p = 0.55)	0.018 (p = 0.70)	0.034 (p = 0.50)	0.021 (p = 0.64)	0.035 (p = 0.47)
Lifetime or current psychopathology (n = 1,611)	-0.014 (p = 0.59)	-0.022 (p = 0.41)	-0.020 (p = 0.42)	-0.011 (p = 0.68)	-0.019 (p = 0.45)	-0.018 (p = 0.49)
<i>Agreeableness (n = 2,093)</i>	<i>-0.052 (p = 0.02)</i>	-0.003 (p = 0.90)	-0.023 (p = 0.30)	-0.038 (p = 0.09)	-0.041 (p = 0.06)	-0.023 (p = 0.31)
No lifetime psychopathology (n = 482)	-0.024 (p = 0.60)	-0.031 (p = 0.51)	-0.006 (p = 0.90)	-0.04 (p = 0.40)	-0.017 (p = 0.72)	-0.039 (p = 0.40)
Lifetime or current psychopathology (n = 1,611)	<i>-0.057 (p = 0.02)</i>	0.000 (p = 0.99)	-0.031 (p = 0.22)	-0.045 (p = 0.09)	-0.049 (p = 0.051)	-0.025 (p = 0.33)
<i>Conscientiousness (n = 2,093)</i>	0.006 (p = 0.79)	<i>0.063 (p = 0.004)</i>	0.026 (p = 0.24)	0.041 (p = 0.07)	0.018 (p = 0.42)	<i>0.057 (p = 0.01)</i>
No lifetime psychopathology (n = 482)	-0.024 (p = 0.60)	0.009 (p = 0.85)	-0.010 (p = 0.83)	-0.014 (p = 0.76)	-0.019 (p = 0.68)	-0.003 (p = 0.95)
Lifetime or current psychopathology (n = 1,611)	0.017 (p = 0.49)	<i>0.064 (p = 0.01)</i>	0.030 (p = 0.23)	0.043 (p = 0.09)	0.026 (p = 0.29)	<i>0.060 (p = 0.02)</i>

<sup>β</sup>-Coefficients and accompanying p values by univariate and multivariate linear regression analysis. Statistically significant associations are presented in italics.

Pooled testosterone level: using the mean standard scores of morning and evening salivary testosterone combined.

<sup>1</sup> Adjusted for age, sex, years of education, smoking status, obesity, menstrual status, SSRI use, time of awakening, and sleep duration <6 h/night (and diagnosis categories when appropriate).

or current psychopathology there were slightly more premenopausal women not using oral contraceptives and fewer men than in the group free of lifetime psychopathology. The mean level of extraversion was lower in the lifetime/current psychopathology group than in the participants without psychopathology.

In subjects with lifetime or current psychopathology there was a larger proportion of current smokers compared with subjects free of lifetime psychopathology (35.9 vs. 23.2%, respectively). Morning salivary testosterone levels were higher than evening levels both in men and in women (table 1). The association between morning and evening salivary testosterone levels was strong ( $\beta = 0.62$ ;  $p < 0.001$ ).

#### Personality Dimensions and Salivary Testosterone

Participants without lifetime depression/anxiety (n = 482) had lower levels of neuroticism (mean  $\pm$  standard error, SEM = 27.0  $\pm$  0.3 vs. 38.5  $\pm$  0.2;  $p < 0.001$ ) and higher levels of extraversion, conscientiousness and agree-

ableness than participants with lifetime or current psychopathology (n = 1,611; 41.8  $\pm$  0.3 vs. 35.3  $\pm$  0.2, 45.0  $\pm$  0.3 vs. 41.2  $\pm$  0.2 and 45.4  $\pm$  0.2 vs. 43.7  $\pm$  0.1, respectively; all  $p < 0.001$ ) but did not differ on the level of openness (37.9  $\pm$  0.3 vs. 38.4  $\pm$  0.2;  $p = 0.15$ ).

Associations between salivary testosterone levels and the NEO-FFI personality domains are shown in table 2. As demonstrated, results were very similar for morning versus evening levels. Consequently, throughout the remainder of the paper we will discuss results for the pooled salivary testosterone levels. In adjusted analyses, pooled testosterone levels were negatively associated with the neuroticism domain in subjects with lifetime or current depression/anxiety ( $\beta = -0.093$ ;  $p = 0.001$ ). No association between salivary testosterone and neuroticism was found for subjects with no lifetime depression and/or anxiety. For the personality domains of openness and agreeableness no associations were found with salivary testosterone. The personality domain of conscientiousness was positively associated with pooled testosterone

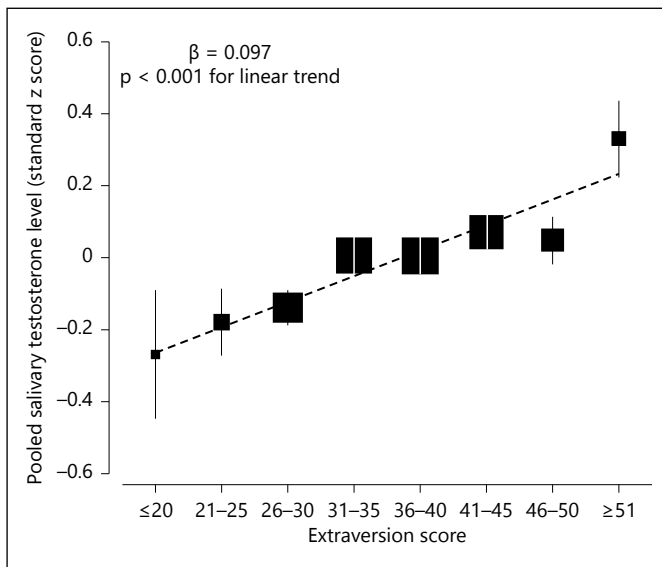
**Table 3.** Associations between salivary testosterone levels and NEO-FFI extraversion item clusters

Variables	Pooled testosterone level		
	unadjusted	adjusted <sup>1</sup>	adjusted <sup>2</sup>
Extraversion: positive affect	0.043 (p = 0.049)	0.056 (p = 0.01)	-0.015 (p = 0.59)
Extraversion: sociability	0.090 (p < 0.001)	0.097 (p < 0.001)	0.077 (p = 0.004)
Extraversion: activity	0.068 (p = 0.002)	0.088 (p < 0.001)	0.063 (p = 0.01)

$\beta$ -Coefficients and accompanying p values by univariate and multivariate linear regression analysis. Pooled testosterone level: using the mean standard scores of morning and evening salivary testosterone combined.

<sup>1</sup> Adjusted for age, sex, years of education, smoking status, obesity, menstrual status, SSRI use, time of awakening, sleep duration <6 h/night, and diagnosis categories.

<sup>2</sup> Additionally adjusted for each of the 2 other extraversion item clusters.



**Fig. 1.** The mean standard scores of pooled morning and evening salivary testosterone levels. The size of each square is proportional to the number of participants. Vertical lines indicate SEM. Mean values are adjusted for age, sex, years of education, smoking status, obesity, menstrual status, and SSRI use (and diagnosis categories when appropriate). The dotted line represents the regression line. The  $\beta$ -coefficient and p value were calculated by multivariate linear regression analysis.

levels in subjects with lifetime or current depression/anxiety ( $\beta = 0.060$ ;  $p = 0.02$ ).

The personality domain of extraversion was positively associated with pooled testosterone levels, both in subjects with lifetime or current depression/anxiety ( $\beta = 0.092$ ;  $p < 0.001$ ) and in subjects without lifetime or current depression/anxiety ( $\beta = 0.094$ ;  $p = 0.04$ ). In figure 1

the relation between salivary testosterone levels and scores on the NEO-FFI extraversion domain is depicted for all 2,093 subjects combined, with the extraversion values categorized to enable visualization of the linear multivariable-adjusted relationship.

Subdividing the personality domain of extraversion into its item clusters of positive affect, sociability and activity (associations were tested for all subjects – both subjects with and subjects without psychopathology) resulted in a strong positive association between sociability and the pooled testosterone levels ( $\beta = 0.097$ ;  $p < 0.001$ ; table 3).

Finally, we performed a multivariate regression analysis including all 3 personality domains that were associated with salivary testosterone (neuroticism, extraversion and conscientiousness). In all participants combined, extraversion remained the only independent statistically significant personality domain that was associated with pooled salivary testosterone levels ( $\beta = 0.079$ ;  $p = 0.006$ ). Associations with neuroticism ( $\beta = -0.024$ ;  $p = 0.42$ ) and conscientiousness ( $\beta = 0.009$ ;  $p = 0.72$ ) were no longer statistically significant. When repeated for both morning and evening testosterone levels in sensitivity analyses, we consistently found that only extraversion – not neuroticism and conscientiousness – was associated with salivary testosterone. When we added a gender  $\times$  extraversion interaction term to the model, we found that the association with extraversion was significantly stronger in women than in men ( $p$  for interaction = 0.04). The  $\beta$ -coefficient for men ( $n = 718$ ) was 0.050, while it was 0.122 for women ( $n = 1,375$ ). Finally, we performed two additional sensitivity analyses. First of all, we adjusted our main analysis for sexual interest (item 22 of the IDS-SR dichotomized into usual or somewhat less interest or pleasure – 78.7%

vs. little or no interest or pleasure – 21.3%). This adjustment hardly affected the relationship between salivary testosterone and extraversion; extraversion remained the only independent statistically significant personality domain that was associated with pooled salivary testosterone levels ( $\beta = 0.072$ ;  $p = 0.01$ ). Secondly, we repeated our final analysis in a sensitivity analysis that excluded the 346 women using oral contraceptives. This showed that the independent association between extraversion and the pooled salivary testosterone levels was somewhat increased in strength ( $\beta = 0.092$ ;  $p = 0.004$ ).

## Discussion

This study demonstrates that salivary testosterone levels are positively associated with scores on the personality dimension of extraversion, both in healthy subjects and in subjects with lifetime or current depression and/or anxiety. Our finding supports the notion that testosterone can be viewed as a psychologically relevant biological trait variable [7]. Notably, although in an earlier study by Sellers et al. [5] the association between testosterone and extraversion was not statistically significant, the strength of the association observed ( $r = 0.09$ ) was very similar to the strength of the association we observed in this study.

As mentioned above, there are theoretical grounds for a relationship between basal testosterone and extraversion. Extraversion can be seen as a construct of motivation for social approach behavior. Several experimental studies have demonstrated an implicit and reflexive effect of testosterone on dominance behavior and social approach behavior [12, 37]. In a study by Terburg et al. [12], testosterone administration was shown to prolong dominant staring into the eyes of threatening faces, demonstrating that testosterone motivates social dominance behavior outside of conscious awareness. Enter et al. [13] have demonstrated that testosterone administration diminished avoidance tendencies to angry faces. Other studies have shown that high testosterone and extraversion are both associated with positive affect, with a lower risk of depression and anxiety, a strong experience of goal-derived reward and high social status ranking [9, 24, 38, 39]. In our opinion our data are complementary to these studies, demonstrating a positive association between testosterone and extraversion in a large epidemiological cohort.

Testosterone was independently associated with 2 of the 3 NEO-FFI item cluster subcomponents – sociability

and activity. Depue and Morrone-Strupinsky [18] have provided a detailed account of the 2-trait structure of extraversion. Following this structure, sociability represents affiliative extraversion, reflecting an enjoyment of close interpersonal bonds, whereas activity represents agentic extraversion, reflecting an enjoyment of leadership roles and assertiveness. Both extraversion traits are related to positive affect [18]. Many studies have supported a link between testosterone and dominance behavior [2], which suggests an association between testosterone and agentic extraversion. However, there is also some, albeit more limited, evidence that the administration of testosterone increases social cooperation and prosocial behavior [40]. In addition, in a recent study by Lang et al. [41], it was demonstrated that extraversion works as a channel both for implicit power motives and implicit affiliation motives. We speculate that the link between testosterone and agentic extraversion could be similar to the link between testosterone and the implicit or explicit desire for high social status. In addition, we speculate that the link between testosterone and affiliative extraversion could be mediated either by implicit affiliation motives or by implicit power motives, as in many human subcultures it may be more advantageous for the maintenance of social status to engage others with agreeableness and enthusiasm rather than with dominant, and sometimes anti-social and aggressive, behavior.

As this is a cross-sectional study the direction of causation cannot be determined. It could be that in individuals with high scores on extraversion, baseline testosterone levels are increased and drive social status attainment or vice versa – in individuals with high testosterone, extraverted behavior is increased and drives social status attainment – or both. Furthermore, some other factor may explain the link between them.

The association between testosterone and the other Big Five traits was also tested in order to elaborate the specificity of the relationship between testosterone and extraversion. An initial negative association between testosterone and neuroticism, as well as an initial positive association between testosterone and conscientiousness, in subjects with lifetime or current depression/anxiety disappeared when extraversion was included in the model. For NEO-FFI personality traits 5 latent classes have been identified, which differ in neuroticism but also extraversion and conscientiousness scores and which are related to the severity of anxiety and depression symptoms [42]. This may explain why the associations for neuroticism and conscientiousness with testosterone disappear when controlling for extraversion and indicates that

the association between testosterone and extraversion is more fundamental.

A strength of our study is that we studied the association between the Big Five personality traits and testosterone in a large cohort study. In earlier research on the association between testosterone and personality characteristics small numbers of participants were studied and results were inconclusive. A second strength is that we subdivided participants into subjects with and without a lifetime diagnosis of depression or anxiety. Our results indicate that extraversion and testosterone are associated both in strata of subjects with and without lifetime depression and/or anxiety. Finally, we collected multiple salivary samples per subject that were subsequently pooled, which is preferable because of the pulsatile release of testosterone. Several earlier studies measured testosterone between noon and 16:00 h [5, 43]. As our samples were taken at different time points (morning samples between mean 07:27 and mean 08:27 h and evening samples between mean 22:03 and mean 23:02 h) our results are not directly comparable with those of Sellers et al. [5] and Josephs et al. [43].

Our study also has some limitations. First, cotton Salivettes were used for the assessment of salivary testosterone. In 1 study spuriously high testosterone was found with cotton-based collection [44], a finding which was not observed in another study [45]. In a study we performed ourselves we observed high correlations in men as well as in women between salivary testosterone levels based on cotton Salivettes and based on a passive drooling device ( $r = 0.87$ ). However, passive drooling devices are currently considered the preferred method for saliva collection [46]. Secondly, we did not monitor compliance with the study protocol other than for the timing of each sample collection, where participants registered sampling times that suggested adequate compliance, and we have not monitored the exact time interval from collection at

the homes of participants until freezing of the saliva samples. A third limitation of our study is that salivary testosterone levels had to be measured after an extra freeze thaw cycle, which may have contributed some error in testosterone measurements, although testosterone has been shown to be very stable [47]. As a fourth limitation, the median number of days between the interview and the saliva sample was 9 days, while ideally measures should have been taken on the same day, although the NEO-FFI scores are known to be stable over time. The test-retest reliability coefficients for extraversion over 1 week to 3 months were as high as 0.91 [48] and remained strong when assessments were even years apart. Finally, a limitation of our study was that we did not assess other sex steroid hormones like estradiol. The relationship between testosterone and personality characteristics might be more complex, for example testosterone might be a third variable, acting as a prohormone for estradiol.

Our results trigger a number of future research questions. First of all, in natural social groups of individuals, preferably from different settings, the relationships between social status ranking, extraversion and testosterone could be tested using multivariate analyses. Secondly, future studies should include measurements of other sex steroids and their interrelationship with testosterone. This appears to be even more relevant, as we observed a significantly stronger association between testosterone and extraversion in women than in men – a finding that requires further research. Finally, in future studies the relationship of testosterone with extraversion and implicit power and affiliation motives should be tested in the same subjects to further unravel their interactions.

## Disclosure Statement

The authors declare that they have no conflicts of interest.

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