The enhancing effects of testosterone in exposure treatment for social anxiety disorder: a randomized proof-of-concept trial

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Individuals with a social anxiety disorder (SAD) show hypofunctioning of the hypothalamus–pituitary–gonadal (HPG) axis, which is linked to social fear and avoidance behavior. As testosterone administration has been shown to facilitate social-approach behavior in this population, it may enhance the effectiveness of exposure treatment. In this proof-of-concept study, we performed a randomized clinical assay in which 55 women diagnosed with SAD received two exposure therapy sessions. Session 1 was supplemented with either testosterone (0.50 mg) or placebo. Next, transfer effects of testosterone augmentation on within-session subjective fear responses and SAD symptom severity were assessed during a second, unenhanced exposure session (session 2) and at a 1-month follow-up, respectively. The participants having received testosterone showed a more reactive fear pattern, with higher peaks and steeper reductions in fear levels in session 2. Post-hoc exploration of moderating effects of endogenous testosterone levels, revealed that this pattern was specific for women with high basal testosterone, both in the augmented and in the transfer session. In contrast, the participants with low endogenous testosterone showed reduced peak fear levels throughout session 1, again with transfer to the unenhanced session. Testosterone did not significantly affect self-reported anxiety. The effects of testosterone supplementation on fear levels show transfer to non-enhanced exposure, with effects being modulated by endogenous testosterone. These first preliminary results indicate that testosterone may act on important fear mechanisms during exposure, providing the empirical groundwork for further exploration of multi-session testosterone-enhanced exposure treatment for SAD.
Exposure intervention
The participants engaged in two 90-min public-speaking exposure sessions delivered one week apart in accordance with the protocols developed by Rodebaugh and colleagues [10, 13]. The sessions were standardized with respect to exposure length (6–8 min), preparation time (max. 5 min), reaction of the experimenter (neutral), and the availability of notes and speech topic. On the morning of the first day, the participants received psychoeducation about SAD and exposure, with the first session starting after 4 h. In both sessions, psychoeducation was repeated and personalized harm expectancies and goals were assessed. Then, the participants presented their prepared speech in front of a therapist, two confederates, and a camera. They reviewed their videotaped performance afterward together with the therapist. The therapists were psychology students in their last year of training (BA and MA level) trained and supervised by experienced, board-certified psychologists (M. H.M.H. and M.K.). To guarantee adherence to the protocol, the therapists were instructed to fill out a checklist of all protocol components and to report any deviations from the protocol. The checklists and reports on deviations showed that 96.3% of the sessions were delivered in accordance with the protocol.

Outcome measures
Within-session fear (primary outcome). Participants rated their fear levels on a subjective units of distress (SUD) scale ranging from 0: No fear to 100: Extreme fear [44]. SUDs were collected after psychoeducation (initial SUD), immediately prior to each exposure session (baseline SUD), immediately prior to the speech (start SUD), every 2 min during, and immediately after the speech (endpoint SUD).

Symptom severity (secondary outcome). Social anxiety symptoms were assessed with the Social Phobia Scale (SPS; [45]), a self-report measure assessing the fear of being observed or watched during social or performance situations. The scale has shown good internal consistency ([45], ɑ = 0.94; Dutch translation; [46], ɑ = 0.86). Participants completed the SPS at baseline, after the second exposure session (post-treatment) and at the 1-month follow-up (FU) assessment.

Saliva samples. To determine endogenous testosterone levels, saliva samples were collected (2 ml passive drool saliva by Salicap; Hamburg, Germany) at eight time points (Fig. 1): (1) at baseline, (2) prior to T/P intake, (3) prior to exposure session 1, (4) immediately after speech delivery in session 1, (5) 30 min after speech delivery in session 1, (6) prior to exposure session 2, (7) immediately after speech delivery in session 2, and (8) 30 min after speech delivery in session 2. Participants were asked to conform to certain directives regarding food and drink intake to prevent pollution of the saliva samples. Samples were stored at −20°C until radio-immune assays were performed at Dr. Kirschbaum’s laboratory (Dresden, Germany); for descriptions of the methodology, see refs. [47, 48].

Procedure. After having provided their informed consent, participants were screened online for eligibility, to which end they filled out the LSAS and answered general screening questions (e.g., age, treatment status, infertility, menstrual cycle). Eligible participants were telephoned for further screening (MINI, check in/exclusion criteria), after which they learned whether they would be participating in the study (see supplement for details). All other assessments and the exposure sessions took place at the treatment facility. After enrollment (see Fig. 1 for timing and procedure), participants completed the baseline assessment1, with the first exposure session being scheduled within one week. The morning of the session, the participants took a pregnancy test, saliva was collected, and psychoeducation provided, after which the participants completed a non-speech-related SUD and received T/P (administered by a research assistant). After 4 h, during which time participants were instructed to avoid physically and psychologically straining activities and heavy meals, they returned for the exposure session, before which saliva was collected and resting HR was recorded. Another saliva sample was collected immediately upon completion of the speech and 30 min thereafter. During the speech, SUDs and HRs were collected. At the end of the session, the therapist checked for adverse drug effects by asking participants about

1Computerized tasks were also part of the baseline assessment, but outcomes will be reported elsewhere.

Medication and randomization
The pharmacist providing the study solutions randomly assigned participants to testosterone (T) or placebo (P) in blocks of four (no stratification). T was suspended in a clear solution (0.5 ml) with 0.5 mg hydroxypropyl-beta-cyclodextrin, 0.005 ml ethanol 96%, and distilled water. P contained the same ingredients, barring T. Participants held the liquid under their tongues for 60 seconds. In women, this dose yields a sharp increase in plasma testosterone concentrations within 15 min and declines to baseline within 90 min [43]. Pharmacodynamic effects can be assayed 4–6 h after intake [30, 42]. Researchers, therapists, and participants were blinded to the group allocation until the completion of the primary outcome analyses.
Components
1. Saliva sample 1
2. Health screening
3. SPS
4. Saliva sample 2
5. Pregnancy test
6. Exposure rationale
7. Initial SUD score
8. T/P intake (11.00 AM)
9. Saliva sample 3
10. Resting HR
11. Speech preparation
12. Speech (SUS, HR)
13. Videotape review
14. Saliva sample 4
15. Saliva sample 5
16. Saliva sample 6
17. Resting HR
18. Speech preparation
19. Speech (SUDs, HR)
20. Videotape review
21. Saliva sample 7
22. Saliva sample 8
23. SPS

Physical complaints. A week later, the second exposure session took place, with all steps being identical to those of the first session barring administration. After a 30-min break, participants took the post-exposure assessment comprising the SPS and a computer task (reported elsewhere). We asked all participants to refrain from using alcohol, drugs, or medication (except from their stable dose of psychotropic medication) during testing days. One month later, participants once again completed the SPS online. This study was registered in the Dutch Trial Register: https://www.trialregister.nl/trial/6238 and at EudraCT (2014–201404475–23).

Timable procedures of the study protocol. SPS Social Phobia Scale, SUD subjective units of distress, T testosterone, P placebo, HR heart rate. Since pregnancy was a reason for exclusion, the pregnancy test was to ascertain that none of our participants was pregnant prior to the start of the testosterone-enhanced session.

Statistical analyses
To test the effects of testosterone augmentation on subjective fear, we used mixed models. A sample size of 52 participants was deemed necessary to detect group differences with at least a moderate effect size and a power of 80%. We tested the acute effects of the enhancement (session 1) and transfer to unenhanced exposure (session 2) separately. Its effects on SAD symptoms (SPS scores) were tested in an additional model (see below). Moreover, we explored augmentation effects on HR in similar models as subjective fear (see supplementary materials for details). We used the Lme4 package in R [49] and p-values were calculated using the likelihood ratio tests (Afxex package) [50]. Independent continuous predictors were centered and sum-to-zero contrasts used. Consistent with the recommendations for mixed models [51], we report unstandardized effect sizes (estimates).

RESULTS
Attrition
One participant receiving placebo dropped out before the first exposure due to illness (see participant section). Another participant in the same group dropped out during the first session (3.6%). All other participants completed both sessions and the follow-up.

Sample characteristics
The data of 54 participants were analyzed (M_age = 23.31, SD = 5.64, range = 18–43; Table 1). There were no significant between-group differences on any of the baseline measures. The manipulation was successful; compared to the placebo group, testosterone levels after testosterone administration (sample 3) were significantly higher in the enhanced group, moreover blinding was successful, participants were unaware if they received T or P (Table 1).

Adverse events
The testosterone and placebo arms did not differ with respect to adverse events; no serious events were reported in either group (for details see supplement).

Acute effects of testosterone augmentation (session 1)
Fear. Before reporting on the critical transfer session (2), we first describe the acute effects of testosterone on fear scores in session 1. Fear scores decreased over time (linear and quadratic), with exposure resulting in the expected within-session reduction: Estimate(linear) = −81.96 (16.76), F(1,51) = 23.89, p < 0.001; Estimate(quadratic) = −85.12(13.99), F(1,51) = 36.95, p < 0.001. The interaction between time (linear or quadratic) and group was not significant: p-values > 0.562 (see Supplementary Fig. S2A).

In the post-hoc model including baseline testosterone levels, the effects of time were confirmed: p-values < 0.049. There was no significant time × group interaction, p-values > 0.562 (see Supplementary section), but the effect for time(quadratic) × group × baseline-T effect was significant: Estimate = 2.26(0.94), F(1,48) = 5.72, p = 0.021. As to fear patterns as a function of endogenous testosterone, in the placebo group, fear was not moderated by baseline testosterone: Estimate = 0.82(1.14), F(1,24) = 0.52, p = 0.476. In contrast, in the testosterone group, fear patterns in the participants with higher baseline testosterone were relatively more reactive,
showing higher peaks followed by stronger reductions, than in those with lower values, where fear responses were characterized by relatively blunted peaks followed by weaker reductions: Estimate\(= -3.73(1.48), F(1,24) = 6.32, p = 0.019\) (Fig. 2A). Inclusion of age or hormonal contraceptives did not improve the fit of any of the models, so these were dropped from the analyses.

### Transfer effects of testosterone augmentation (session 2)

**Fear.** Next, we tested effects for the critical unenhanced session (2). Fear reduced over time\(^2\): Estimate\(= 62.95(15.42), F(1,50) = 16.66, p < 0.001\), Estimate\(= -48.32(11.03), F(1,50) = 19.18, p < 0.001\), Estimate\(= -36.76(8.90), F(1,50) = 17.01, p < 0.001\). Critically, there was a group \(\times\) time\(\times\)quadratic interaction: Estimate\(= 23.68(11.03), F(1,50) = 4.61, p = 0.037\). Compared to participants having received placebo, the participants in the testosterone group showed a more reactive fear pattern (higher SUDs) with a steeper decline at the end of the session (Supplementary Fig. S2B).

The post-hoc observation that testosterone administration had resulted in steeper fear reductions in participants with high testosterone (session 1) was again made in the second, non-enhanced session, with fear levels showing a similar time \(\times\) group \(\times\) Baseline-T interaction: Estimate\(= 1.53(0.74), F(1,47) = 4.22, p = 0.045\). In the placebo group, session-2 fear levels followed the same quadratic pattern regardless of baseline testosterone: Estimate\(= 0.77(0.82), F(1,23) = 0.94, p = 0.357\). In the testosterone group, they showed higher peaks followed by stronger reductions for participants with high baseline testosterone, whereas for those with low baseline testosterone peak fear levels flattened: Estimate\(= -2.29(1.27), F(1,23) = 3.26, p = 0.084\) (Fig. 2B).

**Post-hoc exploration of heart rate in sessions 1 and 2\(^3\).** Our results so far suggest that testosterone may have an acute impact on exposure mechanisms, boosting a steeper fear-decline in individuals with high baseline testosterone levels in session 1, which could be relevant for the subsequent transfer to session 2. To deepen our understanding of potential mechanisms affected during session 1, we post-hoc explored whether psychophysiological reactivity (HR) mimics the acute effects of testosterone administration on fear levels. HR patterns largely mimicked those of the subjective fear patterns in session 1: There was a non-significant trend toward a time\(\times\)group \(\times\)baseline-T interaction: Estimate\(= 0.80(0.42), F(1,44) = 3.64, p = 0.063\). In the placebo group HR decline followed the same slope regardless of baseline testosterone: Estimate\(= 0.04(0.03), F(1,23) = 0.56, p = 0.461\), while in the testosterone group HR reduced more for the participants with higher baseline testosterone levels: Estimate\(= -0.12(0.06), F(1,21) = 3.89, p = 0.061\). These acute psychophysiological effects did not transfer to the non-enhanced transfer session, indicating that they may support the acute fear reactivity, but that it is the subjective fear pattern that is longer-term affected (for full analyses see supplement and Supplementary Fig. S3).

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\(^2\)The residuals of the models for the SUDs in session 2 and the SAD symptom scores showed one standardized value >3; therefore, the models were re-run without this outlier. Since our primary outcomes were similar, the results presented include all data points.

\(^3\)We initially modeled linear, quadratic, and cubic time terms in all HR analyses but dropped the cubic term as it did not improve the model fit.
Testosterone enhancement did not significantly change SAD symptoms, as there was no effect of baseline testosterone.

The effects of testosterone partly coincide with several studies supporting avoidance-reducing and social-approach-facilitating properties of the hormone [25–27]. Moreover, the SUD patterns (increase prior to a decrease) are in line with Emotional Processing Theory (EPT) positing that fear needs to be activated first, and only after prolonged exposure, fear levels will drop. Such reactive pattern is deemed essential for learning and, hence, transfer in the long run [34, 35]. By boosting initial engagement with the feared stimulus, testosterone may affect important learning mechanisms reinforcing transfer (e.g., initial engagement to the feared stimulus in session 1 that transfers to fear levels in a second unenhanced session). Such interpretation is in line with the threat-approach boosting effects of testosterone in patients with social anxiety disorder [27, 31].

Then again, acute testosterone-augmentation effects depended on endogenous testosterone levels. This is consistent with evidence showing that individual differences in basal testosterone and proxies of fetal testosterone exposure (2D:4D ratio) moderate the effects of exogenous testosterone on various pertinent behavioral processes, including social approach, aggression, dominance, and risk-taking [37–39, 52, 53].

The interaction between endogenous testosterone levels and exogenous testosterone administration was interesting and deepen our understanding of the primary results, in that exclusively the participants with low baseline concentrations having received testosterone reported blunted peak fear levels. This is in line with earlier findings regarding the anxiolytic properties of testosterone [30, 54]. Together, these findings suggest that women with relatively low endogenous testosterone show lowered threat response following testosterone supplementation that transfers to the non-enhanced session. In contrast, although the women with higher basal testosterone reported similar fear levels at the end of the enhanced session, they arrived there via a different, more fear-reactive route that appeared to be transferred to the unenhanced session. Arguably, the testosterone-induced effects (e.g., higher peak fear) in women with higher endogenous levels could be interpreted as negative. However, in theoretical accounts of exposure therapy (i.e., EPT [35]...
and inhibitory learning theory [36, 55]) high fear levels during (initial) exposure sessions are deemed beneficial for a good response, prompting the hypothesis that, it may facilitate essential exposure mechanisms in those with high basal levels. In the present proof of concept study, we cannot yet verify such qualification of patterns as beneficial or not, particularly as our single session-enhancement did not result in lower SUD levels at the end of the second exposure, in the testosterone compared to placebo group. We can only make speculations based on theoretical grounds and clearly, treatment protocols with more exposure sessions are needed to further elucidate the effects of exogenous testosterone on fear activation and reduction within and across exposure sessions.

That endogenous testosterone moderates the effects of exogenous testosterone may be explained by trait factors, including individual differences in the sensitivity of the androgen receptor (AR), where relative AR insensitivity has been reported for people with low basal concentrations [56, 57]. Moreover, testosterone administration can lead to AR down-regulation in hypogonadal mice and human males, while long-lasting effects of endogenous testosterone may upregulate its expression [58].

The observed effects of exogenous testosterone on fear levels did not generalize to SAD symptoms. Although we extend previous observations that a single dose of testosterone can affect threat-behavior in SAD in an experimental context [27, 31], to fear-reactivity in a clinical context, we do not observe an effect on clinical outcomes. This may be a result of the fact that our symptom outcome measure (SPS) only has one item measuring speech anxiety. We recommend future studies to use a measure more sensitive to changes in speech anxiety. On the other hand, research testing other pharmacological enhancers demonstrated that repeated doses yielded better exposure outcomes than did a single dose [14, 59] So, future investigations comprising more testosterone-enhanced sessions are necessary to establish whether testosterone can improve SAD symptoms. As to the strengths of our study, we can say that with a comparative randomized clinical assay we were able to establish that the administration of a single dose of testosterone was safe and tolerable; there were no adverse events or augmentation-related drop-out. Moreover, by comparing effects in two successive sessions, we were able to examine the direct effects of the enhancement and their transfer in a relatively quick and cost-effective manner. However, since we only included women because the administration method we used has as yet only been applied in women [42], we cannot say whether our findings will generalize to men. Also, due to inclusion restrictions (e.g., birth control types, pregnancy) and because women with relatively low endogenous testosterone were relatively underrepresented, it remains to be tested whether findings generalize to a broader group and replication in a larger, more varied sample is needed. Furthermore, although all our participants met the SAD criteria, their baseline severity scores were somewhat lower than those reported in other exposure enhancement studies [11, 14, 60]. Even though our findings show that exogenous testosterone already exerts effects in a population with relatively mild symptoms, it needs to be shown whether they generalize to more severely impaired populations.

To conclude, testosterone-enhanced exposure differentially affects in-session fear levels, partly depending on baseline testosterone levels of individuals with SAD. It reduced self-reported peak fear levels in individuals with low baseline testosterone, and increased reactive patterns in individuals with high baseline testosterone. Because both patterns may be relevant for long-term extinction learning, we hope this study inspires an investigation of the longer-term effects of repeated testosterone-enhancements in SAD.

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