**Gonadal hormones modulate the HPA-axis and the SNS in response 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 and alpha-amylase reactivity. Moreover, controlling for sex hormones also diminished the differential pattern of cortisol reactivity in each experimental group. Further correlation analyses revealed differences between groups in the association between sex hormones and stress biomarkers. The present findings point to a modulatory role for sex hormones on HPA and SNS stress reactivity and emphasize the need for control of sex hormone fluctuations when examining cortisol and alpha-amylase reactivity to stress.

**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. The HPA axis and the SNS work in coordination to generate 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 constraints (Keller, El-Sheikh, Granger, & Buckhalt, 2012).

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 between women in various hormone-level groups (e.g., luteal or follicular phases of the menstrual cycle, menopause; 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.

Salivary alpha amylase (sAA), a digestive enzyme found in the oral cavity can serve as marker for SNS activity (Nater, & Rohleder, 2009) while cortisol secretion is the end product of the HPA axis (Hidalgo et al., 2012). Elevated levels of both these markers 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; Allen et al., 2014).

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). Although early studies on cortisol reactivity as a result of stress in humans based on gender yielded equivocal results, demonstrating either no difference by gender or higher cortisol reactivity in men compared with women (Kirschbaum, Wust, & Hellhammer, 1992), further investigations revealed that stressor-induced cortisol response in women was dependent on their estrogen levels. Levels of cortisol in women in the luteal phase and men were comparable and/or higher than those of women in the follicular phase or those who used oral contraceptives (OC), when estrogen levels are higher (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). The authors suggested, therefore, 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 in postmenopausal women have demonstrated elevated levels of cortisol in response to stress in comparison with older men, providing 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, a decrease, or no change in cortisol levels following hormone (estrogen combined with progesterone) replacement therapy (see, for example, Burleson et al., 1998; Edwards & Mills, 2008; Pluchino et al., 2005). 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.

Testosterone has been 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. However, it should be noted that 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 hormones on cortisol reactivity to stress, there are only a few studies that have addressed the potential impact of sex hormones on SNS activation as reflected by sAA levels. No differences in baseline sAA levels were found 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), or between men, OC women, and women in the follicular phase in response to the TSST procedure (Hidalgo et al., 2012). On the other hand, pregnant women (who have higher/lower levels of ???) showed lower sAA reactivity to the TSST procedure (Nierop et al., 2006) and untreated postmenopausal women did not show any sAA reactivity following an exercise test. Postmenopausal women subjected to hormone-replacement-therapy (estrogen plus progestin replacement treatment; Patacchioli et al., 2015) demonstrated levels of sAA comparable to (???).

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 compares them with both cortisol and sAA levels that occur as a result of 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), differences between stress-marker levels and group (men, OC women, luteal phase women) will be significant, whereas, in adjusted models (controlling for sex hormones), differences 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 a negative correlation 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 by advertisements aimed at the student body of the Max Stern Yezreel Valley College (Israel) and neighboring communities. 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: no serious medical, gynecological, or hormonal problems; non-smokers; no ADHD or learning disabilities. In addition, women to be included in the OC group were all using pills containing 25 mg of estrogen (Ethinylestradiol) and 75 mg of progestin (Gestodene). These doses are considered moderate and are commonly prescribed. 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 Max Stern Yezreel Valley College 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 Max Stern Yezreel Valley College psychology department between 8 AM and 10 AM on a single day. 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 (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.

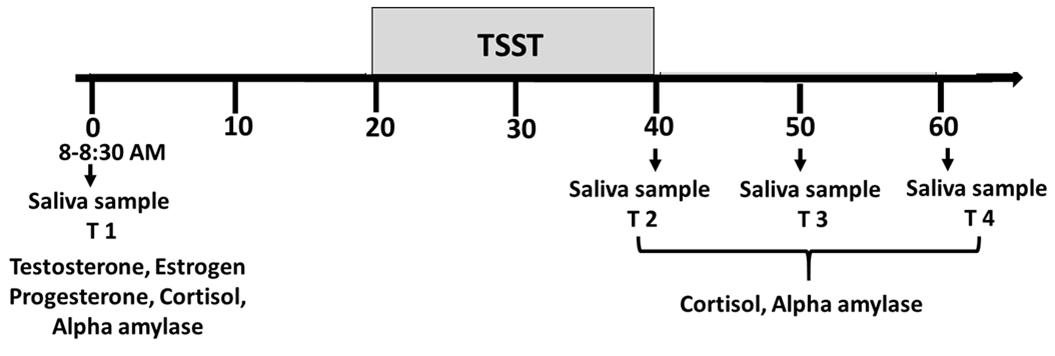


Figure 1. Study design

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 (aside for water), or 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, Cortisol Saliva ELISA, Testosterone Saliva ELISA, Progesterone Saliva ELISA, Alpha Amylase Saliva ELISA, all from IBL International GMBH, Hamburg, Germany). 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. The kits were validated in our laboratory according to good laboratory practice (GLP) guidelines, complying with ISO 9001 certification and JCI accreditation standards.

**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. In total, the procedure, including the preparation phase, took approximately 20 minutes.

**State-Trait Anxiety Inventory (STAI)**

A few days prior to their experimental session, participants completed an STAI. The STAI (Spielberger et al., 1983) consists of 20 items assessing state anxiety (statements relating to the participant’s sense of anxiety at that point in time) and 20 items assessing trait anxiety (statements relating to the participant’s sense of anxiety in general). Participants indicated agreement with each statement on a 4-point scale. The questionnaire was translated into Hebrew by Teichman and Melnick (1979); its internal consistency in the current study was α=.78.

**Statistical analyses**

Cortisol, sAA, and sex hormones (estrogen, progesterone, and testosterone) were not normally distributed and were thus subject to square root 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 to stress, 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’.

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.

**Results**

Table 1 presents the characteristics and sex hormone levels of the study groups. The groups did not differ in their average ages, years of education, or BMI. 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].

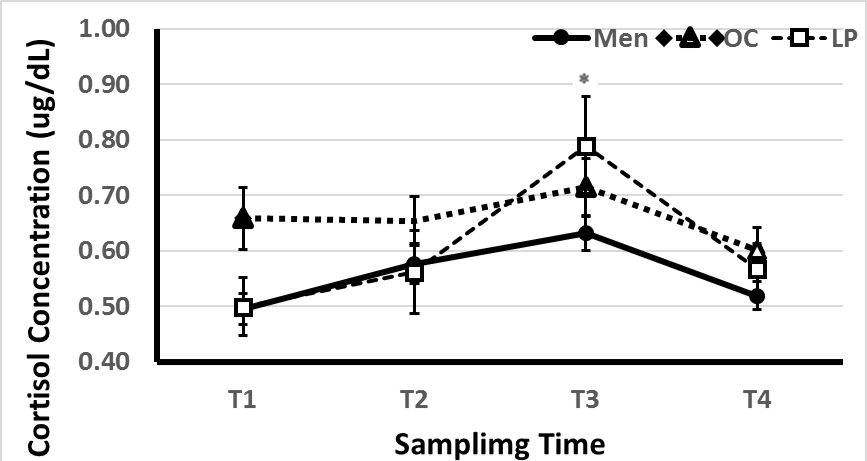
Table 1. Characteristics of the study sample

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Men (N=21)** | **OC (N=20)** | **LP (17)** | **F** |
| **Age** | **26.38 (2.33)** | **23.6 (2.04)** | **24.29 (2.17)** | **8.94\*** |
| **Trait Anxiety** | **46.62 (1.24)** | **45.85 (0.90)** | **46.06 (0.61)** | **0.86** |
| **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. Trait anxiety was measured by the State-Trait Anxiety Inventory (STAI). Abbreviations: OC: oral contraceptives; LP: luteal phase. Data presented as mean ± SEM. \* ?????, \*\*p<0.01

*Cortisol reactivity*

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



Salivary cortisol concentrations before and following the TSST procedure. The figure presents data collected from 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 versus group interaction was also found. These statistical effects diminished when sex hormones (estrogen, progesterone, and testosterone) were controlled. \*P<0.05. Depicted values are means of the corrected cortisol levels (square root) and error bars represent SEM.

A two-way repeated-measures analysis of variance demonstrated no significant main effect for the group [*F* (2, 20) =.55, *p* = .585; *η2p* = .05]. A significant main effect was found for time [*F* (3, 20) = 12.55, *p* = .000; *η2p* = .39], 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) = 3.16, *p* = .021; *η2p* = .24]. However, in re-analyses controlling for sex hormones (estrogen, progesterone and testosterone), the main effect for time [*F* (3, 20) =.69, *p* = .508; *η2p* = .04] and the interaction effect for time X group [*F* (6, 20) = 1.68, *p* = .178; *η2p* = .17] disappeared. Covariation effects were found to be significant for time X group X estrogen [*F* (9, 20) = 2.59, *p* = .031; *η2p* = .29], marginally significant for time X group X testosterone [*F* (9, 20) = 1.96, *p* = .090; *η2p* = .24], and significant for time X group X progesterone [*F* (9, 20) = 1.90, *p* = .100; *η2p* = .23].

Pearson correlations were conducted to further examine the different patterns of modulation that the sex hormones had on cortisol reactivity 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. Estrogen was positively correlated with a change in the LP group (*r* = .81, *p* = .047) only. Further parallel analysis of non-responders revealed an opposite pattern: estrogen was *negatively* correlated with the change between T1 and T3 in the LP group (*r* = -.57, *p* = .028). In order to examine possible alignment between physiological measurements and the self-experienced state anxiety of responders and non-responders, an independent sample t-test was performed. This showed a significant difference in the state-anxiety score of the LP group responders and non-responders [*t*(15) = 2.36, *p* = .004], with responders exhibiting higher levels of state-anxiety (*M* = 47.4, *SD* = 4.28) as opposed to non-responders (*M* = 39.58, *SD* = 4.40).

*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) =.48, *p* = .622; *η2p* = .02], and no significant time X group interaction was found [*F* (6, 50) = 4.90, *p* = .624; *η2p* = .02]. A main effect for time was found [*F* (3, 50) = 18.60, *p* = .000; *η2p* = .27]. Post-hoc analysis revealed that sAA levels at T1 were lower than at other times, with levels at T4 significantly higher than at T3. In re-analyses controlling for sex hormones (estrogen, progesterone and testosterone), the main effect for time was still significant [*F* (3, 50) = 6.08, *p* = .006; *η2p* = .12]. However, the effect of group [*F* (2, 45) =.71, *p* = .497; *η2p* = .03], and the interaction effect for time X group [*F* (6, 50) = 1.55, *p* = .207; *η2p* = .06] were insignificant. 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.

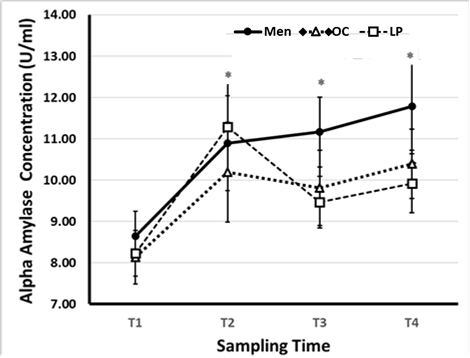
Pearson correlations were conducted to further examine the different patterns of modulation that the sex hormones had on sAA in each group. sAA reactivity was calculated to be the T2 minus T1 scores. In males, testosterone, estrogen, and progesterone were negatively correlated with sAA reactivity (r = -.44, p = .028; r = -.53, p = .012; r = -.42, p = .047, respectively); in OC women, estrogen only had a negative correlation to sAA levels (r = -.58, p = .006).

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<0.05. Depicted values are means of the corrected alpha amylase levels (square root) 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 both systems, controlling for testosterone, estrogen, and progesterone diminished the impact of stress on cortisol and sAA reactivity. Moreover, 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 assumption 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 responses of the two main stress-response systems in humans are modulated by sex hormones.

The specific pattern by which sex hormones influence cortisol reactivity to stress was examined through correlations between hormones in each group. As in previous studies (e.g., Reschke-Hernández, Okerstrom, Bowles Edwards, & Tranel, 2017), the analysis was conducted only on participants who showed stress-induced elevations of cortisol (i.e. ‘responders’). Indeed, in the current study, interesting differences emerged between responders and non-responders. First, while no difference was found between the study groups in their trait anxiety, responders had higher levels of state anxiety in comparison to non-responders, providing psychological validation to the physiological measures of stress reactivity demonstrated by elevated levels of cortisol upon reaction to the TSST. Second, among LP participants, the association between estrogen and cortisol reaction was positive for responders but negative for non-responders. Furthermore, among LP participants non-responders had significantly higher levels of estrogen in comparison to responders (p = .013). 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).

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. Further correlation analyses revealed that in men, sex hormones were negatively correlated with sAA reactivity to stress. That is, higher levels of testosterone, estrogen, and progesterone were associated with lower sAA reactivity. Furthermore, estrogen was negatively associated with sAA reactivity in the OC group. That is, in women using OC, higher levels of estrogen were associated with lower levels of stress reactivity. This is line with a previous study demonstrating lower levels of sAA following TSST in pregnant women in comparison to non-pregnant women (Nierop et al., 2006). However, other studies addressing individual differences in sAA reactivity to stress through comparison of different hormones-level groups provided mixed results (Kivlighan and Granger, 2006; Patacchioli et al., 2015). In light of the present findings and the scarcity and inconsistency of research on the subject, the role of sex hormones in SNS reactivity modulation needs to be further explored.

Limitations and Future Directions

The present study has certain limitations. First, although accustomed in related studies (e.g., Hidalgo et al., 2012) given the small *N*, caution should be exercised in interpreting the results. Second, data collection took place between 8 to 10 AM in order to measure individual variations in high levels of testosterone as well as cortisol. Nevertheless, previous studies chose different time windows, such as later in the afternoon, during which basal and stress-induced cortisol secretion patterns are different due to circadian rhythms. Third, 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). Fourth, 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.

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 show that sex hormones modulate seem to modulate both HPA and SNS responses to stress, as evidence 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 genders 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.

References

Allen, A. P., Kennedy, P. J., Cryan, J. F., Dinan, T. G., & Clarke, G. (2014). Biological and psychological markers of stress in humans: Focus on the Trier Social Stress Test. *Neuroscience & Biobehavioral Review, 38*, 94–124.

Bale, T.L., & Epperson, C.N. (2015). Sex differences and stress across the lifespan. *Nature Neuroscience, 18*, 1413–1420.

Bosch, J. A., de Geus, E. J., Carroll, D., Goedhart, A. D., Anane, L. A.,van Zanten, J. J., et al. (2009). A general enhancement of auto-nomic and cortisol responses during social evaluative threat. *Psychosomatic Medicine, 71*, 877—885.

Bosch, J. A., de Geus, E. J., Veerman, E. C., Hoogstraten, J., & Nieuw Amerongen, A. V., (2003). Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. *Psychosomatic Medicine, 65*, 245–258.

Burleson, M. H., Malarkey, W,B., Cacioppo, J. T., et al. (1998). Postmenopausal hormone

replacement: effects on autonomic, neuroendocrine, and immune reactivity to brief psychological stressors. *Psychosomatic Medicine, 60*, 17–25.

Chatterton, Jr., R. T., Vogelsong, K. M., Lu, Y. C., & Hudgens, G. A. (1997). Hormonal responses to psychological stress in men preparing for skydiving. *Journal of Clinical Endocrinology & Metabolism, 82,* 2503–2509.

Edwards, K. M. & Mills, P. J. (20080. Effects of estrogen versus estrogen and progesterone on cortisol and interleukin-6. *Maturitas, 61*, 330–333.

Friedmann, B., & Kindermann, W. (1989). Energy metabolism and regulatory hormones in women and men during endurance exercise. *European Journal of Applied Physiology & Occupational Physiology, 59,* 1–9.

Handa, R. J., & Weiser, M. J. (2014). Gonadal steroid hormones and the hypothalamo– pituitary–adrenal axis. *Frontiers in Neuroendocrinology, 35*, 197–220

Hidalgo, V., Villada, C., Almela, M., Espin, L., Gomez-Amor, J., & Salvador, A. (2012). Enhancing effects of acute psychosocial stress on priming of non-declarative memory in healthy young adults. *Stress, 15,* 329-338.

Juster, R. P., Raymond, C., Desrochers, A. B., Bourdon, O., Durand, N., Wan, N., Pruessner, J. C., & Lupien, S. J. (2016). Sex hormones adjust “sex-specific” reactive and diurnal cortisol profiles. *Psychoneuroendocrinology, 63,* 282-290.

Kajantie, E., & Phillips, D. I. W. (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology, 31,* 151–178

Keller, P. S., El-Sheikh, M., Granger, D. A., & Buckhalt, J. A. (2012). Interactions between salivary cortisol and alpha-amylase as predictors of children's cognitive functioning and academic performance. *Physiology and Behavior, 105,* 987-995.

Kivlighan, K.T. & Granger, D.A. (2006). Salivary alpha-amylase response to competition: relation to gender, previous experience, and attitudes. *Psychoneuroendocrinology 31*, 703—714.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., & Hellhammer, D.H. (1999). Impact of gendermenstrual cycle phase, and oral contraceptives on the activity of the hypothalamus–pituitary–adrenal axis. *Psychosomatic Medicine, 61*, 154–162.

Kirschbaum, C., Pirke, K-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test' – A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*, 76–81.

Kirschbaum, C., Wust, S., & Hellhammer, D. (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic Medicine, 54*, 648–657.

Kitay, J. I. (1965). Depression of adrenal corticosterone production in oophorectomized rats. *Endocrinology, 77*, 1048–1052.

Kudielka, B.M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: a review. *Biologocal Psychology, 69,* 113-132.

Laine, M., Pienihakkinen, K., Ojanotko-Harri, A., & Tenovuo, J. (1991). Effects of low-dose oral contraceptives on female whole saliva. *Archives of Oral Biology, 36*, 549—552.

Lund, T. D., Rovis, T., Chung, W. C. J., & Handa, R. J. (2005). Novel Actions of Estrogen Receptor-β on Anxiety-Related Behaviors. *Endocrinology, 146,* 797-807.

Nater, U. M., & Rohleder, N. (2009). Salivary alpha-amylase as a noninvasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology 34*,486–496.

Nater, U.M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., & Ehlert, U. (2005).

Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journl of Psychophysiology, 55*, 333–342.

Nierop, A., Bratsikas, A., Klinkenberg, A., Nater, U.M., Zimmermann, R., & Ehlert, U., (2006). Prolonged salivary cortisol recovery in second-trimester pregnant women and attenuated salivary {alpha}-amylase responses to psychosocial stress in human pregnancy. *The Journal of Clinical Endocrinology & Metabolism, 91*, 1329—1335.

Otte, C., Hart, S., Neylan, T.C., Marmar, C.R., Yaffe, K., & Mohr, D.C. (2005). A meta- analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology 30,* 80–91.

Oyola, M. G., & Handa, R. G. (2017). Hypothalamic–pituitary–adrenal and hypothalamic– pituitary–gonadal axes: sex differences in regulation of stress responsivity. *Stress*.

Patacchioli, F. R., Ghiciuc, C. M., Bernardi, M., Dima-Cozma, L. C., Fattorini, L., Squeo, M. R., Galoppi, P., Brunelli, R., Ferrante, F., Pasquali, V., & Perrone, G. (2015). Salivary α -amylase and cortisol after exercise in menopause: influence of long-term HRT. *Climacteric, 18,* 528-535.

Pluchino, N., Genazzani, A. D., Bernardi, F., et al. (2005). Tibolone, transdermal estradiol or

oral estrogen-progestin therapies: effects on circulating allopregnanolone, cortisol

and dehydroepiandrosterone levels. *Gynecological Endocrinology, 20*, 144–9.

Reschke-Hernández, A. E., Okerstrom, K. L., Bowles Edwards, A., & Tranel, D. (2017). Sex and stress: Men and women show different cortisol responses to psychological stress induced by the Trier Social Stress Test and the Iowa Singing Social Stress Test. *Journal of Neuroscience Research, 95,* 106-114.

Rivier, C., & Rivest, S. (1991). Effect of stress on the activity of thehypothalamic–pituitary– gonadal axis: peripheral and central mechanisms. *Biology of Reproduction, 45*, 523– 532.

Rohleder, N., Nater, U.M.,Wolf, J.M., Ehlert, U., & Kirschbaum, C. (2004). Psychosocial stress induced activation of salivary alpha-amylase: an indicator of sympathetic activity? Annals of the New York Academy of Sciences, 1032, 258–263.

Rossi, A.S., & Rossi, P.E. (*1980). Body time and social time: Mood patterns by menstrual cycle phase and day of week.* In J. Parsons, editor: The psychology of sex differences and sex roles (pp. 269-303). New York: Hemisphere.

Schommer NC, Hellhammer DH, & Kirschbaum C. (2003). Dissociation between reactivity of the hypothalamus–pituitary–adrenal axis and the sympathetic-adrenal-medullary system to repeated psychosocial stress. Psychosomatic Medicine, 65, 450–460.

Schultheiss, O. C. & Zimni, M. (2015). Associations Between Implicit Motives and Salivary

Steroids, 2D:4D Digit Ratio, Mental Rotation Performance, and Verbal Fluency. *Adaptive Human Behavior and Physiology, 1,* 387-407.

Skoluda, N., Strahler, J., Schlotz, W., Niederberger, L., Marques, S., Fischer, S., Thoma, M. V., Spoerri, C., Ehlert, U., & Nater, U. M. (2015). Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity. *Psychoneuroendocrinology, 51,* 227-236.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Teichman, Y., & Melnick, H. (1979). *STAI - A questionnaire for the Assessment of State and Trait Anxiety: A Hebrew Manual for the Researcher.* Tel-Aviv, Israel: Tel-Aviv University.

Tenovuo, J., Laine, M., Soderling, E., & Irjala, K. (1981). Evaluation of salivary markers during the menstrual cycle: peroxidase, protein, and electrolytes. *Biochemical Medicine, 25*, 337—345.

Tilbrook, A. J., Turner, A. I., & Clarke, I. J. (2000). Effects of stress on reproduction in non- rodent mammals: the role of glucocorticoids and sex differences. *Reviews of Reproduction, 5*, 105–113.

Toufexis, D., Rivarola, M. A., Lara, H., & Viau, V. (2014). Stress and the Reproductive Axis. *Journal of neuroendocrinology, 26*, 573-586.

Viau, V. (2002). Functional cross-talk between the hypothalamic–pituitary–gonadal and - adrenal axes. *Journal of Neuroendocrinology, 14*, 506–513.

Viau, V., & Meaney, M. J. (2004). Testosterone-dependent variations in plasma and

intrapituitary corticosteroid binding globulin and stress hypothalamic–pituitary–adrenal activity in the male rat. *Journal of Endocrinology, 181,* 223–231.

Viau, V., Soriano, L., & Dallman, M. F. (2001). Androgens alter corticotropin releasing hormone and arginine vasopressin mRNA within forebrain sites known to regulate activity in the hypothalamic–pituitary–adrenal axis. *Journal of Neuroendocrinology, 13,* 442–452.