**A role for gonadal hormones in HPA-axis and SNS reactivity to psychosocial stress**

**Abstract**

Exposure to stress activates both the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). A growing body of research points to the contribution of sex hormones (testosterone, estrogen, and progesterone), the end products of the hypothalamus-pituitary-gonadal (HPG) axis, in modulating stress reactivity. The present study aimed at investigating the potential modulating role of sex hormones on HPA and SNS reactivity to psychosocial stress. The reactivity, induced by the Trier Social Stress Test, was analyzed by measuring the levels of cortisol and alpha-amylase (markers for SNS activity) in four saliva samples each of 21 men and 37 women (17 not using oral contraceptives and in their luteal phase, and 20 women using oral contraceptives). In addition, basal sex hormones were sampled prior to the psychosocial stress exposure. Results revealed that controlling for testosterone, estrogen, and progesterone diminished the impact of stress on cortisol reactivity and on alpha-amylase reactivity. Moreover, controlling for sex hormones also diminished the differential pattern of cortisol reactivity in each experimental group among responders. Furthermore, correlation analyses revealed differences between groups in the association between sex hormones and alpha-amylase. The present findings point to a modulatory role for sex hormones in HPA and SNS reactivity and emphasize the need for control of sex hormone fluctuations when examining cortisol and alpha-amylase reactivity to stress.

Keywords: sex hormones, cortisol, alpha-amylase, TSST

**Significance statement**

Stress activation, known for its role in the etiology of mental illness, is hypothesized to be modulated by sex hormones, but evidence to such modulation is scarce. The present study demonstrates that sex hormones influence the activation of the two major stress systems, the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). These results emphasize the need to control for sex hormone fluctuations when examining the stress reactivity of the HPA axis or the SNS in normal as well as in clinical samples.

**Introduction**

Evolution is driven by the ability of organisms to cope with threats (i.e. stressors) and to reproduce. As each of these functions require considerable physiological resources, it is not surprising that the neuroendocrine systems that regulate them are interrelated (Juster et al., 2016), allowing for mutual modulation according to specific environmental and internal conditions. Specifically, reproduction is regulated by the secretion of sex steroids (testosterone, estrogen, and progesterone) via the hypothalamic-pituitary-gonadal (HPG) axis, while stress response is regulated by the sympathetic nervous system (SNS) and by the hypothalamic-pituitary-adrenal (HPA) axis.

A considerable body of research has focused on the effect of the HPG axis on the HPA axis by comparing HPA activity between men and women in various hormone-level groups (e.g., luteal or follicular phases of the menstrual cycle, menopause; Andreano, Arjomandi, & Cahill, 2008; Hidalgo et al., 2012; Juster et al., 2016). However, direct research on the modulation of physiological stress reactivity by sex hormones is scarce, and studies into possible HPG-SNS interactions are almost completely absent from the literature. Therefore, the aim of the present study was to extend our understanding of the interconnectivity between the HPG axis and both the HPA axis and SNS in reaction to stressors.

Exposure to stress results in activation of several physiological pathways including HPA axis and SNS. The HPA axis and the SNS work in coordination in order to generate the physiological changes associated with stress response. However, each is assumed to be activated in response to different situational demands and under differential contextual and personal constrains (Keller, El-Sheikh, Granger, & Buckhalt, 2012). Stress triggers the HPA axis to a hormonal cascade, ending in cortisol secretion (for a meta-analysis exploring the impact of stress on cortisol release, see Michaud, Matheson, Kelly, & Anisman, 2008). Stress also triggers the SNS to release catecholamines such as norepinephrine and epinephrine with salivary alpha amylase (sAA), a digestive enzyme found in the oral cavity, serving as the marker for SNS activity (Nater, & Rohleder, 2009).. Elevated levels of both sAA and cortisol have been indicated following various stressors, such as parachute jumping (Chatterton et al., 1997), physical exercise (Friedmann & Kindermann, 1989), and psychological challenges (Bosch et al., 2003). A psychosocial stress procedure widely used in laboratory settings is the Trier Social Stress Test (TSST; Kirschbaum Pirke, & Hellhammer, 1993), which consists of a free speech task and a mental arithmetic task in front of an audience. The TSST has been shown to elicit acute increases of both sAA and cortisol (Allen et al., 2014; Nater et al., 2005; Rohleder et al., 2004).

Historically, most studies on stress reactivity have been conducted solely on male participants to avoid potential variability resulting from female HPG-axis cyclic fluctuations. In the last two decades, however, an increasing number of stress physiology studies have included female participants in various hormonal states (Juster et al., 2016). Early human studies investigating changes in cortisol reactivity because of stress yielded equivocal results, demonstrating either no difference by sex (Rohleder, Wolf, & Kirschnaum, 2003) or higher cortisol reactivity in men compared with women (Almela et al., 2011; Kirschbaum, Wust, & Hellhammer, 1992; Merz, 2017; Preuß & Wolf, 2009; for reviews, see Kudielka, Hellhammer, & Wüst, 2009 and Liu et al., 2017). However, further investigations revealed that stressor-induced cortisol response in women was dependent on their estrogen levels. Levels of cortisol in men and women in the luteal phase were comparable or higher than those of women in the follicular phase or those who used oral contraceptives (OC), conditions in which estrogen levels are higher (Espin et al., 2013; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Consequently, Kirschbaum et al. (1999) suggested that estrogen levels modulate cortisol levels, and that higher estrogen levels stimulate the production of cortisol-binding globulin, resulting in the removal of free cortisol levels from circulation. Further investigations demonstrating elevated cortisol response to stress in postmenopausal women provided additional support for this suggestion (Otte et al., 2005).

Evidence for the potential role of progesterone in modulating the HPA axis is scarce and focuses mainly on menopausal women. The findings are ambiguous, as various studies have indicated an increase (Burleson et al., 1998), a decrease (Pluchino et al., 2005), or no change (Edwards & Mills, 2008) in cortisol levels following hormone (estrogen combined with progesterone) replacement therapy. Recently, Juster and colleagues (2016) explored the role of sex hormones in modulating the HPA by comparing cortisol levels (following exposure to the TSST procedure) between men, OC women, cycling women, and postmenopausal women. They found that higher levels of basal progesterone were associated with lower levels of cortisol in men. Animal studies provided evidence to the role of progesterone on the HPA-axis in revealing progesterone receptors in several limbic and corticolimbic structures serving the HPA-axis (Lupien, McEwen, Gunnar & Heim, 2009), including the thalamus, hypothalamus, amygdala, hippocampus, and prefrontal cortex (Guerra-Araiza, Coyoy-Salgado, & Camacho-Arroyo, 2002; Guerra-Araiza, Villamar-Cruz, Gonzalez-Arenas, Chavira, & Camacho-Arroyo, 2003; Kato et al., 1994).

Testosterone has been also studied to shed light on crosstalk between the HPA and HPG axes in stress reactivity. Animal studies, such as the restraint stress in rats, demonstrated that basal testosterone affects HPA reactivity to certain stressors (e.g., Viau, 2002). Juster and colleagues (2016) found that testosterone was negatively correlated with cortisol reactivity to the TSST in menopausal women. In addition, due to aromatization of testosterone to estrogen in the brain, testosterone can also exert estrogenic effects (Kudielka & Kirschbaum, 2005).

Akin to research on the modulation of sex on cortisol reactivity to stress, there are only few studies that have addressed the potential impact of sex hormones on SNS activation. Merz (2017) measured stress-induced activation of the SNS using the cold-pressor test by measuring the systolic and diastolic blood pressures. This study demonstrated that men had higher systolic blood pressure compared with women. The study also showed that women using OC had higher systolic and diastolic blood pressures compared to women in the follicular phase and higher diastolic blood pressure compared to women in the luteal phase. Studies investigating the impact of sex hormones on SNS activation, as reflected by sAA levels, found no differences in baseline sAA levels between men and between women at various menstrual phases (Tenovuo, Laine, Soderling, & Irjala, 1981), or between men and women using OC (Laine, Pienihakkinen, Ojanotko-Harri, & Tenovuo, 1991). On the other hand, studies exploring intra- and inter- sex differences in sAA response to stress produced mixed results. For example, a few studies demonstrated no differences in sAA reactivity between men and women exposed to a competition challenge (Kivlighan and Granger, 2006); between men, OC women, and women in the follicular phase in response to the TSST procedure (Hidalgo et al., 2012); or between women in the follicular phase and the luteal phase following a modified version of a public speech task (Hlavacova, Solarikova, Marko, Brezina, & Jezova, 2017). On the other hand, pregnant women (who have higher levels of estrogen and progesterone) showed lower sAA reactivity to the TSST procedure in comparison with non-pregnant women (Nierop et al., 2006). Nierop et al. hypothesized that the reduced SNS reactivity to stress during pregnancy might protect both the mother and her offspring from excessive levels of sAA. Further support for this notion was demonstrated by another study focusing on menopause. Del Rio et al. (1998) showed that estrogen and progesterone administration reduced cardiovascular and catecholamine responses to mental stress (colored word test) in menopausal women. In contrast, in another study, postmenopausal women subjected to hormone-replacement-therapy (estrogen plus progestin replacement treatment) showed increased sAA production following exercise as opposed to untreated postmenopausal women (Patacchioli et al., 2015). This inconsistency may be partially explained by the divergent stress procedures used in these studies (i.e., psychosocial, mental, physical).

The present study aimed at investigating the role of the sex hormones (testosterone, estrogen, and progesterone) in modulating stress reactivity of both the HPA axis and the SNS by examining their markers, cortisol and sAA, respectively. To the best of our knowledge, the potential impact of sex hormones on both systems has not been directly and systematically studied to date. As mentioned, there is only one study on the HPA-HPG axes crosstalk that directly measured hormone levels (Juster et al., 2016), which revealed sex-specific associations between sex hormones and cortisol reactivity, but it did not study the effect of sex hormones on sAA reactivity. Therefore, the present study measured basal levels of sex hormones and examined their potential modulation on both cortisol and sAA levels in response to the TSST procedure. Based on previous findings (Juster et al., 2016; Patacchioli et al., 2015), we hypothesized that stress reactivity (reflected by changes in the levels of the stress markers cortisol and sAA) will be modulated by sex hormones. That is, in unadjusted models (not controlling for sex hormones), interactions between stress-marker levels and group (men, OC women, luteal phase women) will be significant, whereas, in adjusted models (controlling for sex hormones), interactions will not be significant. Furthermore, accumulated data (limited as it is) suggests an inhibitory effect of sex hormones on stress reactivity. Thus, we hypothesized that the modulatory role of sex hormones will be reflected by negative correlations between levels of all three sex hormones and stress reactivity.

**Methods**

**Participants**

The study sample included 58 young (M = 24.81, SD = 2.47) men (N=21) and women (N=37). Of the female participants, 20 were taking oral contraceptives (Oral Contraceptives group; OC). The other 17 were not using oral contraceptives and were at the mid luteal phase (day 21) of their menstrual cycle at the time of study (Luteal Phase group; LP). Participants were recruited from among college students by advertisements. All participants were undergraduate students from various departments: behavioral sciences (psychology, education), social sciences (sociology and anthropology, information systems, economics, accounting, and management), and communications. After signing an informed consent form, the volunteers completed a questionnaire regarding their health, habits, and demographic details to verify that they met the inclusion criteria. The exclusion criteria were as follows: individuals with serious medical, gynecological, or hormonal problems; smokers; and attention deficit hyperactivity disorder (ADHD) and learning disabilities by self-report. All male participants met the inclusion criteria. In addition, women were pre-screened to verify they met inclusion criteria. Women to be included in the OC group were all tested during the on-phase of pill intake and were using pills containing 25 mg of estrogen (Ethinylestradiol) and 75 mg of progestin (Gestodene). These doses are considered moderate and are commonly prescribed, and these doses are considered as having antiandrogenic properties (Montoya, & Bos, 2017). All women were using pills for at least one year. The women included in the LP group had not been using oral contraceptives for at least six month prior to the study, had a regular menstrual cycle, and were not pregnant or lactating. These participants were monitored for at least 3 months prior to the study in order to verify the regularity of their cycle, and were summoned to the research laboratory on the 21st day of their cycle using the day of onset of the last menstruation as a reference point (Rossi and Rossi, 1980).

The Institutional Ethics Review Board approved the complete study protocol. Each volunteer that was accepted to the study received 100 NIS compensation.

**Experimental Procedure**

Experimental sessions took place in the laboratory of the psychology department between 8 AM and 10 AM on a single day. This sampling schedule, in which testosterone levels were highest, was chosen to measure individual differences in testosterone levels. All participants gave their first saliva sample at least one hour following awakening. The study design allowed all participants to undergo all the procedures in a single experimental session that was composed of the following three consecutive stages (see fig. 1): A) completion of a set of cognitive tasks such as: memory and visuospatial abilities (data not included) (20 minutes); B) the Trier Social Stress Test procedure (20 minutes); and C) completion of a set of cognitive tasks similar to stage A (20 minutes). The participants provided saliva samples at four assessment points: T1 (baseline: 8-8:30 AM), T2 (immediately following the TSST), T3 (T2+ 10 minutes), and T4 (T3+ 10 minutes). For the T1 sample, participants provided 5 ml of saliva, used for evaluating levels of testosterone, estrogen, and progesterone, as well as baseline levels of cortisol and sAA. For the remaining samples, participants provided 2 ml of saliva, used for evaluating levels of reactive cortisol and sAA.



Figure 1. Study design

The experimental session was composed of the following consecutive stages: A) pre-stress baseline; B) TSST procedure; C) post-stress period. Participants provided saliva samples at the four assessment points indicated as T1-T4. Participants provided 5 ml of saliva at T1 (for evaluating levels of testosterone, estrogen, progesterone, cortisol, and sAA. For T2-T4, participants provided 2 ml of saliva (for evaluating levels of reactive cortisol and sAA).

**Saliva sampling procedure and biochemical analysis**

The participants were instructed to refrain from eating, drinking (except water), and smoking for at least one hour prior to the experimental session. Prior to each saliva sampling, participants were instructed to chew on a piece of parafilm for several seconds to increase saliva secretion. They then deposited a sample of saliva in a SaliCap sampling vial (IBL International GMBH, Hamburg, Germany).

Saliva samples were stored at -20°C immediately upon collection and until the laboratory tests were performed. For each biochemical analyte, tests were performed using commercial CE-IVD-approved ELISA kits: 17 beta estradiol saliva ELISA (mean intra-assay CV% = 4.8, mean inter-assay CV% = 3.4, assay sensitivity = 0.4 pg/mL), cortisol saliva ELISA (mean intra-assay CV% = 4.8, mean inter-assay CV% = 8.1, assay sensitivity =0.005 µg/dL), testosterone saliva ELISA (mean intra-assay CV% = 5.7, mean inter-assay CV% = 9.1, assay sensitivity = 2.0 pg/mL), progesterone saliva ELISA (mean intra-assay CV% = 5.2, mean inter-assay CV% = 7.0, assay sensitivity = 3.1 pg/mL), alpha amylase saliva ELISA (mean intra-assay CV% = 4.6, mean inter-assay CV% = 6.2, assay sensitivity = 3.6 U/mL), all from IBL International GMBH, Hamburg, Germany. All tests were performed in an SQII ELISA processor (AESKU Systems, Wendelsheim, Germany). All tests were performed in the Endocrinology Laboratory of Emek Medical Center, an ISO 9001 (2015 version) certified and Join Committee International (JCI) accredited facility. All analytical kits used in the study were previously validated in the laboratory according to good laboratory practice standards. All tests were run in an SQII ELISA processor (AESKU Systems, Wendelsheim, Germany). A calibration curve using standard duplicates was performed for each analyte in every run.

**Trier Social Stress Test**

Psychological stress was induced by employing the TSST procedure (Kirschbaum, Pirke, Hellhammer, 1993). This procedure consists of a stress task that includes 5 minutes of free speech (a simulated job interview for the participant’s ‘dream job’) and 5 minutes of a mental arithmetic task, both conducted in front of a committee composed of a man and a woman sitting at a distance of 1.5 m and a video camera. At the beginning of the procedure, the participants were instructed by the committee regarding the task at hand, were notified that the performance will be recorded for subsequent behavioral analysis, and then taken to a second room in which they had 10 minutes to formulate the speech alone. Next, the participants entered the committee room in which they carried out the free speech task and the arithmetic task (counting backwards in steps of 13 starting with the number 1,022). Both jury members provided the instructions in turns, and the gender of the starting member was counterbalanced. In total, the procedure, including the preparation phase, took approximately 20 minutes.

**Statistical analyses**

Cortisol, sAA, and sex hormones (estrogen, progesterone, and testosterone) were not normally distributed and were thus subject to log 10 transformation that normalized their distribution. Differences between groups in the levels of sex hormones and trait anxiety were analyzed via one-way analysis of variance (ANOVA).

Due to the large variability observed among participants in their cortisol reactivity and in order to focus on the factors particularly associated with stress induced elevations in stress biomarkers, the sample was divided into responders and non-responders according to Schommer, Hellhammer, & Kirschbaum (2003) and Hidalgo et al. (2012). Participants who demonstrated an increase in salivary cortisol from T1 (baseline level) to T3 (10 minutes following completion of the TSST) were considered ‘responders’ as in previous similar studies (Reschke-Hernández, Okerstrom, Bowles Edwards, & Tranel, 2017). The distribution of responders across experimental groups were assessed through the Pearson Chi-square test, and responders were equally distributed in each group [*χ2*(2) = 2.70, *p* = .259].

Levels of cortisol (responders only) and sAA (for the whole sample) were analyzed via repeated measures analysis of variance (time X group) with sex hormones as covariates.

For all the ANOVA tests, whenever Mauchly's test indicated a violation of sphericity assumption, Greenhouse-Geisser corrections were used. Post-hoc comparisons were performed using Bonferroni adjustments for multiple comparisons of *p* values.

Pearson's correlations (1-tailed) were calculated in order to examine the association between baseline sex hormone concentrations and cortisol and sAA reactivity. To this end, cortisol and sAA reactivity were calculated as the change in the scores of each from their baseline values, at T3 and T2, respectively. These different time points were selected as the SNS releases catecholamines immediately at the onset of a stressor, while the HPA releases of glucocorticoids is slower(Cornelisse, van Stegeren, & Joels, 2011; Kloppe, Garcia, Schulman, Ward, & Tartar, 2012). In fact, cortisol reaches peak levels, only 21–45 minutes following the onset of a stressor. For meta-analyses, see Dickerson & Kemeny, 2004 and Goodman, Janson, & Wolf, 2017.

**Results**

Table 1 presents sex hormone levels of the study groups. The one-way ANOVA revealed a significant difference between the groups in levels of testosterone [*F* (2, 55) =17.18, *p* <0.01; *η2p* = .39] and progesterone [*F* (2, 51) =30.89, *p* <0.01; *η2p* = .55], with post hoc tests revealing that men had higher levels of testosterone than women in either the OC or LP groups, and women in the LP group having higher levels of progesterone than men and OC women. Though one-way ANOVA revealed no significant differences between the groups in estrogen levels [*F* (2, 53) =2.07, *p* = .136; *η2p* = .07], a comparison between men and women in both groups verified higher levels of estrogen among women (1.51±0.04) compared to men (1.40±0.04) [*F* (1, 56) =33.67, *p* <0.01; *η2p* = .38]. Participants of the different groups did not differ in their level of trait anxiety [*F* (2, 55) =0.86, *p* = .136; *η2p* = .01].

Table 1. Characteristics of the study sample

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Men (N=21)** | **OC (N=20)** | **LP (17)** | **F** |
| **Testosterone** | **11.65 (0.80)** | **5.83 (0.44)** | **7.20 (0.55)** | **17.18\*\*** |
| **Progesterone** | **4.07 (0.09)** | **3.78 (0.13)** | **10.77 (0.95)** | **30.89\*\*** |
| **Estrogen** | **1.40 (0.04)** | **1.47 (0.06)** | **1.56 (0.07)** | **2.07** |

Sex hormone data following square root transformation. Abbreviations: OC: oral contraceptives; LP: luteal phase. Data presented as mean ± SEM. \*\**p*<.01

*Cortisol reactivity*

A two-way repeated-measures analysis of variance demonstrated no significant main effect for group [*F* (2, 51) =.93, *p* = .402; *η2p* = .03] and no significant time X group interaction [*F* (6, 51) = 1.48, *p* = .223; *η2p* = .05]. A significant main effect was found for time [*F* (3, 51) = 18.49, *p* < .001; *η2p* = .26], with post-hoc analysis revealing that cortisol levels at T1 were higher than other cortisol measurements (T2, T4). However, in re-analyses controlling for sex hormones (estrogen, progesterone and testosterone), the main effect for time [*F* (3, 51) =.58, *p* = .521; *η2p* = .01] disappeared.

Next, separate analyses for cortisol reactivity were conducted with responders and non-responders. Eleven of the male participants (52.4%), seven of the OC participants (35%), and five (29.4%) of the LP participants showed increased cortisol secretion at T3 (10 min following TSST completion) compared to T1 (baseline) and were thus considered ‘responders’. In contrast, cortisol levels among non-responders tended to decline following the TSST procedure (T2: M = .55, SD = .12; T3: M = .52, SD = .13). The difference in basal cortisol levels between responders and non-responders was significant [*t*(56) = 3.83, *p* < .001], with non-responders demonstrating higher cortisol levels at the baseline (*M* = 1.16, *SD* = .25) in comparison to the cortisol levels of the responders (*M* = .91, *SD* = .22).

Fig 2 compares the effects of the TSST procedure on cortisol secretion between the respondents of the three study groups.

Figure 2. Cortisol concentrations

Salivary cortisol concentrations before and following the TSST procedure. The figure presents data collected from responders i.e., the participants demonstrating increased cortisol levels 10 minutes following the TSST procedure (T3). Participants included 11 men, 7 women using oral contraceptives (OC), and 5 women in the luteal phase (LP) of their menstrual cycle. A two-way repeated-measure ANOVA demonstrated no significant main effect for the group, but a significant main effect for time, with post-hoc analysis revealing that cortisol level at T3 was higher than baseline level (T1). A significant time X group interaction was also found. These statistical effects diminished when sex hormones (estrogen, progesterone, and testosterone) were controlled. \**P*<.05. Depicted values are means of the corrected cortisol levels, and error bars represent SEM.

For responders, a two-way repeated-measures analysis of variance demonstrated no significant main effect for the group [*F* (2, 20) =.73, *p* = .493; *η2p* = .07]. A significant main effect was found for time [*F* (3, 20) = 15.79, *p* < .001; *η2p* = .44], with post-hoc analysis revealing that cortisol levels at T3 were higher than baseline levels (T1) or other cortisol measurements (T2, T4). A significant time X group interaction was also found [*F* (6, 20) = 3.24, *p* < .01; *η2p* = .25]. Post-hoc analyses using Bonferroni adjustments for multiple comparisons revealed that cortisol levels in men at T3 and T2 were higher than the baseline levels (T1). In OC women, cortisol levels at T3 were higher than the levels at T2 and T4, and the cortisol levels at T2 were higher than levels at T4. Also, for women in the LP, cortisol levels at T3 were higher than the levels at T4 (for all *p* < .05). However, in re-analyses controlling for sex hormones (estrogen, progesterone and testosterone), the main effect for time [*F* (3, 20) =.54, *p* = .659; *η2p* = .04] and the interaction effect for time X group [*F* (6, 20) = .36, *p* = .745; *η2p* = .03] disappeared.

For non-responders, a two-way repeated-measures analysis of variance demonstrated no significant main effect for the group [*F* (2, 30) =.57, *p* = .573; *η2p* = .04] and no significant time X group interaction [*F* (6, 30) = 2.20, *p* = .082; *η2p* = .13]. A significant main effect was found for time [*F* (3, 30) = 99.18, *p* < .001; *η2p* = .77], with post-hoc analysis revealing that cortisol levels at T1 were higher than other cortisol measurements (T2, T3, T4). However, in re-analyses controlling for sex hormones (estrogen, progesterone, and testosterone), the main effect for time [*F* (3, 20) =.75, *p* = .472; *η2p* = .03] disappeared.

Pearson correlations were conducted to further examine the different patterns of modulation that the sex hormones had on cortisol reactivity in the total sample and in each group. As T3 was the only time point in which cortisol significantly increased compared to baseline, we calculated the measure of cortisol reactivity to be the T3 minus T1 scores. In the total sample, the change in cortisol levels from T1 to T3 was not significantly correlated with testosterone but showed a negative correlation with estrogen and progesterone (*r* = -.33, *p* < .01, *r* = -.39, *p* < .01, respectively). A separate analysis of the correlations between sex hormones and cortisol reactivity among responders and non-responders revealed that estrogen and progesterone were negatively correlated with the change in cortisol levels in non-responders but not in responders (see Table 2).

Table 2

*Correlations between sex hormones (T, E, P) and cortisol reactivity for responders and non-responders*

|  |  |  |
| --- | --- | --- |
|  | *Responders (N=23)**r p* | *Non-responders (N=35)**r p* |
| Testosterone  |  -.10 .330  |  -.18 .164 |
| Estrogen  |  .05 .419  |  -.37 .019 |
| Progesterone  |  .31 .088 |  -.49 .002 |

*sAA reactivity*

The pattern of sAA reactivity for each study group is depicted in figure 3. In a two-way repeated-measures analysis of variance, no significant main effect was found for group [*F* (2, 50) =.38, *p* = .686; *η2p* = .02], and no significant time X group interaction was found [*F* (6, 50) =.74, *p* = .615; *η2p* = .03]. A main effect for time was found [*F* (3, 50) = 56.66, *p* < .001; *η2p* = .54]. Post-hoc analysis revealed that sAA levels at T2 were higher than at other times, with levels at T4 significantly higher than at T3 (see Figure 3). However, in re-analyses controlling for sex hormones (estrogen, progesterone and testosterone), the main effect for time disappeared [*F* (3, 50) = 1.18, *p* = .320; *η2p* = .03] , and the effects of group [*F* (2, 45) =.46, *p* = .638; *η2p* = .02], and time X group interaction [*F* (6, 50) =.95, *p* = .420; *η2p* = .02] remained not significant. Covariation analysis showed a trend towards a time X group X estrogen interaction [*F* (9, 48) = 2.22, *p* = .061; *η2p* = .12]. However, no significant interaction for time X group X testosterone [*F* (9, 49) = .72, *p* = .608; *η2p* = .04] or for time X group X progesterone [*F* (9, 47) = 1.39, *p* = .270; *η2p* = .08] were found.

Pearson correlations were conducted to further examine the different patterns of modulation that the sex hormones had on sAA in each group. sAA reactivity was calculated to be the T2 minus T1 scores. No significant correlations were found (*p* > .05).

Figure 3. Alpha amylase concentrations

Figure 3. Alpha Amylase concentrations

Salivary alpha amylase concentrations before and following the TSST procedure. A two-way repeated-measures ANOVA demonstrated no time X group interaction, but a significant main effect for group, and a significant main effect for time. Post-hoc analysis revealed that alpha amylase levels increased after the TSST. These statistical effects diminished when sex hormones (estrogen, progesterone and testosterone) were controlled for. \*Significant difference from T1; *P*<.05. Depicted values are means of the corrected alpha amylase levels, and error bars represent the SEM.

**Discussion**

The aim of the present study was to explore the role of sex hormones in modulating stress reactivity of the HPA axis and SNS, by examining their biomarkers, cortisol, and sAA, respectively. For the HPA system, controlling for testosterone, estrogen, and progesterone diminished the impact of stress on cortisol reactivity. As in previous studies (e.g., Reschke-Hernández et al., 2017), the analysis of the effects of the stress procedure on cortisol levels was conducted with a distinction between participants who showed stress-induced elevations of cortisol (i.e. responders) and those who did not (i.e., non-responders). Controlling for sex hormones also diminished the differential pattern of cortisol reactivity in each experimental group (men, LP, OC). These findings are in line with a previous study demonstrating that sex differences in cortisol reactivity to stress were only significant in unadjusted models, that is, when sex hormones were not controlled (Juster et al., 2016), and with the assertion that sex hormones underline the differences between men and women in stress-coping mechanisms (Bale, & Epperson, 2015). To date, support for this hypothesis has been provided mostly by animal studies due to the challenges associated with controlling for sex hormone fluctuations in humans (Oyola, & Handa, 2017). Therefore, the present findings provide important verification of the notion that the activation of the main stress-system in humans is modulated by sex hormones.

The specific pattern by which sex hormones influence cortisol reactivity to stress was examined through correlations between hormones in the entire sample and separately for responders and non-responders. First, baseline cortisol levels were significantly higher in non-responders compared to responders. This result agrees with previous studies reporting that the basal cortisol levels were negatively correlated with cortisol reactivity (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004), suggesting that the lack of stress-induced elevations among non-responders was due to a ceiling effect (Tsumura, Sensaki, & Shimada, 2015). The correlation analyses revealed that the association between estrogen and progesterone with cortisol reactivity for the entire sample was negative. Further analyses conducted separately for responders and non-responders revealed that this effect was driven by the non-respondent group. In other words, for non-responders, higher levels of estrogen and progesterone were associated with lower levels of cortisol change following stress exposure. In contrast, no significant correlations were found for responders. These preliminary findings suggest the existence of estrogen-related differential biological constructs that may influence responsiveness to stressful events. Previous animal and human studies provide support for this assertion by demonstrating that estrogens has both anxiogenic and anxiolytic properties. These dual functions of estrogen are explained by the existence of two distinct estrogen receptor systems, each playing a critical role in regulating different functions (Lund, Rovis, Chung, & Handa, 2005). Thus, the specific balance between opposing estrogen-receptor systems may underlie the differential influence of estrogen on cortisol reactivity in responders and non-responders, as well as the inconsistency in previous findings on the effects of estrogen on the response to psychosocial stress (Kajantie & Phillips, 2006). Further evidence for the potential role of sex hormones on stress reactivity comes from studies focusing on their modulatory activity within stress neural circuitry (for example, Goldstein et al., 2005; Jacobs et al., 2015). Jacobs et al. (2015) have demonstrated that estrogen regulates neural activity within subcortical regions (amygdala, hippocampus, and hypothalamus) in stress circuity in women in response to a mild visual stress task. Higher blood oxygen level-dependent activation in these regions was demonstrated under low estrogen levels, and this activation was attenuated when estrogen levels elevated.

The present findings showed that higher levels of progesterone were associated with lower levels of cortisol reactivity to stress. This result agrees with a previous study (Juster et al., 2016) showing that higher levels of basal progesterone were associated with lower levels of cortisol in men. However, in the present study, this association was found in non-responders, whereas for responders, the association was positive (but not significant). Previous studies have demonstrated that higher levels of progesterone were associated with higher levels of cortisol secretion in reaction to stress. Roca et al. (2003) have demonstrated that progesterone administration increased HPA-axis response to exercise. Herrera, Nielsen, and Mather (2016) found that higher baseline progesterone levels during the low progesterone follicular phase were associated with higher cortisol in response to stress after the cold pressor test. Inconsistency in results may derive from differences between ovarian progesterone and adrenal progesterone. While the adrenals are the main source of progesterone in males, both ovaries and the adrenal are the source of progesterone in women (Wirth, Meier, Fredrickson, & Schulthesis, 2007). An association between ovarian and adrenal progesterone across the menstrual cycle in women was also demonstrated (Herrera et al., 2016). Future studies should further investigate the role of progesterone in modulating the HPA-axis reactivity to stress in men and women across the menstrual cycle.

The mechanism by which the HPA and HPG axes interact have not been completely resolved (Handa & Weiser, 2014). Current perspectives on the mechanisms by which the axes interact address their relationship as bidirectional (Viau, 2002). Hence, while some studies focused on the regulation of cortisol through sex hormones, demonstrating the involvement of testosterone and estrogen in modulating adrenal (Kitay, 1965), pituitary (Viau & Meaney, 2004), and hypothalamus functions (Viau, Soriano, & Dallman, 2001), others addressed the patterns by which the HPA axis regulates gonadal functions. The latter studies demonstrated that activation of the HPA axis under conditions of chronic stress has an inhibitory effect upon gonadal hormone secretion (Rivier, & Rivest, 1991; Tilbrook, Turner, & Clarke, 2000; Toufexis, Rivarola, Lara, & Viau, 2014). In terms of the HPA-HPG interaction, the present study was unidirectional, examining only the influence of basal sex hormones on cortisol reactivity to stress, and not vice versa. Thus, further exploration is still needed to shed light on the specific patterns by which HPA activity modulates the secretion of specific sex hormones.

To the best of our knowledge, the current study is the first to directly address the potential modulation of SNS stress reactivity (measured via sAA levels) by sex hormones. We found that in adjusted models for sex hormones, sAA reactivity to stress was diminished as compared with non-adjusted models. This finding agrees with the pattern found for cortisol reactivity demonstrating that sex differences in cortisol reactivity to stress were only significant in unadjusted models, that is, when sex hormones are not controlled. The notion that sex hormones modulate the SNS response to stress is not new. Previous studies examining intra- and inter-sex differences in SNS reactivity to stress produced inconsistent findings. This inconsistency, however, may be due to different stress procedures used in these studies (i.e., psychosocial, mental, physical), different age-groups, and varying hormonal statuses. A direct examination of the role of sex hormones in modulating SNS reactivity to stress in empirical literature is absent; therefore, the present findings demonstrating the interconnectivity between the HPG-axis and the SNS is important for further investigations exploring individual differences in stress reactivity. Future studies should elaborate the current investigation to uncover the modulating patterns of each gonadal hormone on SNS reactivity to various stress conditions.

 Examining the reactivity pattern of the two biomarkers for stress show unique reactivity patterns for each biomarker. While a peak was observed for cortisol on T3 followed by a decline in cortisol levels, for sAA, an immediate elevation was apparent, and conservation of elevated levels was demonstrated. First, the differences between the two main stress systems in the time-points following stress agree with previous findings (e.g., Kloppe et al., 2012). The SNS releases catecholamines immediately at the onset of a stressor, whereas the slower-acting HPA-axis releases glucocorticoids 21–45 minutes following the onset of a stressor (For metaanalyses, see Dickerson & Kemeny, 2004 and Goodman, Janson, & Wolf, 2017). Second, the differences in time-course between cortisol and sAA levels may be explained by differences in sensitivity level of these two biomarkers to mental stress. In our study, participants were assessed in cognitive tasks following the psychosocial stressor (i.e., TSST). It has been suggested that sAA is sensitive to other stressors and can account for the conservation of sAA levels even in time-points T3 and T4. Noto, Sato, Kudo, Kurata, and Hirota (2005) showed that sAA, but not cortisol levels, increased significantly following a mental arithmetic task. Thus, the SNS and HPA-axis differ in their response time-frame and in their sensitivity to many stressors.

Limitations and Future Directions

The present study has certain limitations. First, the current study included a relatively small sample size. Although related studies (e.g., Hidalgo et al., 2012) included similar sample sizes, interpretation of results obtained from a limited number of participants should be taken with caution. Second, data collection took place between 8 to 10 AM, central time segment of the human daily schedule that is understudied in the context of stress reactivity. However, this is a time window in which diurnal cortisol levels are highest (Ghiciuc et al., 2011). Thus, the high baseline levels of cortisol could account for the relatively high number of non-responders in the current study. Moreover, cortisol levels tend to rise sharply following awakening, reaching a maximum within 30 minutes before beginning to decline (i.e. "cortisol awakening response”; CAR) (Ghiciuc et al., 2011). In the current study increases in cortisol levels following the TSST could not be explained by the CAR as all participants gave their first saliva sample at least one hour following awakening. In any case, the impact of sex hormones on stress reactivity at different time points throughout the day remains to be examined. Third, due to the short time pulsating dynamics of sex hormones secretion, the use of a single sample, as in the current study, may lead to considerable variability that complicates interpretation. However, the significant results obtained despite this variability attest the importance of sex hormones in the stress response. Nevertheless, using multiple saliva samples in future studies will yield more accurate assessments of the hormonal levels and may increase statistical power. Fourth, for women not using OC, the luteal phase of the menstrual cycle was chosen, in order to capture higher levels of both estrogen and progesterone (Schultheis & Zimni, 2015). Other studies, however, chose different phases, for example, the follicular phase, in order to capture the highest estrogen levels (Hidalgo et al., 2012). Fifth, modern life mainly challenges humans with psychosocial stressors. Therefore, the present study used the most validated measure of psychosocial stress, namely, the TSST, which has been proven to elicit the highest psychological and physiological responses to stress (Skoluda et al., 2015). Nevertheless, it is assumed that various stressors elicit differentiated responses and physiological mechanisms (Bosch et al., 2009). Thus, future studies should broaden the sampling schedule, include additional hormone-levels groups (i.e. menopausal women HRT- and non-HRT users), and test the impact of other stressors. Sixth, no control group was part of the present study. Given that the current study is the first to report the modulation of sex hormones directly on the SNS (alongside the HPA-axis), the present findings are preliminary, and future studies should further explore these relationships using a larger sample including stress as well as control conditions.

In summary, the present study examined both the HPA axis and the SNS as two main systems of stress reactivity, thereby extending previous studies exploring the role of sex hormones in modulating the stress response. The present findings point to the role of sex hormones in HPA and SNS responses to stress, as evident by the levels of cortisol and sAA. Furthermore, the present findings demonstrate that these modulation mechanisms are not unified for men and women, and are differentiated within sex as a function of hormone levels. Thus, it is suggested that future studies control for sex hormones when examining stress reactivity of the HPA axis or the SNS.

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