Title: Exogenous testosterone enhances cortisol and affective responses to social-evaluative stress in dominant men

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Abstract

Stress often precedes the onset of mental health disorders and is linked to negative impacts on physical health as well. Prior research indicates that testosterone levels are related to reduced stress reactivity in some cases but correlate with increased stress responses in other cases. To resolve these inconsistencies, we tested the causal influence of testosterone on stress reactivity to a social-evaluative stressor. Further, prior work has failed to consider status-relevant individual differences such as trait dominance that may modulate the influence of testosterone on responses to stressors. Participants (n = 120 males) were randomly assigned to receive exogenous testosterone or placebo (n = 60 testosterone treatment group) via topical gel prior to a well-validated social-evaluative stressor. Compared to placebo, testosterone significantly increased cortisol and negative affect in response to the stressor, especially for men high in trait dominance (95% confidence intervals did not contain zero). The findings suggest that the combination of high testosterone and exposure to status-relevant social stress may confer increased risk for stress-mediated disorders, particularly for individuals high in trait dominance.

1.1 Introduction

Stress is a leading contributor to poor health and mortality: Exposure to chronic or severe stress predicts increased risk for cardiovascular disease, psychiatric conditions such as depression and substance use disorders, and infectious disease (McEwen, 2004; Hammen, 2005; Stephens & Wand, 2012). Cortisol, a steroid hormone released as part of the hypothalamic-pituitary-adrenal (HPA) axis response to stress, mediates adaptive responses to stressors in the short term – for example, by stimulating gluconeogenesis to provide energy to respond to and recover from a stressor (McEwen, 2004). But in the context of chronic or severe stressors, cortisol can negatively impact physical and mental health via dysregulation of immune system activity and neurotoxic effects within the central nervous system (McEwen, 2004; Cohen et al., 2012; Sapolsky, 2000). Stress also heightens negative affect, which is an independent pathway that predicts poor mental and physical health (Hammen, 2005; Kiecolt-Glaser et al., 2002).

Because of these potential consequences for physical and mental health, the systems that modulate responses to stressors are of great importance for understanding relationships between stress, health, and well being. Prevailing theories propose that the sex hormone testosterone should reduce stress responses, but the causal effect of testosterone on stress reactivity in humans remains unclear. In animal (e.g., rodent) models of stress, testosterone reduces cortisol reactivity to stress (Viau & Meaney, 2004) and reduces fear behavior (Aikey et al., 2002). Consistent with this stress-buffering account, in humans, testosterone suppresses cortisol responses to pharmacological stimulation of the HPA axis in men (Rubinow et al., 2005), and reduces unconscious attention to fearful faces in women (van Honk et al., 2005). Yet other studies indicate that testosterone correlates with *increased* cortisol and negative affect in response to situations that threaten social status, like losing a competition (Mehta et al., 2008; Zilioli &

Watson, 2013) or being relegated to a low-ranking social position (Josephs et al., 2006). This correlational evidence is convergent with theorizing that testosterone directs the pursuit and maintenance of social status (Mazur & Booth, 1998) and suggests that testosterone may enhance acute stress responses when the stressor relates to social status, like during a social evaluation for a high-status job.

Despite this correlational evidence, the causal effects of testosterone on responses to status-relevant social stress have not been adequately tested in healthy young adults[1]. Further, the few correlational studies that have examined testosterone and social stress have provided mixed evidence linking testosterone levels to both increased (Juster et al., 2016) and decreased (Stephens et al., 2016) cortisol output. In order to clarify this inconsistent correlational evidence, we provide a direct causal test of testosterone's impact on cortisol and negative affect responses to stress using a status-relevant social stressor. We hypothesize that testosterone alters these stress responses, but because of the mixed correlational evidence, we are agnostic with regards to the direction of the effect. Moreover, given the increasing rate at which testosterone is prescribed (Baillargeon et al., 2013), administering testosterone prior to a social-evaluative stressor will provide much needed insight into testosterone's potential impact on stress and health.

Prior research also indicates that testosterone's effects on status-relevant behavior depend on trait dominance, an individual difference factor relevant to concern for status attainment, but the interactive effects of testosterone and dominance on stress responses is unknown. High levels of trait dominance, marked by a propensity to use force, fear, or intimidation to gain high-ranking positions within social groups (Cheng et al., 2013), correlate with increased

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[1] But *cf.* Rohleder et al. (2002), for a small sample of older men that found no difference in cortisol responses to an acute stressor several days after a single dose of testosterone vs. placebo.

cardiovascular stress reactivity (Lee & Hughes, 2014) and accentuate the behavioral effects of testosterone in status-relevant situations. For example, exogenous testosterone increases men's aggression after provocation (Carré et al., *in press*) and increases women's competitive behavior after winning a contest (Mehta et al., 2015), but only in individuals high in self-reported trait dominance. These behavioral results suggest that trait dominance may also exacerbate the influence of high testosterone levels on responses to status-relevant stressors.

2. Materials and Methods

2.1 Participants

Male participants (n = 120) between the ages of 18-40 (*Mean* = 22.50 years; *SE* = 0.33) were recruited via emailing campus listservs and by placing flyers on and near campus. Interested parties were screened for medical conditions that would prevent participation in the study, including immune, endocrine, neurological, or mental health conditions, and alcohol or drug abuse (see *Supplemental Materials*). The University of Oregon's Institutional Review Board approved all methods.

2.2 Protocol

2.2.1 Participants and Recruitment. After passing screening, the participant chose a day (Monday–Saturday) to attend a six-hour laboratory session, which began between 9:00–11:00 AM. These times were chosen so that the stressor would occur between 1:00–3:00 PM to control for diurnal endocrine variation. Participants were asked to refrain from eating or drinking anything except water or brushing their teeth 1.5 hours prior to the start of the session. Upon arrival at the session, experimenters obtained informed consent from the participant (see **Figure 1** for a study timeline of saliva sampling and self-report data collection). Participants were paid $60 for completing the laboratory session.

2.2.2 Gel Application and Blinding. This study was executed as a between-groups, placebo-controlled experimental design. Testosterone and placebo doses (n = 60 in each group) were randomly ordered prior to data collection by members of our laboratory who were unaffiliated with this research. Participants were given a sealed enveloped which either revealed that the gel was testosterone or placebo – a single-blind condition – or simply stated that he had an equal chance of receiving testosterone or placebo – a double-blind condition. These blinding conditions were implemented to control for the expectancy effects (i.e., 'conventional wisdom') of receiving testosterone (Eisenegger et al., 2010). A laboratory member uninvolved in data collection prepared the envelopes prior to the start of data collection, thus the experimenter in the laboratory session never knew whether the vial contained testosterone or placebo, or to which blinding condition the participant was assigned.

Under the supervision of the experimenter, the participant rubbed increments of the gel onto his own shoulders and upper arms. The participant was then given several minutes to read the contents of the envelope, during which the gel dried.

2.2.3 Pharmacological Manipulation. The testosterone gel (AbbVie, Chicago, IL) consisted of a 150-mg dose of testosterone in addition to pharmacologically inactive ingredients (see *Supplemental Materials*). The placebo gel contained the same inactive ingredients as the testosterone gel; the lack of testosterone was the only difference between the gels. The testosterone dose and time course for this protocol was based on prior topical-testosterone-administration research that showed serum testosterone concentrations peaked 3 hours after a

150-mg testosterone dose (Eisenegger et al., 2013)[2]. Prior research has also shown physiological and neural reactivity 3-6 hours after testosterone administration[3] (Tuiten et al., 2000, Radke et al., 2015). In order to execute our protocol during peak concentrations and within a 6-hour time period, this project utilized a 150-mg dose of testosterone approximately four hours (Mean=3.98 hours, SE=0.015 hours) prior to the social-evaluative stressor.

2.2.4 Social-Evaluative Stressor. The social-evaluative stressor (the Trier Social Stress Test, or TSST; Kirschbaum et al., 1993) consisted of a mock job interview for a high-status, managerial position followed by a verbal mental math task. The job was described in a printed document and was designed to be representative of an early-career position consisting of managing a small team (i.e., twelve student employees) in a campus business office. Two panelists wearing white lab coats conducted the interview. These panelists were trained to maintain neutral affect and behavior throughout the task. For a majority of participants (72.5%), the panel was mixed gender; the remaining panels were composed of two male or two female panelists (24.2% male panelists; 3.33% female panelists; see *Supplemental Materials* for evidence that panelist gender did not alter results).

2.2.5 Other tasks. The study protocol contained three decision-making paradigms prior to the social-evaluative stressor in order to maximize data obtained from each participant undergoing exogenous testosterone administration; all will be analyzed and reported elsewhere.

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[2] See Bird et al. (2016) and Carré et al. (2015) for work published after this data was collected that shows that peak testosterone concentrations occur earlier, approximately sixty minutes after topical gel administration.

[3] This prior research was conducted on women using sublingual testosterone and so may have questionable value for experimental studies involving topical testosterone in men. As above, we recommend Bird et al. (2016) and Carré et al. (2015) for more up-to-date methods for topical testosterone administration.

Participants could earn bonus money based on their performance in two of these tasks, though the exact amount earned was not revealed until the end of the laboratory session.

2.3 Questionnaires

2.3.1 Dominance and Prestige Scale. Trait dominance was indexed from a scale that measures dominance aspects of status-seeking motivation, related to obtaining status via force, fear, or intimidation, and prestige motivations, which are related to obtaining status via competence, social skills, or respect (Cheng et al., 2013). The survey – which has been shown to be valid and reliable (Cheng et al., 2010) – consists of 17 items related to dominance (e.g., "I try to control others rather than permit them to control me.") and prestige (e.g., "Members of my peer group respect and admire me.") on a scale from 1 (not at all) to 7 (very much). Dominance (Cronbach's $\alpha = .68$) and prestige items (Cronbach's $\alpha = .83$) were averaged and normalized within each subscale.

2.3.2 Positive and Negative Affect. The PANAS-X general negative and general positive affect subscales were used to measure affect responses. Participants responded on a 1 (not at all or very little) to 4 (quite a bit) scale. Negative (average Cronbach's $\alpha = 0.89$) and positive (average Cronbach's $\alpha = 0.84$) items were averaged for each time point according to published guidelines (Watson & Clark, 1994). We also conducted exploratory analyses on the subscales that underlie general negative affect (fear, hostility, guilt, sadness) and general positive affect (joviality, self-assurance, attentiveness).

2.4 Saliva Sampling and Endocrine Assays

The cortisol response to stress was determined from saliva samples collected immediately before (i.e, pre-TSST) and +0, +20, and +40 minutes after the stressor (see *Supplementary*

Materials for analyses of samples that occurred prior to gel application (morning baseline) and

three hours after gel application, **Figures S1**-**S3**). For saliva collection, participants were

instructed to drool 2 mL of saliva into polypropylene centrifuge tubes. Saliva samples were

assayed in duplicate for cortisol and testosterone in our laboratory using commercially available

enzyme immunoassay (EIA) kits (DRG International; see *Supplemental Materials* for details).

The average intra-assay coefficients of variation (CVs) were 4.68% (cortisol) and 6.55%

(testosterone); the inter-assay CVs were 14.8% (cortisol) and 16.1% (testosterone) averaged

across low and high control samples.

Testosterone administration resulted in testosterone levels that exceeded the EIA kits'

maximum (5250 pg/mL) in 34.4% of samples within the testosterone group (17% of all samples;

no samples in placebo condition were above threshold). As a conservative estimate of the

testosterone concentrations, we replaced these unknown values with the EIA kit's maximum

value, 5250 pg/mL. If one of the duplicate samples was within the kit's range, we averaged that

known value with the maximum. All analyses were chosen *a priori* to focus on testosterone

treatment as a categorical variable. This data replacement strategy therefore provides a low-end

estimate of testosterone concentration in order to confirm that testosterone treatment sufficiently

raised testosterone concentrations. Given this conservative estimate, any significant differences

can be extrapolated to be true for a test if the actual concentrations were known (see

Supplemental Materials for further justification of this approach).

 2.5 Analytical Plan

Multi-level models were constructed to examine Time x Testosterone vs. Placebo (T/P)

and Time x T/P x Dominance effects on cortisol and negative affect responses to social-

evaluative stress (see *Supplementary Materials* for full models). T/P condition was effects coded

(Testosterone = 1, Placebo = -1). All analyses controlled for participant blinding condition (see *Supplemental Materials* for analyses of blinding and). Ninety-five percent confidence intervals (95%CIs) of the model estimates were used to determine the magnitude and direction of the effects. Relying on these model interpretations avoids some of the issues inherent to interpreting p-values as part of null hypothesis testing (Cummings, 2014).

In order to confirm interpretations of the multilevel models of the cortisol response, we conducted a GLM analysis on area-under-the-curve with respect to increase (AUC_I), a measure of cortisol reactivity that takes into account all four samples (Pruessner et al., 2003). Simple slope analyses were used to decompose interactions (Preacher et al., 2006).

3. Results

3.1 Preliminary Analyses

Four participants (n = 2 from testosterone treatment group) did not complete the social-evaluative stressor and were excluded from analyses. Two additional participants were missing a single sample – one participant left the laboratory prior to completing the TSST+40 sample and one participant's sample was improperly aliquoted during the assay process – but these participants were left in the analyses as multilevel models are generally able to account for singular missing data points. Outliers (>3 SD) for negative affect were found at each time point (Baseline: n = 2; Pre-TSST: n = 3; Post-TSST: n = 1); these values were Winsorized to a score 3 SD above the mean for each time point. See Table S1 for descriptive statistics and correlations (*Supplemental Materials*).

As expected, the testosterone gel substantially increased testosterone concentrations (mean of post-gel testosterone concentrations, Testosterone group: M = 2959.87 pg/mL, *95%CI*[2472.46, 3447.28]; Placebo group: M = 164.00 pg/mL, *95%CI*[122.99, 205.01]; see

Supplementary Materials, **Figure S3**). No differences in pre-TSST cortisol or baseline affect were found between treatment groups or in exploration of interactions between treatment group and trait dominance (see *Supplementary Materials*).

3.2 Cortisol Response to Stress

Examining the impact of exogenous testosterone on cortisol concentrations across time revealed a Time x Testosterone/Placebo (T/P) condition interaction, such that exogenous testosterone increased cortisol responses to the social-evaluative stressor compared to placebo (Time x T/P: $B = 0.020$, *95%CI*[0.001, 0.038]; $Time^2$ x T/P: $B = -0.013$, *95%CI*[-0.031, 0.005]; **Figure 2A**, **Table S2**, *Supplementary Materials*).

This Time x T/P interaction was moderated by trait dominance (Time x T/P x Dominance: $B = 0.020$, *95%CI*[0.002, 0.038]; $Time^2$ x T/P x Dominance: $B = -0.021$, *95%CI*[-0.039, -0.003]; **Figure 2B**). Decomposing these interaction terms with simple slope analyses revealed that high dominant men given testosterone showed a robust increase in cortisol due to the stressor (Time: $B = 0.101$, 95%CI[.064, 0.137]; $Time^2$: $B = -0.132$, 95%CI[-0.169, -0.097]) compared to high dominant men given placebo, who displayed a relatively flat cortisol response (Time: $B = 0.022$, 95%CI[-0.014, 0.058]; $Time^2$: $B = -0.066$, 95%CI[-0.101, -0.030]). Low trait dominant men given testosterone (Time: $B = 0.064$, 95%CI[0.028, 0.100]; $Time^2$: $B = -0.084$, 95%CI[-0.120, -0.049]) or placebo (Time: $B = 0.065$, 95%CI[0.028, 0.101]; $Time^2$: $B = -0.100$, 95%CI[-0.137, -0.064]) were essentially equivalent in terms of their cortisol response to the stressor.

Follow-up analyses on cortisol AUC_I confirmed this overall pattern of results: Testosterone treatment predicted increased AUC_I ($B = 0.073$, 95%CI[0.007, 0.358]), but this was moderated by trait dominance ($B = 0.077$, 95%CI[0.013, 0.141]; **Table S3**; see *Supplemental*

Materials for simple slope analyses). For men high in trait dominance, testosterone increased

cortisol AUC_I compared to placebo; no differences were evident for low trait dominant men

(**Figure 3;** see *Supplemental Materials* for reactivity and recovery analyses, **Figure S4**). Trait

prestige levels did not interact with T/P to predict cortisol levels (**Tables S4**).

3.3 Positive and Negative Affect Responses

Testosterone increased negative affect in anticipation of the stressor compared to placebo

(Time x T/P: $B = 0.050$, *95%CI*[-0.010, 0.111]; $Time^2$ x T/P: $B = -0.044$, *95%CI*[-0.088, -0.001];

Figure 4A). Testosterone's causal increase of negative affect across time was also moderated by

trait dominance (Time x T/P x Dominance: $B = 0.080$, *95%CI*[0.021, 0.139]; $Time^2$ x T/P x

Dominance: $B = -0.036$, *95%CI*[-0.079, 0.007]; **Figure 4B**; **Table S5**, *Supplementary*

Materials). Simple slope analyses revealed that for men high in trait dominance, testosterone

(Time: $B = 0.324$, 95%CI[0.206, 0.443]; $Time^2$: $B = -0.229$, 95%CI[-0.315, -0.143]) increased

negative affect in response to the stressor compared to placebo (Time: $B = 0.066$, 95%CI[-0.051,

0.183]; $Time^2$: $B = -0.068$, 95%CI[-0.153, 0.017]). Low trait dominant men given testosterone

(Time: $B = 0.148$, 95%CI[0.029, 0.267]; $Time^2$: $B = -0.160$, 95%CI[-0.246, -0.073]) or placebo

(Time: $B = 0.209$, 95%CI[0.090, 0.329]; $Time^2$: $B = -0.144$, 95%CI[-0.231, -0.058]) showed

increased negative affect in response to the stressor, but were essentially equivalent in terms of

their negative affect in response to the stressor. Thus, testosterone enhances negative affect in

response to a forthcoming social stressor and sustains this negative response to the stressor for

individuals high in trait dominance.

Follow-up analyses revealed that the effects of testosterone among high-dominance men

were also seen on two specific subscales of negative affect, fear and hostility (**Table S6, Figure**

S5). Trait prestige levels were not found to moderate the effect of testosterone on negative affect in response to the stressor (**Table S7**).

Positive affect decreased in response to the social-evaluative stressor (Time: B = -0.437, *95%CI*[-0.503, -0.371]), but neither testosterone nor the interaction of testosterone and trait dominance moderated this effect (all 95%CIs contain zero; **Table S8**).

4. Discussion

This study provides causal evidence that exogenous testosterone increases cortisol concentrations and negative affect in response to a social-evaluative stressor, especially for individuals with high trait dominance. For an individual high in trait dominance – who is already predisposed to being concerned with status and accustomed to wielding social or even physical force to obtain it – exogenous testosterone administration motivates strong concern for his status, making him vigilant for cues to potential threats. Indeed, testosterone increases neural reactivity and behavioral responses to threatening interpersonal cues that may signal an impending social challenge, like angry faces (Goetz et al., 2014; Hermans et al., 2008; Terburg et al., 2012). This increased concern for status during a social evaluation may be driving the dominant individual with high testosterone levels to feel more negative affect and elicit a stronger physiological response to the stressor. The present work therefore advances theory on testosterone and social status (Mazur & Booth, 1998) and challenges medical assumptions of testosterone's stress-suppressant effects by showing that testosterone's influence on susceptibility to status threat extends to acute social-evaluative stress. Future work that investigates the effects of testosterone on stress responses must (i) consider the social context in which the stressor exists and (ii) account for individual differences in relevant psychosocial constructs like trait dominance.

In addition to these psychosocial explanations, careful consideration must be given to the potential biological mechanisms by which testosterone increases stress responses. Testosterone has been linked to increased activity in brain areas sensitive to threatening stimuli, such as the amygdala (Goetz et al., 2014). In animal research the amygdala is a key neural component that promotes HPA responses to stress (Herman et al., 2005), with limited work suggesting it may influence human responses well (Dedovic et al., 2009). Testosterone is also associated with reduced connectivity within frontal-limbic neural circuitry, a pattern thought to indicate decreased neural regulation of affect and behavioral responses to threat (Volman et al., 2011; van Wingen et al., 2010). Although currently untested, an individual with high testosterone levels who is high in trait dominance in the midst of a social evaluation may therefore experience increased activation and reduced regulation of these neural threat responses as part of an exacerbated response to the stressor.

4.1 Future Directions

The present study provides clear evidence of testosterone's direct effects on stress responses and the moderating influence of trait dominance, but several facets must be explored in future work. First, this study used a prescription-strength dose of testosterone in order to supersede naturally occurring testosterone levels in eugonadal men. Future work must ensure testosterone's causal effects are robust at naturally occurring concentrations, which could be accomplished by blocking gonadal endocrine functioning prior to administering testosterone to normal physiological ranges (Goetz et al., 2014).

Second, although we provide evidence of testosterone's direct effects on stress responses, the broader gonadal endocrine system should continue to be explored with regards to stress responses. For example, some of testosterone's effects within the central nervous system depend

on local conversion to estrogen metabolites such as estradiol (Naftolin, 1994). Further, estradiol administered to men has been shown to increase HPA-axis responses to stress (Kirschbaum et al., 1996). Thus the effects reported here may depend on estradiol conversion, but future research must rigorously test this dependency in humans by blocking testosterone conversion to estradiol or antagonizing estrogen receptors.

Third, based on the extant literature (Carré et al., *in press*; Mehta et al., 2015; Slatcher et al., 2011), we focused on trait dominance but future work should examine other possible moderators relevant to stress, testosterone, and social-status motivations. These putative moderators may include Type A/B personality, which may relate to trait dominance and correlates with stress-linked health outcomes (Pittner et al., 1983), as well as other factors that have been previously shown to moderate testosterone's behavioral effects, like trait impulsivity (Carré et al., *in press*) or 2D:4D digit ratio, a presumptive index of prenatal androgen exposure (van Honk et al., 2011).

Finally, the present study was limited to men due to constraints on the use of prescription-strength testosterone, but future research must consider the effects of testosterone and dominance on women's stress responses. Prior work has shown that neural responses to threat are similar for men and women given exogenous testosterone (Hermans et al., 2008; Goetz et al., 2014) and, more broadly, that the interactive effects of exogenous testosterone and trait dominance alter women's status-relevant behavior (Mehta et al., 2015). But women's status-seeking motivations may be more dependent on other sex hormones, such as estradiol (Stanton & Schultheiss, 2007), suggesting that a broader examination of gonadal hormones is warranted for men and women.

In summary, future work must investigate the extent to which stress responses are causally enhanced by physiologically normal ranges of testosterone and other gonadal hormones like estradiol, while exploring an array of status-relevant moderators of stress responses in men and women.

4.2 Conclusions

These results have important implications for understanding testosterone's role in stress and health and may provide mechanistic insights for the clinical science of stress-linked disorders. Stress often precedes the onset of psychiatric conditions, like depression and substance use disorders (Hammen, 2005; Stephens & Wand, 2012). Individuals high in trait dominance with high testosterone levels may therefore be susceptible to stress-linked disorders like mood or substance abuse disorders due to increased reactivity to social stress. In support of this inference, dominance motivations have been theorized to share common etiology for externalizing psychopathologies with known links to stress exposure, such as drug and alcohol abuse (Johnson et al., 2012). In comparison to dominance, evidence of testosterone's relationship with mental health is mixed: One large study found that above-average testosterone levels correlated with depressive symptoms, though controlling for protective psychosocial factors like marriage or employment attenuates this relationship (Booth et al., 1999a). But this same work and other research shows that lower testosterone levels (i.e., hypogonadism) are also associated with depressive symptomology (Booth et al., 1999a; Ford et al., 2016; Giltay et al., 2017), which testosterone treatment may improve (Snyder et al., 2016). Given this evidence of increased symptomatology at high and low testosterone levels, the present results may be one end of a U-shaped relationship between testosterone and stress. Future work must therefore clarify the clinical significance of testosterone by continuing to examine the causal and moderating

pathways by which these biological and psychosocial factors affect the onset and course of mental health disorders via alterations in stress responses.

In terms of physical health, exogenous testosterone is increasingly prescribed to treat hypogonadism (Baillargeon et al., 2013) and is associated with improved cardiovascular health (Alexander et al., 2017). Some limited evidence indicates that endogenous testosterone may relate to physical health in an inverted U-shape, with increased health complaints at high and low concentrations (Booth et al., 1999b), while other evidence suggests exogenous testosterone may actually boost the risk of non-fatal heart attacks (Finkle et al., 2014; *cf.* Corona et al., 2014 for alternative explanations of these findings). To date, none of this work has considered the potential ramifications testosterone may have for stress-related health conditions when considered within a psychosocial context. Our findings show that in response to a social stressor, testosterone increases cortisol levels and negative affect, which are both theorized pathways for downstream negative consequences of stress such as poor cardiovascular health (McEwen, 2004; Cohen et al., 2012; Sapolsky, 2000; Kiecolt-Glaser et al., 2002) and increased risk for acute cardiac events such as a heart attack (Steptoe & Kivimäki, 2012). It should be noted that an acute stress response in itself is not unhealthy. For example, cortisol and negative affect could provide metabolic energy and motivation to gain a high status position within a stressful social setting. But in the long term, over repeated stressors, testosterone's causal increase of these stress responses may represent a liability to health and well-being, particularly for dominant individuals (McEwen, 2004). These results advocate strongly for the inclusion of psychosocial variables like trait dominance in future biomedical and clinical studies on the effects of testosterone on stress and stress-linked health outcomes.

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Table/Figure Legends

Figure 1: Timeline of the experimental protocol for the present report. All timings are approximate and are represented in minutes relative to the end of the social-evaluative stressor. "TSST intro." represents the instructions for the social-evaluative stress procedure. Two saliva samples were collected prior to the TSST-relevant samples: At baseline (prior to gel application) and three hours after gel application (see Supplemental Materials).

Figure 2: Cortisol response to social-evaluative stress. All values are estimated marginal means from relevant models and all error bars are 95% confidence intervals. **Panel A.** Time x Testosterone vs. Placebo (T/P) effect on cortisol response. **Panel B.** Time x T/P effect on cortisol response graphed at ±1 SD trait dominance.

Figure 3: Cortisol response to stress as indexed by AUC_I. All values are estimated marginal means from relevant models and all error bars are 95% confidence intervals. **Panel A.** Main effects of T/P on cortisol AUC_I. **Panel B.** Interactive effects of T/P x trait dominance on cortisol AUC_I. * = group means differ with 95% confidence (i.e., interval of one group's mean does not include the other group's mean).

Figure 4: Negative affect response to social-evaluative stress. All values are estimated marginal means from relevant models and all error bars are 95% confidence intervals. In each graph, "Pre-TSST" was measured after giving instructions for the social-evaluative stress task but before beginning the task and is therefore a measure of anticipatory negative affect. **Panel A.** Time x T/P on negative affect. **Panel B.** Time x T/P effect on negative affect graphed at ±1 SD trait dominance.