

The gonadal response to social stress and its relationship to cortisol

Belinda Pletzer, Eefje S. Poppelaars, Johannes Klackl & Eva Jonas

To cite this article: Belinda Pletzer, Eefje S. Poppelaars, Johannes Klackl & Eva Jonas (2021): The gonadal response to social stress and its relationship to cortisol, *Stress*, DOI: [10.1080/10253890.2021.1891220](https://doi.org/10.1080/10253890.2021.1891220)

To link to this article: <https://doi.org/10.1080/10253890.2021.1891220>



© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



Published online: 12 Mar 2021.



Submit your article to this journal [↗](#)



Article views: 366



View related articles [↗](#)



View Crossmark data [↗](#)

The gonadal response to social stress and its relationship to cortisol

Belinda Pletzer^{a,b} , Eefje S. Poppelaars^a , Johannes Klackl^a  and Eva Jonas^a 

^aDepartment of Psychology, University of Salzburg, Salzburg, Austria; ^bCentre for Cognitive Neuroscience, University of Salzburg, Salzburg, Austria

ABSTRACT

The cortisol response to social evaluative stress has been well characterized. However, data regarding changes in gonadal hormones after stress are still scarce and inconsistent. The majority of studies have focused on testosterone reactivity to stress in men, while estradiol responses or gonadal stress responses in women have hardly been investigated. Furthermore, it has not been evaluated whether sex hormone reactivity to stress differs between men and women and the relationship between cortisol and gonadal reactivity to stress is still unclear. To address these questions, we re-analyzed saliva samples collected from 37 men and 30 women in their luteal cycle phase before and repeatedly after social-evaluative stress. Both, testosterone and estradiol levels were assessed. In both men and women, testosterone was significantly reduced after stress. Testosterone levels were at their lowest after 20 minutes, but did not return to baseline until 35 minutes after stress. Across the whole sample, estradiol was significantly increased after stress with two separate peaks after 15 and 30 minutes. Follow-up analyses revealed that 41 participants actually responded with a decrease in estradiol levels to stress, with lowest levels after 20 min, while the remaining participants responded with an increase in estradiol levels. These gonadal stress responses appear to be largely independent of the cortisol response to stress. These results demonstrate that the endocrinological stress response is not restricted to the HPA axis and stress responsivity of gonadal hormones is not simply driven by cortisol. Accordingly, the stress responsivity of gonadal hormones and their association to psychological variables is an additional avenue to explore in both men and women.

ARTICLE HISTORY

Received 19 March 2020
Accepted 11 February 2021

KEYWORDS

Social evaluative stress; testosterone; estradiol; cortisol; psychosocial stress; sex differences

1. Introduction

The endocrine response to acute stressors is well characterized, with an immediate response of the sympathetic nervous system (SNS; peak in adrenaline), followed by a slower response of the hypothalamo-pituitary-adrenal (HPA) axis with a peak in corticosteroids (CORT; cortisol in humans) after about 15 minutes (see Bali & Jaggi, 2015 for a review).

1.1. Modulation of the HPA axis by gonadal hormones



Several studies in humans suggest an increased or decreased cortisol response depending on the levels of gonadal hormones (see Toufexis et al., 2014 for a review). For instance, a blunted cortisol response is often observed in women as compared to men (e.g. Kudielka & Kirschbaum, 2005; Uhart et al., 2006; see Kajantie & Phillips, 2006 for a review; see Liu et al., 2017 for a meta-analysis; but see Kirschbaum et al., 1999; Rohleder et al., 2001; Wolf et al., 2001). However, during the luteal phase of women, sex difference in the cortisol response were diminished (Kirschbaum et al., 1999; Kudielka et al., 2004; Wolf et al., 2001; Rohleder et al., 2001; but see Tersman et al., 1991; Duchesne et al., 2012), since a higher cortisol response was observed during the high-hormone

luteal phase of the menstrual cycle compared to low hormone phases (Kirschbaum et al., 1999; Altemus et al., 2001; Espin et al., 2013; Montero-López et al., 2018; Stephens et al., 2016; but see: Duchesne et al., 2012; Maki et al., 2015). Likewise, estrogen treatment increases the cortisol response in pre-menopausal women and transwomen (Kirschbaum et al., 1996; Fuss et al., 2019; but see Komesaroff et al., 1999; Kudielka et al., 1999 for opposite results in peri- and post-menopausal women).

Apart from estrogens, also male gonadal hormones may contribute to the sex difference in the cortisol response to various stressors. In men and transmen, testosterone administration has been shown to diminish the cortisol response to physiological CRH stimulation (Fuss et al., 2019; Rubinow et al., 2005), but increases the cortisol response to psychosocial stressors (Knight et al., 2017). Accordingly, the effects of basal testosterone levels on the cortisol response to stress appear to depend on the type of the stressor.

1.2. Modulation of the HPG axis by stress and adrenal hormones

Vice versa, an up- or downregulation of gonadal hormones, depending on cortisol levels, has also been described.

CONTACT Belinda Pletzer  Belinda.Pletzer@sbg.ac.at  Department of Psychology, University of Salzburg, Hellbrunnerstr. 34, Salzburg 5020, Austria

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Chronic elevation of cortisol downregulates the HPG axis and reduce gonadal hormone levels in both men and women (Aakvaag et al., 1978; Roney & Simmons, 2014). However, also acute stressors have been shown to impact sex hormone levels, particularly testosterone levels in men (see e.g. Chichinadze & Chichinadze, 2008 for a review). Several studies suggest a decrease in testosterone after physical stressors, like exercise (Fernández-García et al., 2002; Gomez-Merino et al., 2002; Maner et al., 2008; Oltmanns et al., 2005). However, some studies also demonstrate an increase in testosterone levels in response to stressors (Bedgood et al., 2014), particularly in competitive situations (Crewther et al., 2018; Gladue et al., 1989; Gonzalez-Bono et al., 1999), in dominant individuals or winners (Bernhardt et al., 1998; Chichinadze & Chichinadze, 2008; Crewther et al., 2018; Schultheiss et al., 2005; Zitzmann & Nieschlag, 2001).

Competition introduces a social-evaluative element to physical stress. Accordingly, social stressors, like the TSST, may be more likely to elicit testosterone elevations than physical stressors. However, the results obtained with the TSST are inconsistent (Bedgood et al., 2014; Juster et al., 2016; Lennartsson et al., 2012; Schoofs & Wolf, 2011). It is possible that these inconsistencies result from variability in participants' assessment of the competitiveness of the stress situation (Chichinadze & Chichinadze, 2008). The TSST does not only involve social evaluation, but also a cognitive element in the form of arithmetic. Accordingly, participants' assessment of their numerical skills may also play a role in how threatening or competitive the situation is perceived. Removing the arithmetic element from the stress provocation procedure may provide an opportunity for the unconfounded assessment of the gonadal response to social stress.

1.3. Open questions and the current study

In summary, the following modes of interaction between the adrenocortical and gonadal stress response have been hypothesized:

1. Baseline levels of gonadal hormones determine the strength and direction of the cortisol response (Kajantie & Phillips, 2006).
2. Baseline levels of cortisol determine the strength and direction of the gonadal stress response (e.g. Bedgood et al., 2014).
3. The cortisol response triggers a change in gonadal hormone levels (Chichinadze & Chichinadze, 2008).

It is however also possible, that gonadal hormone levels respond to stress independently of the cortisol response, and while doing so moderate the cortisol response to stress or vice versa. So far it remains unclear which of these modes of interactions is predominant. Also, most studies evaluating the gonadal response to stress have focused on testosterone levels, while estradiol levels have rarely been evaluated (Herrera et al., 2016; Schoofs & Wolf, 2011). Furthermore, no study so far has directly compared the gonadal response to stress

between men and women, despite the apparent sex differences in HPG functionality.

To address these questions, we analyzed testosterone and estradiol levels from saliva samples which were previously obtained to assess the cortisol response to an impromptu public speaking task—a social-evaluative stress paradigm similar to the TSST, but devoid of the cognitive challenge related to mental arithmetic. Our previous results show that this stressor reliably raises cortisol levels in both men and women with a peak 20 minutes after the stress manipulation ends (Poppelaars et al., 2019), as is typical for social stressors (Labuschagne et al., 2019). Based on the previous literature and the potential interaction modes between HPA and HPG axis outlined above, we chose an exploratory approach to establish:

1. Whether baseline sex hormones relate to the cortisol response to social-evaluative stress.
2. Whether estradiol and testosterone levels increase or decrease after stress, and if so.
3. Whether the gonadal stress response relates to baseline cortisol levels or the cortisol response to stress.

As the previous literature does not allow to draw clear conclusions on the directionality of these effects, no directional hypotheses were formulated.

2. Methods

This manuscript reports additional analyses on the sample described in Poppelaars et al. (2019). Poppelaars et al. (2019) assesses the association between different stress response stages, including the cortisol response, and personality characteristics. However, while it is well known that gonadal hormones may modulate the cortisol response to stress (see Kajantie & Phillips, 2006 for a review), the literature on changes in gonadal hormones is still scarce and largely inconsistent. Accordingly, we decided to re-analyze the samples collected during the study described in Poppelaars et al. (2019) for estradiol and testosterone in order to additionally evaluate the gonadal response to social-evaluative stress. Thus, the approach of the current study is exploratory and results should be interpreted with caution. From the measures collected by Poppelaars et al. (2019) only those included in the current analyses will be described in detail in the following. In order to not unduly inflate the probability of false positives, we focus the manuscript on endocrinological measures only.

2.1. Participants

As described in Poppelaars et al. (2019), of the 85 university students who participated in the experiment, 37 men (age: $M = 22.8$, $SD = 2.6$) and 30 women (age: $M = 22.9$, $SD = 2.8$) fulfilled the inclusion criteria, i.e. they were 18–35 years old, right-handed, had normal or corrected-to-normal vision, were heterosexual, free of psychiatric and endocrinological disorders, free of medication, and not regular smokers or drinkers.

Female participants did not use oral hormonal contraception or an intrauterine device for the past three months, were not currently pregnant or breast-feeding, had a regular menstrual cycle (21–35 days, Fehring et al., 2006) and were tested in their luteal cycle phase (day 3–10 before onset of next menses, high progesterone). The average cycle duration in the final sample was 27.6 days ($SD=3.2$) and the average testing day was day 20.5 ($SD=3.6$). Progesterone levels were in a plausible range of 42.6 and 527.5 pg/mL, with an average of 194.7 ($SD=121.9$).

2.2. Ethics statement

The study was approved by the ethics committee of the University of Salzburg. All methods conform to the Declaration of Helsinki. All subjects gave their informed written consent to participate in the study.

2.3. Protocol to induce social-evaluative stress

Social-evaluative stress was induced by an impromptu public speaking task in front of a prerecorded audience. The task is an adaptation of the Leiden public speaking task (Westenberg et al., 2009). It has been shown to considerably increase salivary cortisol levels (Poppelaars et al., 2019; Westenberg et al., 2009) and differs from the Trier social stress test in two important aspects: (i) the audience is prerecorded and thus constant for all participants, and (ii) no mental arithmetic has to be performed, thereby reducing the cognitive challenge of the situation. After baseline measurements, participants had to give a five-minute speech in English about their positive and negative qualities in front of a life-size-projected prerecorded audience and a camera. No participant was a native English speaker, but all participants were competent enough to participate, as assessed by a general English language competence test (www.cambridgeenglish.org/test-your-english/general-english/). Before the speech, participants had five minutes to prepare, but were not allowed to take any notes. Participants could see themselves in the camera-finder during the speech. Although participants were told that the audience would evaluate their video on 10 aspects concerning speech delivery, content, and quality, the videos were never evaluated. Participants were informed about the deception during the debriefing. The entire stress manipulation lasted about 18 minutes. As described in Poppelaars et al. (2019), this task resulted in a reliable cortisol response across all participants, which did not differ between men and women.

2.4. Procedure

All experiments were performed in English between 12am and 6pm. It has been shown that during this time-window cortisol levels are low and sensitive to external stimulation (Nicolson, 2008). Before participants arrived at the lab, they were informed that they would perform tasks relating to brain activity, hormones, and heart rate. They signed the

informed consent form and completed a custom health questionnaire to assess exclusion criteria as described in Poppelaars et al. (2019). A first saliva sample was taken to obtain baseline measurements of all hormones, before participants were informed that they had to perform a public speaking task. Further saliva samples were taken immediately after the public speaking task, 10 minutes later and then every five minutes until 35 minutes after the stress manipulation.

2.5. Hormone analysis

Seven saliva samples of 2 mL each were collected by spitting in a tube at baseline (i.e. 15 minutes before the stress manipulations), as well as every 5 minutes after the stress manipulations. To avoid sample contamination or hormone manipulation unrelated to the experiment, participants were instructed not to eat, drink (except water) or exercise 30 min prior to the experiment, and not to smoke on the same day. All samples were immediately frozen at -20°C . Prior to analysis, solid particles were removed by centrifugation for 15 min and 10 min respectively at 3,000 rpm. Cortisol levels had already been analyzed for Poppelaars et al. (2019). For the current manuscript, we additionally analyzed free estradiol and testosterone from the same samples, using DeMediTec salivary ELISA kits (DeMediTec Diagnostics, Kiel, Germany). Please note that this analysis step required an additional freeze-thaw cycle. While salivary steroids appear to be stable after repeated freeze-thaw cycles (Durdiaková et al., 2013; Gandara et al., 2007; Keevil et al., 2014), it is possible that hormone degradation led to a slight underestimation of actual sex hormone values. However the treatment of samples was consistent across participants to ensure the comparability of hormone values. In order to increase reliability, all samples were analyzed in duplicate and samples with intra-assay coefficients of variability above 25% were reanalyzed.

2.6. Statistical analyses

Statistical analysis was performed in RStudio version 1.1.423 with R version 3.1.3 (R Core Team, 2018). Hormone values exceeding the group mean by more than three standard deviations were excluded. This concerned cortisol values of one female participant, estradiol values of one female participant and testosterone values of one male participant.

In a first step, it was assessed whether baseline testosterone or estradiol levels predicted the cortisol reactivity to stress as reported in Poppelaars et al. (2019) using separate correlations for men and women.

In a second step it was addressed whether testosterone and estradiol respond to SET. We used linear mixed effects models, as implemented by the `lme` function of the `nlme` package, to assess whether time had a significant effect on estradiol and testosterone levels. Participant number was modeled as a random factor, while sex and time were modeled as fixed factors (formula: $\text{hormone} \sim 1|\text{PNr} + \text{sex} * \text{timepoint}$). All continuous dependent and independent

variables were scaled (z-standardized) prior to analysis in order to allow an interpretation of effect sized based on standard deviations similar to Cohen's d.

In a third step, it was evaluated how the gonadal response to stress as described in the current manuscript related to the cortisol response to stress as described in Poppelaars et al. (2019). Stress-responsiveness of all hormones (cortisol, testosterone, estradiol) was calculated as area under the curve related to the increase (AUCi) according to the following formula (compare Pruessner et al., 2003; Fekedulegn et al., 2007):

$$\sum_{i=0}^5 \frac{(Horm_i + Horm_{i+1})}{2} \cdot (t_{i+1} - t_i) - Horm_0 \cdot (t_6 - t_0)$$

where $Horm_i$ denotes the i -th hormone measurement and t_i the i -th timepoint. $Horm_0$ denotes the baseline measurement. Accordingly, AUCi is negative if hormones decrease, but positive, if hormones increase.

Correlation analyses were performed separately for men and women in order to assess whether (i) cortisol baseline measurements predicted the gonadal response to stress, and (ii) the cortisol response related to the gonadal response to stress. P-values were corrected for multiple comparisons across all correlations using false discovery rate (FDR). Non-significant associations with an at least moderate effect size of $r > 0.35$ are reported as trends and interpreted as directions for the development of further research questions.

In a final step, exploratory analyses were conducted to characterize different estradiol response profiles.

3. Results

3.1. Relationship of baseline gonadal hormones to cortisol reactivity

The cortisol response, calculated as AUCi, was not related to baseline testosterone or estradiol levels in either men or women (all $|r| < 0.25$, all $p > .19$, compare Table 1).

3.2. Gonadal response to social-evaluative stress

3.2.1. Testosterone

Baseline testosterone levels were significantly higher in men compared to women ($b = 1.49$, $SE_b = 0.14$, $t_{(63)} = 10.75$, $p < .001$). Stress reduced testosterone levels ($b = -0.15$, $SE_b = 0.03$, $t_{(388)} = -5.68$, $p < .001$) irrespective of participants' sex ($b = -0.02$, $SE_b = 0.04$, $t_{(388)} = -0.49$, $p = .62$). Also AUCi for testosterone did not differ significantly between men and women ($t_{(55.22)} = 0.40$, $p = .69$). Pairwise comparisons showed that in both men and women, testosterone reached a minimum 20 minutes after social-evaluative stress ($b = -0.21$, $SE_b = 0.05$, $t_{(63)} = -4.23$, $p < .001$; Figure 1(A)), i.e. at the same time that cortisol levels peak (Poppelaars et al., 2019). Until 35 minutes after stress, testosterone levels did not return to baseline levels ($b = 0.02$, $SE_b = 0.04$, $t_{(63)} = 0.46$, $p = .65$).

3.2.2. Estradiol

Baseline estradiol levels did not differ significantly between men and women ($b = 0.01$, $SE_b = 0.20$, $t_{(64)} = 0.06$, $p = .95$). While this is somewhat unexpected, it is not unusual, since estradiol levels only reach peak-levels in women during the

Table 1. Correlations in men and women.

	Men				Women			
	T baseline	E Baseline	T AUCi	E AUCi	T Baseline	E Baseline	T AUCi	E AUCi
T baseline		-0.16	-0.67***	0.04		0.39	-0.39	0.18
E baseline		0.03	0.18	-0.78***		0.08	-0.22	-0.24
Cort baseline	-0.04	0.36	0.18	-0.28	0.55*	0.08	-0.22	0.13
Cort AUCi	0.09	-0.22	0.13	0.11	0.01	0.11	0.25	-0.22

Note. Significant and trend associations are displayed in bold font. *** = $p_{FDR} < 0.001$; * = $p_{FDR} < 0.05$.

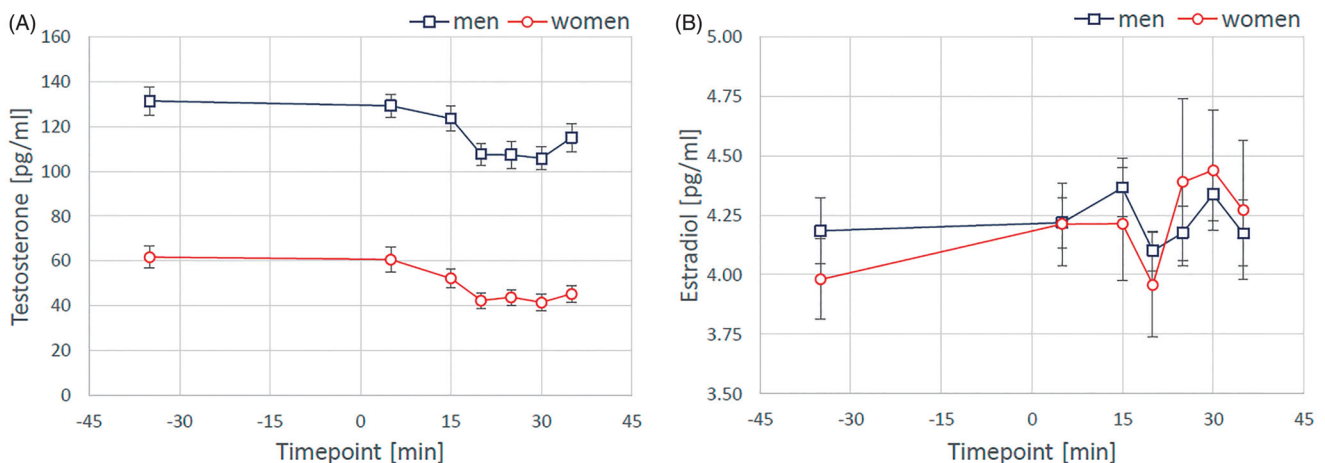


Figure 1. Testosterone and estradiol response to social-evaluative stress. (A) Testosterone decreased significantly in response to stress in both men and women with a minimum 20 minutes after stress cessation (Timepoint 0). (B) Estradiol increased significantly in response to stress in both men and women with two maxima, 15 and 30 minutes after stress cessation (Timepoint 0).

pre-ovulatory cycle phase and there is a high variability in luteal phase estradiol levels (Pletzer et al., 2019; Shultz et al., 2005). Across the whole sample, stress increased estradiol levels ($b = 0.11$, $SE_b = 0.04$, $t_{(394)} = 2.40$, $p = .02$), irrespective of participants' sex ($b = -0.10$, $SE_b = 0.06$, $t_{(394)} = -1.61$, $p = .11$). Also AUCi for estradiol did not differ significantly between men and women ($t_{(60.28)} = 1.02$, $p = .31$). Descriptively, in both men and women, we observed two separate estradiol peaks 15 and 30 minutes after social-evaluative stress, with a local minimum 20 minutes after social-evaluative stress (Figure 1(B)).

Testosterone response, calculated as AUCi, was predicted by baseline testosterone in men ($r = -0.67$, $p_{FDR} < 0.001$; Figure 2(A)), with a similar trend in women ($r = -0.39$, $p < .05$). The estradiol response, calculated as AUCi, was predicted by baseline estradiol only in men ($r = -0.78$, $p_{FDR} < .001$; Figure 2(B)), but not in women ($r = -0.24$, $p = .14$), compare Table 1.

3.3. Interrelation between sex and stress hormones

Testosterone baseline levels were significantly related to cortisol baseline levels in women ($r = .55$, $p_{FDR} < .05$; Figure

3(A)), but not in men ($r = -0.04$, $p = .83$). Estradiol baseline levels were not significantly related to cortisol baseline in either men or women (all $|r| < .36$, all $p > .03$; Figure 3(B)), though a trend association was observed in men ($r = .36$, $p = .036$). Neither the testosterone, nor the estradiol response, calculated as AUCi, was related to cortisol baseline levels or cortisol reactivity in men or women (all $|r| < .30$, all $p > .26$).

3.4. Exploratory analyses: characterizing estradiol response profiles

Since two distinct peaks were observed in the estradiol response to stress, we explored whether this bimodal estradiol response was the result of different estradiol response profiles. A subset of 41 participants was identified, who exhibited a decrease in estradiol levels following stress ($b = -0.09$, $SE_b = 0.04$, $t_{(245)} = -2.22$, $p = .03$). In this group, estradiol was significantly reduced 20 minutes after social-evaluative stress ($b = -0.41$, $SE_b = 0.05$, $t_{(40)} = -9.01$, $p < .001$; Figure 4), but increased back to baseline levels 35 min after social-evaluative stress ($b = 0.27$, $SE_b = 0.07$, $t_{(40)} = 3.56$, $p = .001$). In the remaining 25 participants, estradiol showed a significant increase after social-evaluative stress ($b = 0.21$,

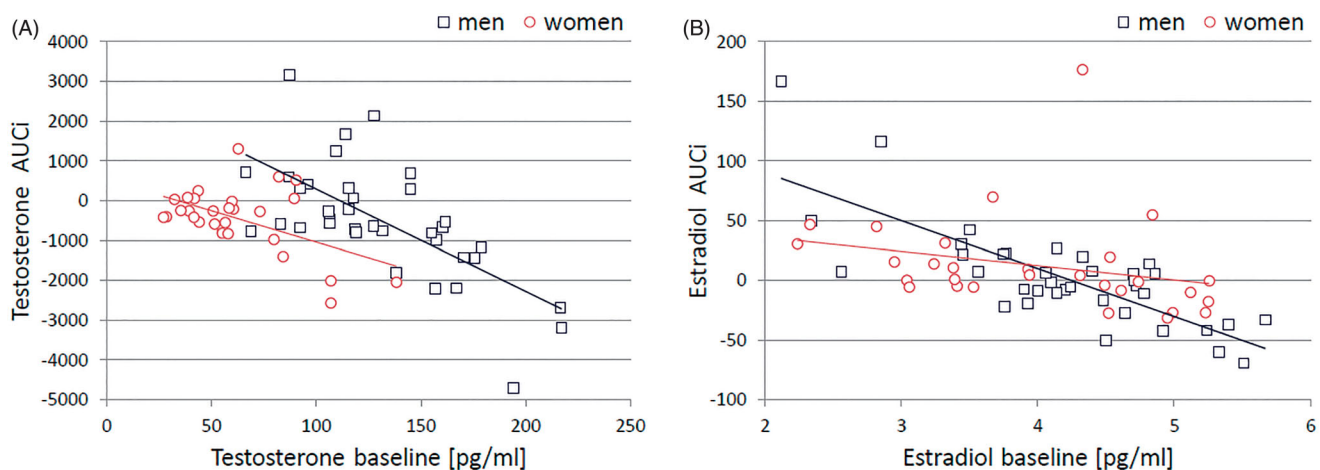


Figure 2. Relationship of sex hormone baseline levels to the gonadal stress response in men and women. (A) Testosterone baseline levels predict the testosterone response to stress in men and women. (B) Estradiol baseline levels predict the estradiol response to stress in men, but not women. Gonadal stress response was calculated as area under the curve related to increase (AUCi).

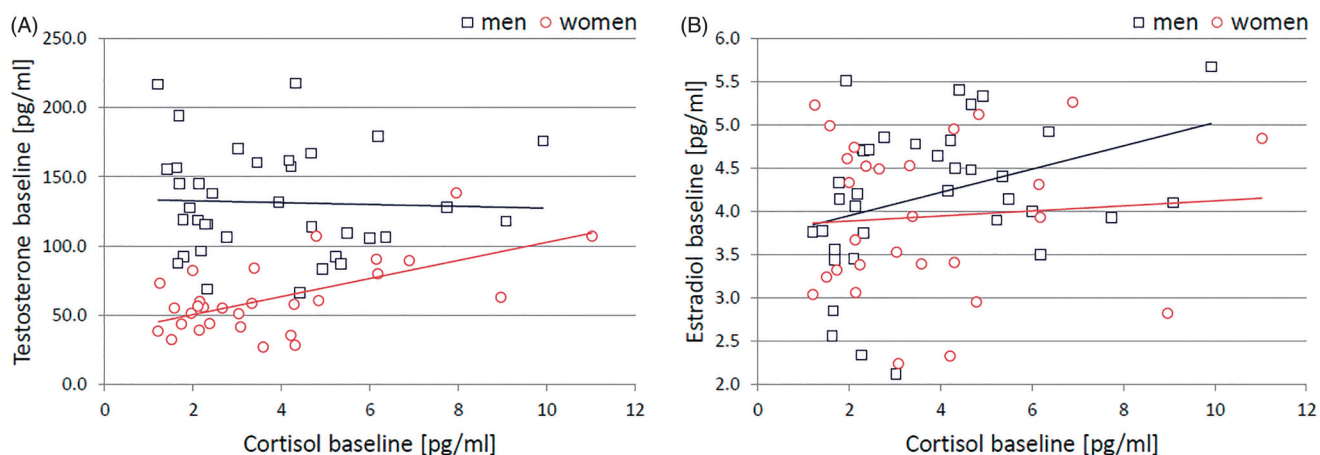


Figure 3. Relationship of cortisol baseline levels to sex hormone baseline levels in men and women. Cortisol relates to testosterone in women, but not men. Estradiol shows a trend relationship to cortisol in men, but not women.

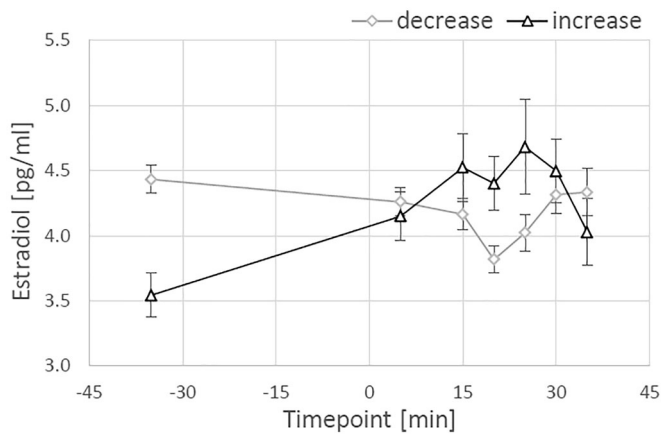


Figure 4. Estradiol response profiles. Exploratory analyses revealed that 62% of participants showed a decreasing estradiol response with a minimum 20 minutes after stress cessation (Timepoint 0). In the remaining 38% of participants, estradiol increased significantly with two maxima 15 and 30 minutes after stress cessation.

$SE_b = 0.04$, $t_{(149)} = 5.09$, $p < .001$; Figure 4), with strong variability in the timing of the peak. Again, two maxima were observed, 15 and 25 minutes after social-evaluative stress, both significantly elevated compared to baseline levels (both $b > 0.37$, both $SE_b < 0.11$, both $t_{(24)} > 3.47$, both $p < .002$). After 20 minutes, a significant decrease was observed ($b = -0.21$, $SE_b = 0.05$, $t_{(24)} = -3.89$, $p < .001$). The estradiol profile was not affected by participant's sex ($X^2 = 0.06$, $p = .80$).

To further characterize these different response profiles, exploratory t-tests were performed to compare endocrinological variables between the two profiles. Apart from higher baseline estradiol levels in participants with a decreasing estradiol profile ($t_{(64)} = 4.60$, $p_{FDR} < 0.001$), no differences in baseline hormone levels, cortisol, or testosterone reactivity were observed between the different estradiol reactivity profiles (all $|t| < 2.06$, all $p_{FDR} > 0.11$). Furthermore, correlation patterns were not different between participants with increasing vs. decreasing estradiol profiles.

4. Discussion

The present study aimed to evaluate the gonadal stress response in men and women, its predictors, and its relationship to the adrenocortical stress response. In the light of a scant literature on the topic, we chose an exploratory approach to this question, re-analyzing samples from a previous study, which focused on the cortisol response to stress. In the following sections, the results will be discussed separately for testosterone and estradiol reactivity.

4.1. Testosterone reactivity to social-evaluative stress

In addition to the well-established cortisol increase after stress, our results showed that testosterone decreased in response to social-evaluative stress in the majority of participants, and irrespective of participants' sex. In both men and women, a minimum testosterone level was reached 20 minutes after social evaluative stress ended, i.e. at the same time that cortisol levels peak (Poppelaars et al., 2019).

The absolute decrease encompassed about 20 pg/mL in both men and women. It should be noted however, that this decrease corresponded to 33% of baseline testosterone in women, but only 15% of baseline testosterone in men, indicating that in relative terms, women exhibited larger testosterone drops than men. The observation of a significant testosterone decrease after stress is in line with studies reporting testosterone decreases after physical and some social-evaluative stressors (Fernández-García et al., 2002; Gomez-Merino et al., 2002; Juster et al., 2016; Maner et al., 2008). Based on previous observations that testosterone elevations may occur after winning and/or in competitive situations (Chichinadze & Chichinadze, 2008; Crewther et al., 2018), it can be speculated that the social-evaluative stress was either experienced as a defeat or as not very competitive. Since competitive behavior and personality traits have been related to the interactive effects of testosterone and cortisol at baseline (dual hormone hypothesis, see Mehta & Prasad, 2015 for a review), future work should explore whether testosterone and cortisol reactivity also interactively modulate participants' behavior during a stress situation.

The strongest predictor of testosterone reactivity was baseline testosterone, irrespective of participants' sex. The higher the baseline testosterone levels, the stronger was the decrease in response to stress. A similar observation in the opposite direction has previously been noted for the HPA axis, where participants with higher basal cortisol levels show a blunted cortisol response to stress, while cortisol reactivity is higher in participants with lower cortisol baseline levels (Buchanan & Tranel, 2008; Kunz-Ebrecht et al., 2003; Pletzer et al., 2010; Takahashi et al., 2004). A physiological explanation may lie in negative feedback within the HPG axis, such that in participants with higher testosterone levels at baseline, a stronger discrepancy is noted by the hypothalamus during the stress state. Since testosterone has previously been related to social dominance (Chichinadze & Chichinadze, 2008), competitiveness (Crewther et al., 2018), and aggression (Romero-Altínez & Moya-Albiol, 2016), one psychological explanation for this response pattern may be that participants with higher levels of the aforementioned personality traits experience the social evaluative situation as more defeating than otherwise.

4.2. Estradiol reactivity to social-evaluative stress

Turning to estradiol reactivity, a small increase with two separate peaks could be identified in the whole sample. This observation is in line with early reports of ACTH-dependent increases in adrenal estradiol production (Wasada et al., 1978). Exploratory subset analyses revealed two separate response profiles, irrespective of participant's sex. While some participants responded to stress with a decrease in estradiol—similar to testosterone—other participants responded to stress with an increase in estradiol. While the decrease was reached after 20 minutes, around the same time as cortisol levels peak (Poppelaars et al., 2019), the increase showed two separate peaks after 15 and 25 minutes. Unfortunately, no determinants of the estradiol response profile could be

identified among the variables explored in the present study apart from baseline estradiol levels, which were higher in the case of a decreasing profile. Furthermore, no predictors of the estradiol response to stress could be identified, apart from baseline estradiol levels in men. It is interesting to note that baseline estradiol levels were a much stronger predictor of estradiol reactivity in men compared to women. The higher the baseline estradiol levels, the more likely was a decreasing profile and the stronger was the decrease in men. This is similar to cortisol response profiles, as previously shown (e.g. Pletzer et al., 2010), potentially indicating that the profiles arise from negative feedback within the HPG axis. The fact that estradiol responds to stress in a bidirectional manner may explain why previous studies were unable to detect a significant estradiol reactivity to stress (Herrera et al., 2016; Schoofs & Wolf, 2011).

As a limitation it may be noted that the assessment of estradiol via ELISAs has its limits, especially in the lower concentration range (Schultheiss et al., 2018). Thus, the lack of predictors for the estradiol response identified in the present study may also be due to a lack of sensitivity of the utilized assessment method. A more concise picture might emerge when utilizing liquid-chromatography-mass spectrometry (LC-MS) for estradiol assessment. It is possible that the limited sensitivity of the ELISA-measurements may also have contributed to the lack of sex differences in basal estradiol levels.

4.3. Interaction between sex and stress hormones

Regarding the three modes of interaction between the HPA and HPG axis postulated in the introduction, it is noteworthy that the cortisol and testosterone responses appeared to be widely independent.

First, the cortisol response was not dependent on baseline testosterone or estradiol levels, which is contrary to previous observations of a stronger cortisol response depending on hormonal status (compare Kajantie & Phillips, 2006 for a review). This observation is in line, however, with the fact that all women in the current study were tested during the same cycle phase and thus had the same hormonal status with respect to their individual hormone profile.

Second, the gonadal response was not predicted by baseline cortisol levels or cortisol reactivity in either men or women. It can thus be speculated that both responses are independently driven by a common source. Indeed, the earlier SNS response to stress may induce a gonadal stress response, as sympathetic nerves have been shown to innervate the gonads and influence their endocrine function via various mechanisms (see Toufexis et al., 2014 for a review). Accordingly, in acute stress situations, a gonadal response to stress may occur simultaneously but independently of the HPA response to stress. Utilizing data from Poppelaars et al. (2019), this question was explored by correlating the gonadal stress responses to the stress response of the pre-ejection period (PEP), a measure of sympathetic nervous system activity. However, no significant association was observed, suggesting that this hypothesis might not hold merit; although it deserves further research.

While gonadal and cortisol responses to stress appear to be independent, baseline cortisol levels predicted baseline testosterone levels in females. It is possible that cortisol affects testosterone reactivity only in the lower range, making changes in men hard to detect due to their higher baseline testosterone levels. Another explanation is that cortisol might influence testosterone production in the adrenal glands to a stronger extent than testosterone production in the gonads. This interpretation could also account for the fact that the absolute testosterone reduction was comparable between men and women, even though men have higher testosterone levels. In women, the testosterone produced by the adrenal glands represents a much higher proportion of the overall testosterone concentration (Burger, 2002) than in men, who produce the majority of their testosterone in the testes. A similar explanation may account for the fact that baseline cortisol relates to baseline estradiol only in men, but not in women.

It is also possible that DHEA, a precursor of testosterone, plays a role in that respect. As DHEA has been discussed as a cortisol antagonist (Alhaj et al., 2006; Hechter et al., 1997), it might be involved in returning cortisol levels to baseline after stress reactivity. In line with that assumption, DHEA elevations have been observed after psychosocial stress, albeit later than the cortisol peak (Izawa et al., 2008; Lennartsson et al., 2012). A higher demand for DHEA following cortisol elevations may contribute to the reduction of testosterone production in the adrenal gland.

5. Conclusion

In summary, our data show that apart from cortisol and irrespective of participant's sex, testosterone and estradiol also respond to social-evaluative stress. However, these responses appear to be largely independent from the cortisol response to stress. Keeping in mind the status of this study as an exploratory re-analysis, these results should be interpreted with caution. Nevertheless, they provide further evidence that the endocrinological stress response is not restricted to the HPA axis and stress reactivity of gonadal hormones is not simply driven by cortisol. Accordingly, the stress reactivity of gonadal hormones and their association to psychological variables is an additional avenue to explore in an attempt to understand healthy and pathophysiological mechanisms underlying the stress response. Importantly, our results show that stress reactivity of gonadal hormones was observed in both men and women.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was funded by the Austrian Science Fund (FWF), projects W1233, P28261 and P32276.

Notes on contributors

Belinda Pletzer is a cognitive neuroscientist interested in the interactions between endocrine system and nervous system. She has a particular interest in women's health, including the effects of sex and gender on brain and behavior.

Eefje S. Poppelaars is a data scientist working in the biotech industry in the Netherlands. She completed her PhD at Salzburg University (Austria) on the topic of stress regulation during social-evaluative threat. Her research interests include (electro/psycho/cardiovascular/endocrine) physiology and social anxiety.

Johannes Klackl is a post-doctoral researcher in the field of social neuroscience. His current main interest is how people respond to threat, and how motivational, emotional and cognitive processes shape different ways of responding.

Eva Jonas is a Professor of Social Psychology. Her work focuses on motivated social cognition, i.e. on information processing which is influenced by motivational aspects and shapes human behavior in social interactions. She is explaining reactions to threats, as well as processes of social influence and the initiation of change. She also aims to investigate neural and physiological processes involved in experiencing and managing threat.

ORCID

Belinda Pletzer  <http://orcid.org/0000-0002-7277-0923>

Eefje S. Poppelaars  <http://orcid.org/0000-0002-7030-0282>

Johannes Klackl  <http://orcid.org/0000-0003-2619-4715>

Eva Jonas  <http://orcid.org/0000-0003-1016-3076>

Data availability statement

The data-set and script used in the analyses are freely available online (<https://osf.io/jwpa4/>).

References

- Aakvaag, A., Bentsdal, Ø., Quigstad, K., Walstad, P., Rønningen, H., & Fonnum, F. (1978). Testosterone and testosterone binding globulin (TeBG) in young men during prolonged stress. *International Journal of Andrology*, 1(1–6), 22–31. <https://doi.org/10.1111/j.1365-2605.1978.tb00573.x>
- Alhaj, H. A., Massey, A. E., & McAllister-Williams, R. H. (2006). Effects of DHEA administration on episodic memory, cortisol and mood in healthy young men: A double-blind, placebo-controlled study. *Psychopharmacology*, 188(4), 541–551. <https://doi.org/10.1007/s00213-005-0136-y>
- Altemus, M., Roca, C., Galliven, E., Romanos, C., & Deuster, P. (2001). Increased vasopressin and adrenocorticotropin responses to stress in the midluteal phase of the menstrual cycle. *The Journal of Clinical Endocrinology & Metabolism*, 86(6), 2525–2530. <https://doi.org/10.1210/jcem.86.6.7596>
- Bali, A., & Jaggi, A. S. (2015). Clinical experimental stress studies: Methods and assessment. *Reviews in the Neurosciences*, 26(5), 555–579. <https://doi.org/10.1515/revneuro-2015-0004>
- Bedgood, D., Boggiano, M. M., & Turan, B. (2014). Testosterone and social evaluative stress: The moderating role of basal cortisol. *Psychoneuroendocrinology*, 47, 107–115. <https://doi.org/10.1016/j.psyneuen.2014.05.007>
- Bernhardt, B. C., Dabbs, J. M., Jr., Fielden, J. L., & Lutter, C. D. (1998). Testosterone changes during vicarious experiences of winning and losing among fans at sporting events. *Physiology & Behavior*, 65(1), 59–62. [https://doi.org/10.1016/S0031-9384\(98\)00147-4](https://doi.org/10.1016/S0031-9384(98)00147-4)
- Buchanan, T. W., & Tranel, D. (2008). Stress and emotional memory retrieval: Effects of sex and cortisol response. *Neurobiology of Learning and Memory*, 89(2), 134–141. <https://doi.org/10.1016/j.nlm.2007.07.003>
- Burger, H. G. (2002). Androgen production in women. *Fertility and Sterility*, 77(4), 3–5. [https://doi.org/10.1016/S0015-0282\(02\)02985-0](https://doi.org/10.1016/S0015-0282(02)02985-0)
- Chichinadze, K., & Chichinadze, N. (2008). Stress-induced increase of testosterone: Contributions of social status and sympathetic reactivity. *Physiology & Behavior*, 94(4), 595–603. <https://doi.org/10.1016/j.physbeh.2008.03.020>
- Crewther, B. T., Obmiński, Z., & Cook, C. J. (2018). Serum cortisol as a moderator of the relationship between serum testosterone and Olympic weightlifting performance in real and simulated competitions. *Biology of Sport*, 35(3), 215–221. <https://doi.org/10.5114/biol-sport.2018.74632>
- Duchesne, A., Tessera, E., Dedovic, K., Engert, V., & Pruessner, J. C. (2012). Effects of panel sex composition on the physiological stress responses to psychosocial stress in healthy young men and women. *Biological Psychology*, 89(1), 99–106. <https://doi.org/10.1016/j.biopsycho.2011.09.009>
- Durdiaková, J., Fábryová, H., Koborová, I., Ostatníková, D., & Celec, P. (2013). The effects of saliva collection, handling and storage on salivary testosterone measurement. *Steroids*, 78(14), 1325–1331. <https://doi.org/10.1016/j.steroids.2013.09.002>
- Espin, L., Almela, M., Hidalgo, V., Villada, C., Salvador, A., & Gomez-Amor, J. (2013). Acute pre-learning stress and declarative memory: Impact of sex, cortisol response and menstrual cycle phase. *Hormones and Behavior*, 63(5), 759–765. <https://doi.org/10.1016/j.yhbeh.2013.03.013>
- Fehring, R. J., Schneider, M., & Raviele, K. (2006). Variability in the phases of the menstrual cycle. *Journal of Obstetric, Gynecologic & Neonatal Nursing*, 35(3), 376–384. <https://doi.org/10.1111/j.1552-6909.2006.00051.x>
- Fekedulegn, D. B., Andrew, M. E., Burchfiel, C. M., Violanti, J. M., Hartley, T. A., Charles, L. E., & Miller, D. B. (2007). Area under the curve and other summary indicators of repeated waking cortisol measurements. *Psychosomatic Medicine*, 69(7), 651–659. <https://doi.org/10.1097/PSY.0b013e31814c405c>
- Fernández-García, B., Lucía, A., Hoyos, J., Chicharro, J. L., Rodríguez-Alonso, M., Bandrés, F., & Terrados, N. (2002). The response of sexual and stress hormones of male pro-cyclists during continuous intense competition. *International Journal of Sports Medicine*, 23(8), 555–560. <https://doi.org/10.1055/s-2002-35532>
- Fuss, J., Claro, L., Ising, M., Biedermann, S. V., Wiedemann, K., Stalla, G. K., Briken, P., & Auer, M. K. (2019). Does sex hormone treatment reverse the sex-dependent stress regulation? A longitudinal study on hypothalamus-pituitary-adrenal (HPA) axis activity in transgender individuals. *Psychoneuroendocrinology*, 104, 228–237. <https://doi.org/10.1016/j.psyneuen.2019.02.023>
- Gandara, B. K., Leresche, L., & Mancl, L. (2007). Patterns of salivary estradiol and progesterone across the menstrual cycle. *Annals of the New York Academy of Sciences*, 1098, 446–450. <https://doi.org/10.1196/annals.1384.022>
- Gladue, B. A., Boechler, M., & McCaul, K. D. (1989). Hormonal response to competition in human males. *Aggressive Behavior*, 15(6), 409–422. [https://doi.org/10.1002/1098-2337\(1989\)15:6<409::AID-AB2480150602>3.0.CO;2-P](https://doi.org/10.1002/1098-2337(1989)15:6<409::AID-AB2480150602>3.0.CO;2-P)
- Gomez-Merino, D., Chennaoui, M., Drogou, C., Bonneau, D., & Guezennec, C. Y. (2002). Decrease in serum leptin after prolonged physical activity in men. *Medicine & Science in Sports & Exercise*, 34(10), 1594–1599. <https://doi.org/10.1097/00005768-200210000-00010>
- Gonzalez-Bono, E., Salvador, A., Serrano, M., & Ricarte, J. (1999). Testosterone, cortisol, and mood in a sports team competition. *Hormones and Behavior*, 35(1), 55–62. <https://doi.org/10.1006/hbeh.1998.1496>
- Hechter, O., Grossman, A., & Chatterton, R. T. Jr. (1997). Relationship of dehydroepiandrosterone and cortisol in disease. *Medical Hypotheses*, 49(1), 85–91. [https://doi.org/10.1016/S0306-9877\(97\)90258-9](https://doi.org/10.1016/S0306-9877(97)90258-9)
- Herrera, A. Y., Nielsen, S. E., & Mather, M. (2016). Stress-induced increases in progesterone and cortisol in naturally cycling women. *Neurobiology of Stress*, 3, 96–104. <https://doi.org/10.1016/j.ynstr.2016.02.006>
- Izawa, S., Sugaya, N., Shirotaki, K., Yamada, K. C., Ogawa, N., Ouchi, Y., Nagano, Y., Suzuki, K., & Nomura, S. (2008). Salivary dehydroepiandrosterone secretion in response to acute psychosocial stress and its correlations with biological and psychological changes. *Biological*

- Psychology*, 79(3), 294–298. <https://doi.org/10.1016/j.biopsycho.2008.07.003>
- Juster, R., Almeida, D., Cardoso, C., Raymond, C., Johnson, P., Pfaus, J., Mendrek, A., Duchesne, A., Pruessner, J., & Lupien, S. (2016). Gonads and strife: Sex hormones vary according to sexual orientation for women and stress indices for both sexes. *Psychoneuroendocrinology*, 72, 119–130. <https://doi.org/10.1016/j.psyneuen.2016.06.011>
- Kajantie, E., & Phillips, D. I. (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology*, 31(2), 151–178. <https://doi.org/10.1016/j.psyneuen.2005.07.002>
- Keevil, B., MacDonald, P., Macdowall, W., Lee, D., & Wu, F. (2014). Salivary testosterone measurement by liquid chromatography tandem mass spectrometry in adult males and females. *Annals of Clinical Biochemistry: An International Journal of Biochemistry and Laboratory Medicine*, 51(3), 368–378. <https://doi.org/10.1177/0004563213506412>
- Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine*, 61(2), 154–162. <https://doi.org/10.1097/00006842-199903000-00006>
- Kirschbaum, C., Schommer, N., Federenko, I., Gaab, J., Neumann, O., Oellers, M., Rohleder, N., Untiedt, A., Hanker, J., Pirke, K. M., & Hellhammer, D. H. (1996). Short-term estradiol treatment enhances pituitary-adrenal axis and sympathetic responses to psychosocial stress in healthy young men. *The Journal of Clinical Endocrinology & Metabolism*, 81(10), 3639–3643. <https://doi.org/10.1210/jcem.81.10.8855815>
- Knight, E. L., Christian, C. B., Morales, P. J., Harbaugh, W. T., Mayr, U., & Mehta, P. H. (2017). Exogenous testosterone enhances cortisol and affective responses to social-evaluative stress in dominant men. *Psychoneuroendocrinology*, 85, 151–157. <https://doi.org/10.1016/j.psyneuen.2017.08.014>
- Komesaroff, P. A., Esler, M. D., & Sudhir, K. (1999). Estrogen supplementation attenuates glucocorticoid and catecholamine responses to mental stress in perimenopausal women. *The Journal of Clinical Endocrinology & Metabolism*, 84(2), 606–610. <https://doi.org/10.1210/jcem.84.2.5447>
- Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology*, 29(1), 83–98. [https://doi.org/10.1016/S0306-4530\(02\)00146-4](https://doi.org/10.1016/S0306-4530(02)00146-4)
- Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. *Biological Psychology*, 69(1), 113–132. <https://doi.org/10.1016/j.biopsycho.2004.11.009>
- Kudielka, B. M., Schmidt-Reinwald, A. K., Hellhammer, D. H., & Kirschbaum, C. (1999). Psychological and endocrine responses to psychosocial stress and dexamethasone/corticotropin-releasing hormone in healthy postmenopausal women and young controls: The impact of age and a two-week estradiol treatment. *Neuroendocrinology*, 70(6), 422–430. <https://doi.org/10.1159/000054504>
- Kunz-Ebrecht, S. R., Mohamed-Ali, V., Feldman, P. J., Kirschbaum, C., & Steptoe, A. (2003). Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. *Brain Behavior and Immunity*, 17(5), 373–383. [Database] [https://doi.org/10.1016/S0889-1591\(03\)00029-1](https://doi.org/10.1016/S0889-1591(03)00029-1)
- Labuschagne, I., Grace, C., Rendell, P., Terrett, G., & Heinrichs, M. (2019). An introductory guide to conducting the Trier Social Stress Test. *Neuroscience and Biobehavioral Reviews*, 107, 686–695. <https://doi.org/10.1016/j.neubiorev.2019.09.032>
- Lennartsson, A. K., Kushnir, M. M., Bergquist, J., Billig, H., & Jonsdottir, I. H. (2012). Sex steroid levels temporarily increase in response to acute psychosocial stress in healthy men and women. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, 84(3), 246–253. <https://doi.org/10.1016/j.ijpsycho.2012.03.001>
- Liu, J. J. W., Ein, N., Peck, K., Huang, V., Pruessner, J. C., & Vickers, K. (2017). Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-analysis. *Psychoneuroendocrinology*, 82, 26–37. <https://doi.org/10.1016/j.psyneuen.2017.04.007>
- Maki, P. M., Mordecai, K. L., Rubin, L. H., Sundermann, E., Savarese, A., Eatough, E., & Drogos, L. (2015). Menstrual cycle effects on cortisol responsivity and emotional retrieval following a psychosocial stressor. *Hormones and Behavior*, 74, 201–208. <https://doi.org/10.1016/j.yhbeh.2015.06.023>
- Maner, J. K., Miller, S. L., Schmidt, N. B., & Eckel, L. A. (2008). Submitting to defeat: Social anxiety, dominance threat, and decrements in testosterone. *Psychological Science*, 19(8), 764–768. <https://doi.org/10.1111/j.1467-9280.2008.02154.x>
- Mehta, P. H., & Prasad, S. (2015). The dual-hormone hypothesis: A brief review and future research agenda. *Current Opinion in Behavioral Sciences*, 3, 163–168. <https://doi.org/10.1016/j.cobeha.2015.04.008>
- Montero-López, E., Santos-Ruiz, A., García-Ríos, M. C., Rodríguez-Blázquez, M., Rogers, H. L., & Peralta-Ramírez, M. I. (2018). The relationship between the menstrual cycle and cortisol secretion: Daily and stress-invoked cortisol patterns. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, 131, 67–72. <https://doi.org/10.1016/j.ijpsycho.2018.03.021>
- Nicolson, N. A. (2008). Measurement of cortisol. In L. J. Luecken & L. C. Gallo (Eds.), *Handbook of physiological research methods in health psychology* (p. 37–74). Sage Publications, Inc. <https://doi.org/10.4135/9781412976244.n3>
- Oltmanns, K. M., Peters, A., Kern, W., Fehm, H. L., Born, J., & Schultes, B. (2005). Preserved inhibitory effect of recurrent hypoglycaemia on the male gonadotrophic axis. *Clinical Endocrinology*, 62(2), 217–222. <https://doi.org/10.1111/j.1365-2265.2005.02203.x>
- Pletzer, B., Jäger, S., & Hawelka, S. (2019). Sex hormones and number processing. Progesterone and testosterone relate to hemispheric asymmetries during number comparison. *Hormones and Behavior*, 115, 104553. <https://doi.org/10.1016/j.yhbeh.2019.07.001>
- Pletzer, B., Wood, G., Moeller, K., Nuerk, H. C., & Kerschbaum, H. H. (2010). Predictors of performance in a real-life statistics examination depend on the individual cortisol profile. *Biological Psychology*, 85(3), 410–416. <https://doi.org/10.1016/j.biopsycho.2010.08.015>
- Poppelaars, E. S., Klackl, J., Pletzer, B., Wilhelm, F. H., & Jonas, E. (2019). Social-evaluative threat: Stress response stages and influences of biological sex and neuroticism. *Psychoneuroendocrinology*, 109, 104378. <https://doi.org/10.1016/j.psyneuen.2019.104378>
- Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, 28(7), 916–931. [https://doi.org/10.1016/S0306-4530\(02\)00108-7](https://doi.org/10.1016/S0306-4530(02)00108-7)
- R Core Team. (2018). *R: A language and environment for statistical computing*. R Core Team.
- Rohleder, N., Schommer, N. C., Hellhammer, D. H., Engel, R., & Kirschbaum, C. (2001). Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosomatic Medicine*, 63(6), 966–972. <https://doi.org/10.1097/00006842-200111000-00016>
- Romero-Martínez, Á., & Moya-Albiol, L. (2016). The use of testosterone/cortisol ratio in response to acute stress as an indicator of propensity to anger in informal caregivers. *The Spanish Journal of Psychology*, 19, E48. <https://doi.org/10.1017/sjp.2016.62>
- Roney, J. R., & Simmons, Z. L. (2014). Elevated psychological stress predicts reduced estradiol concentrations in young women. *Adaptive Human Behavior and Physiology*, 1, 30–40. <https://doi.org/10.1007/s40750-014-0004-2>
- Rubinow, D. R., Roca, C. A., Schmidt, P. J., Danaceau, M. A., Putnam, K., Cizza, G., Chrousos, G., & Nieman, L. (2005). Testosterone suppression of CRH-stimulated cortisol in men. *Neuropsychopharmacology*, 53, 83–107. <https://doi.org/10.1038/sj.npp.1300742>
- Schoofs, D., & Wolf, O. T. (2011). Are salivary gonadal steroid concentrations influenced by acute psychosocial stress? A study using the Trier Social Stress Test (TSST). *International Journal of Psychophysiology*, 80(1), 36–43. <https://doi.org/10.1016/j.ijpsycho.2011.01.008>
- Schultheiss, O. C., Dlugash, G., & Mehta, P. H. (2018). Hormone measurement in social neuroendocrinology: A comparison of immunoassay and mass spectroscopy methods. In O. C. Schultheiss & P. H. Mehta

- (Eds.), *Routledge international handbook of social neuroendocrinology*. Abingdon, UK: Routledge.
- Schultheiss, O. C., Wirth, M. M., Torges, C. M., Pang, J. S., Villacorta, M. A., & Welsh, K. M. (2005). Effects of implicit power motivation on men's and women's implicit learning and testosterone changes after social victory or defeat. *Journal of Personality and Social Psychology, 88*(1), 174–188. <https://doi.org/10.1037/0022-3514.88.1.174>
- Shultz, S. J., Sander, T. C., Kirk, S. E., & Perrin, D. H. (2005). Sex differences in knee joint laxity change across the female menstrual cycle. *The Journal of Sports Medicine and Physical Fitness, 45*(4), 594–603.
- Stephens, M. A., Mahon, P. B., McCaul, M. E., & Wand, G. S. (2016). Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones. *Psychoneuroendocrinology, 66*, 47–55. <https://doi.org/10.1016/j.psyneuen.2015.12.021>
- Takahashi, T., Ikeda, K., Ishikawa, M., Tsukasaki, T., Nakama, D., Tanida, S., & Kameda, T. (2004). Social stress-induced cortisol elevation acutely impairs social memory in humans. *Neuroscience Letters, 363*(2), 125–130. <https://doi.org/10.1016/j.neulet.2004.03.062>
- Tersman, Z. I. T. A., Collins, A., & Eneroth, P. (1991). Cardiovascular responses to psychological and physiological stressors during the menstrual cycle. *Psychosomatic Medicine, 53*(2), 185–197. <https://doi.org/10.1097/00006842-199103000-00008>
- Toufexis, D., Rivarola, M. A., Lara, H., & Viau, V. (2014). Stress and the reproductive axis. *Journal of Neuroendocrinology, 26*(9), 573–586. <https://doi.org/10.1111/jne.12179>
- Uhart, M., Chong, R. Y., Oswald, L., Lin, P. I., & Wand, G. S. (2006). Gender differences in hypothalamic-pituitary-adrenal (HPA) axis reactivity. *Psychoneuroendocrinology, 31*(5), 642–652. <https://doi.org/10.1016/j.psyneuen.2006.02.003>
- Wasada, T., Akamine, Y., Kato, K. I., Ibayashi, H., & Nomura, Y. (1978). Adrenal contribution to circulating estrogens in woman. *Endocrinologia Japonica, 25*(2), 123–128. <https://doi.org/10.1507/endocrj1954.25.123>
- Westenberg, P. M., Bokhorst, C. L., Miers, A. C., Sumter, S. R., Kallen, V. L., van Pelt, J., & Blöte, A. W. (2009). A prepared speech in front of a pre-recorded audience: Subjective, physiological, and neuroendocrine responses to the Leiden Public Speaking Task. *Biological Psychology, 82*(2), 116–124. <https://doi.org/10.1016/j.biopsycho.2009.06.005>
- Wolf, O. T., Schommer, N. C., Hellhammer, D. H., McEwen, B. S., & Kirschbaum, C. (2001). The relationship between stress induced cortisol levels and memory differs between men and women. *Psychoneuroendocrinology, 26*(7), 711–720. [https://doi.org/10.1016/S0306-4530\(01\)00025-7](https://doi.org/10.1016/S0306-4530(01)00025-7)
- Zitzmann, M., & Nieschlag, E. (2001). Testosterone levels in healthy men and the relation to behavioural and physical characteristics: Facts and constructs. *European Journal of Endocrinology, 144*(3), 183–197. <https://doi.org/10.1530/eje.0.1440183>