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Effects of sex and menstrual cycle phase on cardiac response and alpha- amylase levels in psychosocial stress.

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Highlights

- Follicular women exhibited blunted alpha-amylase reactivity to the TSST
- Luteal women and men showed higher alpha-amylase reactivity to the TSST
- Men showed higher LF/HF response to the TSST compared to the control condition

- No condition differences were found in the LF/HF responses of the two groups of women
- Sex and menstrual cycle factors should be considered in the stress response

ABSTRACT

The impact of sex and the menstrual cycle phase on the autonomic response to psychosocial stress remains controversial. This study explored autonomic nervous system activity through salivary alpha-amylase, heart rate, and heart rate variability responses to the Trier Social Stress Test (TSST) in healthy young people. The sample was composed of 25 men, 26 women in the luteal phase, and 25 women in the follicular phase, from 18 to 25 years of age. Participants were exposed to the TSST or a control condition. The results indicate that women in their follicular phase showed a blunted alpha-amylase response to stress compared to men and women in the luteal phase. In addition, men showed higher sympatho-vagal activity in the stress condition compared to the two groups of women. These results confirm that sex and the menstrual cycle phase are potential modulators of autonomic nervous system reactivity to psychosocial stress.

Keywords:

Salivary alpha-amylase; psychosocial stress; sex differences; menstrual cycle; heart rate variability.

1. INTRODUCTION

Many studies have shown that men and women differ in the way they respond to stressors, based on their endocrinological and behavioral responses (Taylor et al., 2000), and this difference seems to be reflected in the sex- specific prevalence rates for several diseases (Gobinath et al., 2017). Thus, women suffer more often from autoimmune illnesses associated with anxiety and depression, whereas men are more susceptible to developing cardiovascular disease (Kajantie & Phillips, 2006). A stressful stimulus results in the activation of several physiological pathways, including the autonomic nervous system (ANS) and the hypothalamic– pituitary–adrenal axis (HPA), which seem to be affected by sex. Therefore, studying sex differences in stress reactivity could help us to find a mechanism that explains why some diseases are more common in men and others in women.

A large amount of research has been published on sex-differences in ANS and HPA-axis responses to stress. Differences have been found between men and women on several baseline or pre-stress cardiovascular measures, such as heart rate (HR) and blood pressure (Suarez et al., 2004), as well as differences in the effects of sex and hormonal status on the physiological response to acute psychosocial stress (Stark et al., 2006; Wolf et al., 2001). In addition, a higher salivary cortisol response has been found in men than in women in reaction to social stress (Hidalgo et al., 2014; Kirschbaum et al., 1999; Stephens et al., 2016; Uhart et al., 2006). The role of sex hormones is an issue that needs to be considered to better explain why some studies show no sex differences in stress responsiveness. For example, the number of cortisol responders to social stress decreases during the follicular phase and in post-menopausal women, compared to women in the luteal phase (Villada et al., 2017), possibly because women in the luteal phase show higher levels of progesterone and estrogens than in other phases of the menstrual cycle or even during the postmenopausal period (Gordon &

Girdler, 2014).

Some authors have also suggested that estrogens have a diminishing effect on sympathetic activity, but the effect of progesterone is still unclear (Kajantie & Phillips, 2006). Moreover, the circulating levels of allogpregnolone (a neuroactive metabolite of progesterone) seem to be involved in the sensitivity to stress. Specifically, the larger allopregnolone levels after stress induction (aversive movies) have been related to a higher HR response, greater negative affect, and lower phasic activity in the amygdala and medial Prefrontal Cortex (mPFC) in women in the luteal phase than in women in the follicular phase (Ossewarde et al., 2010).

Despite the relevance of sex differences in the autonomic stress response and its interaction with stress hormones, only a few studies have focused on sex differences in salivary alpha-amylase (sAA) levels or responses, with ambiguous results (Kivlighan & Granger, 2006; Nater et al., 2006; Takai et al., 2007). sAA has been proposed as an indirect marker of the sympatho-adrenal medullar (SAM) response to different stressors (Filaire et al., 2010; Nater & Rohleder, 2009). In recent years, sAA activity has received considerable attention (Nater et al., 2005, 2006; Rohleder & Nater, 2009; van Stegeren, Wolf, & Kindt, 2008), and its response profile to stress has been studied in several age groups, including children (Räikkönen et al., 2010; Spinrad et al., 2009), adolescents (Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010; Susman et al., 2010), young people (Nater et al., 2005, 2006; Rohleder, Wolf, Maldonado, & Kirschbaum, 2006; Schoofs, Hartmann, & Wolf, 2008) and older adults (Almela et al., 2011; Strahler, Mueller, Rosenloecher, Kirschbaum, & Rohleder, 2010). Discussion about whether sAA is a good index for sympathetic nervous system (SNS) activity is still ongoing because the available literature is inconclusive. Positive associations between stress-induced sAA responses and markers of SNS activity have been observed, for example, in plasma concentrations of norepinephrine (Thoma, Kirschbaum, Wolf, &

Rohlededer, 2012) and in measures of the sympatho-vagal balance, with increases in heart rate (HR) (Nater et al., 2006) and decreases in the cardiac parasympathetic activity (Bosch, Geus, Veerman, Hoogstraten, & Nieuw Amerongen, 2003). However, other studies have reported no associations between stress-induced sAA levels and plasma noradrenaline (Petrakova et al., 2015) or cardiovascular measures (Strahler et al., 2010).

Another currently debated aspect is whether there are sex differences in the sAA response to acute stress. Although no sex differences in basal sAA levels were found in some studies (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007), others reported greater noradrenergic arousal (higher sAA at baseline) in men compared to women (Carr, Scully, Webb & Felmingham, 2016). Several studies have reported that men and women have a comparable sAA responsivity to standardized stressors such as the cold pressor stress (CPS) test (van Stegeren et al., 2008) or the Trier Social Stress Test (TSST) (Almela et al., 2011; Hidalgo et al., 2012; Hlavacova et al., 2017; Maruyama et al., 2012; Schoofs & Wolf, 2011; Thoma et al., 2012). However, Smeets (2010) reported sex differences in the acute sAA response to the TSST, with a higher sAA response in men than in women (women were tested only in the luteal phase). More recently, Carr et al. (2016) found higher sAA reactivity to stress in young women (tested in several phases of the menstrual cycle) than in men, in response to the CPS test. Therefore, in light of these mixed findings, it seems necessary to take into account the women's menstrual cycle phase when studying the noradrenergic response to acute stress.

The impact of sex on other biomarkers of the stress response, such as HR or heart rate variability (HRV), also remains controversial. Several studies have reported no sex differences in HR responses to the TSST (Kirschbaum et al., 1999; Kelly, Tyrka, Anderson, Price, & Carpenter et al., 2008; Villada, Hidalgo, Almela & Salvador, 2018), whereas other studies have found an enhanced HR response in women (Kudielka, Buske-Kirschbaum,

Hellhammer, & Kirschbaum, 2004; Smeets, 2010). Results from studies on the effects of sex hormones on HR responses are unclear. Some studies on cardiac variability across phases of the menstrual cycle suggest that estrogen may attenuate HR reactivity (Kajantie & Phillips, 2006). However, years later, one study showed that women tested in the luteal phase exhibited a greater HR response to the TSST than men, but not greater than women in the follicular phase (Childs et al., 2010). Moreover, no differences have been found in the HR and HRV parameters in response to social stress in young women in different phases of the menstrual cycle (Gordon & Girdler, 2014; Pico-Alfonso et al., 2007; Villada et al., 2014).

HRV, a measurement of the beat-to-beat difference in HR, is mediated by the parasympathetic and sympathetic cardiac nerves and reflects the capacity for the parasympathetic inhibition of autonomic arousal. There is evidence indicating that this balance may be affected by various psychiatric conditions, as well as by the presence of stressful stimuli (Berntson and Cacioppo, 2004), showing an increased response of the sympathetic component and a decrease in the parasympathetic response during the stress condition (Kim et al., 2018; Nater et al., 2006). Regarding sex differences in HRV, a recent meta-analysis showed that, in general, HRV in women is characterized by a relative dominance of vagal, parasympathetic activity, despite a higher mean HR (Koening and Thayer et al., 2016). In addition, some studies report that HRV displays physiological changes throughout the menstrual cycle (Brar et al., 2015; Uckuyu et al., 2013; Vishrutha et al., 2012). Vishrutha et al. (2012) concluded that the HF component of HRV was higher in the follicular phase, whereas the LF component was higher in the luteal phase. These results suggest a parasympathetic predominance during the follicular phase and greater sympathetic activity in the luteal phase (Brar et al., 2015; Vishrutha et al., 2012), although other studies did not find differences in HRV related to the menstrual cycle phase (Das et al., 2015; Leich et al., 2003; Nakagawa et al., 2006). Thus, this relationship between the menstrual cycle and

autonomic activity is still an unresolved question (Das et al., 2015).

Due to a relative lack of knowledge and consistency regarding the role of sex and the menstrual cycle phase in ANS markers of the stress response, the current study aims to clarify the sex and menstrual cycle phase differences in the ANS response to stress. To address this response, we integrated the sAA response with cardiac markers (HR and HRV) in a comprehensive range of complementary indicators of ANS activity. Because personality traits, specifically trait anxiety, can play an important role in the psychophysiological stress response (Chida & Hamer, 2008), in order to control individual differences, a measure of trait anxiety was included. Based on previous literature, we expect a sympathetic activation in response to the TSST, compared to the control condition, measured through sAA, HR, and the ratio of low and high frequency HRV bands (LF/HF), and a decrease in the root mean square of successive differences (RMSSD) as a parasympathetic modulation. In relation to sex differences and the effects of the menstrual cycle phase in the response to stress, due to mixed results in the literature, we aim to explore the full profile of stress responses in women in different phases of the menstrual cycle.

2. METHODS

2.1.Participants

The final sample was composed of seventy-six healthy university students, 25 men (mean age = 19.56; SD = 1.80) and 51 women (mean age = 18.90; SD = 1.48). From the women's subgroup, 26 were tested in the luteal phase of the menstrual cycle (mean age = 19; SD = 1.62), and 25 were tested in the follicular phase of the menstrual cycle (mean age = 18.80; SD = 1.35).

To recruit participants, informative talks were held in various campus classrooms. Volunteers were interviewed by trained psychologists, and they completed an extensive questionnaire to check whether they met the study prerequisites. From the initial sample of 119

volunteers, 20 women were excluded because they had spontaneous anovulatory cycles, 13 subjects were not selected because of the exclusion criteria, and 10 subjects were eliminated due to a variety of problems during the experimental procedure. The exclusion criteria were: alcohol or other drug abuse, visual or hearing problems, presence of cardiovascular, endocrine, neurological, or psychiatric disease, and the presence of a stressful life event during the past year. To obtain this information participants were asked about the presence or not of any important (positive or negative) event in the past year that they considered stressful and that changed their daily life significantly: e.g. traffic accident, change of habits, winning the lottery, death of someone close). Each event was evaluated based on the impact (positive vs. negative) and based on the magnitude: from 1 (not relevant) to 4 (very relevant). Subjects were also excluded if they were using any medication directly related to emotional or cognitive function, or one that was able to influence hormonal and sAA levels, such as glucocorticoids, beta-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, and psychotropic substances. Vitamins, sporadic use of painkillers, and natural therapies were allowed. Because there is no conclusive data available on the impact of the use of oral contraceptives on sAA (Rohleder & Nater, 2009), women using oral contraceptives were excluded from participation. None of the participants were habitual smokers, although in each condition, five participants reported sporadic smoking (less than 10 cigarettes a week).

Subjects in the final sample were randomly assigned to one of two conditions: the TSST procedure versus a control condition. The TSST group was made up of 13 men, 13 women in the luteal phase (4th to 8th day before the onset of the new menstrual cycle), and 11 women in the follicular phase (5th to 8th day after the onset of the new menstrual cycle). The subjects in the control condition were 12 men, 13 women in the luteal phase, and 14 women in the follicular phase.

The menstrual cycle phase was calculated using two estimation procedures. First, in order to establish the date of each subject's appointment, all the cycles were converted to a standard 28-day cycle, taking as reference points the day of onset of the last menstruation and the real length of the studied cycle (Rossi & Rossi, 1980). Second, to confirm the previous estimation and estimate the ovulation point, Basal Body Temperature (BBT) was recorded daily during two complete menstrual cycles by means of sublingual temperature, taken for 5 min before getting up. To analyze the BBT, the "smoothed curve" (SMC) method was used, as described by McCarthy and Rockette (1983, 1986).

Participants who met the criteria were contacted by telephone and asked to attend a session that took place in a laboratory at the Faculty of Psychology. Before each session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session.

Additionally, they were instructed not to brush their teeth at least 1 h prior to the session, and to drink only water, without eating, smoking, or taking any stimulants, such as coffee, cola, caffeine, tea or chocolate, 2 h prior to the session, because these factors have been shown to modify sAA activity (Rohleder & Nater, 2009). The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Research Committee of the University of Murcia. All the participants received verbal and written information about the study and signed an informed consent.

2.2.Procedure

Experimental sessions were held in the laboratory at the university between 2pm and 5pm (the sequence is presented schematically in Figure 1). This study employed a between–subjects design where participants were tested individually and in a single session. After arriving at the laboratory, participants' weight and height were measured, and the HR

measurement device was connected and activated. Experimental sessions consisted of several phases of equal durations in both conditions. Each session took approximately 1 h to complete. In the last part of the session, all the participants completed the Inventory of Situations and Responses of Anxiety (ISRA).

[INSERT FIGURE 1 HERE]

2.2.1. Stress condition

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated, and baseline measures were obtained for HR and HRV, salivary alpha-amylase**,** anxiety and mood. After the habituation phase, the introduction phase started. In this phase, the participants were informed about the procedure for the stress task. They received the instructions in front of an audience in the same room where the task took place. Next, the participants returned to the first room and started the preparation for the TSST (10 min duration).

The TSST was performed according to the description provided by Kirschbaum et al. (1993). This test consists of a 10-min preparation phase that includes instructions for the speech, 5 min of free speech (a simulated job interview), and a 5 min mental arithmetic task (serial subtractions as quickly and accurately as possible) in front of a committee composed of a man and a woman.

Once it was over, the participants returned to the first room, and they had 20 min to recover while they answered the trait anxiety questionnaire. Then, the HR and HRV recorders were turned off.

2.2.2. Control condition

The control condition was designed to be as similar as possible to the stress condition in mental workload and overall physical activity, but without the main components capable of provoking stress, such as evaluative threat and uncontrollability (Dickerson & Kemeny, 2004). The control task was composed of a preparation phase of 10 min, where the participants read a chapter from a book with neutral content, followed by 5 minutes of reading aloud and an arithmetic task that involved counting by one for 5 minutes. The control condition was performed in the same room as the stress condition, but all the stress-producing elements were removed prior to starting it (video camera, tape recorder, committee, and microphone). The timing of the saliva samples, the questionnaires used, and the phase durations were the same for both conditions.

2.3.Measures

2.3.1. Salivary Alpha-amylase (sAA)

sAA concentrations were estimated from saliva samples obtained using Salivettes (Sarstedt, Nümbrecht, Germany) during a 55 min period with six assessment points: t−5, t0, t+10, t+15, t+20 and t+40 min, from the start of the stressor (see Figure 1). Participants were instructed to introduce the cotton swab into their mouths for 1 min, not chew the cotton because it may affect salivary protein composition as well as the flow rate (Bosch et al., 2011), and move the swab around in a circular pattern to collect saliva from all the salivary glands (Rohleder and Nater, 2009). Saliva samples were frozen at −20 ◦C from the end of the session until the analyses took place. The concentration of alpha-amylase in saliva was measured by an enzyme kinetic method following the protocol specified in Rohleder, Wolf, Maldonado and Kirschbaum (2006). Inter- and intra-assay variation was below 10%.

2.3.2. Heart Rate (HR) and Heart Rate Variability (HRV)

HR and HRV were measured using an HR monitor (Suunto, model T6, Suunto Oy,Vantaa, Finland), which consists of a chest belt for heart beat detection and transmission and a "watch" for data collection and storage. Heartbeat detection is performed with an accuracy of 1 ms, and these types of monitors have shown good validity (Roy, Boucher, & Comtois, 2009). After eliminating the artifacts, the HR and HRV means for each 5-min phase (habituation, preparation, speech, arithmetic, and recovery) were computed. HR was recorded in real-time and expressed as beats per minute (bpm). To evaluate HRV, we used two parameters: the root mean square of successive differences (RMSSD in ms) from time domain analysis, reflecting the short-time HRV, predominantly as a response to changes in parasympathetic tone; and the ratio between low and high frequency components (LF/HF ratio) of HRV spectra as an index of vagal balance (Task Force, 1996).

2.3.3. Trait Anxiety

The Inventory of Situations and Responses of Anxiety (ISRA, Cano-Vindel & Miguel-Tobal., 1999) is a self-report questionnaire that measures, based on Lang's model (Lang, 1968), different components (cognitive, physiological, and motor) of trait anxiety on three subscales containing 24 items each (Cronbach's alpha ranges from $\alpha = 0.95$ to 0.98). A total score is obtained by simply adding together the scores on the three subscales.

2.4. Statistical Analyses

Data were tested for normal distribution and homogeneity of variance using the Kolmogorov–Smirnov and Shapiro-Wilk tests before the statistical procedures were applied. These analyses revealed significant deviations from normality on all the absolute sAA levels. Therefore, they were log-transformed prior to analyses, and all subsequent analyses were conducted with log-transformed data.

One-way ANOVAs were conducted to evaluate possible differences between groups on demographic and anthropometric variables and trait anxiety scores (ISRA). ANOVAs for repeated measures were used to analyze sAA levels and HR and HRV parameters (RMSSD, LF/HF), with group (luteal vs. follicular vs. men) and condition (TSST vs. control) as between-subjects factors. We employed time as within-subjects factor (For sAA: t−5, t0, t+10, t+15, t+20 and t+40 min; for HR- HRV parameters: habituation, preparation, speech, arithmetic task and recovery. Furthermore, the area under the curve with respect to the increase (AUCi) was calculated using the trapezoid formula specified in Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003) for sAA, HR, RMSSD, and LF/HF. Univariate ANOVAs were computed to compare the two conditions and groups on all the physiological indices.

All the results were corrected using the Greenhouse-Geisser procedure, where appropriate. Post-hoc comparisons were performed using Bonferroni adjustments for multiple comparisons. As a measure of the effect size, we report Partial Eta Squared (η^2_p) values.

3. RESULTS

3.1. Demographic and anthropometric variables

The main characteristics of the groups are shown in Table 1. Significant differences were found only on BMI, with men showing higher BMI than both groups of women. All the groups showed similar scores on the ISRA total and each of its subscales, with no significant differences.

[INSERT TABLE 1 HERE]

3.2. Biochemical and cardiovascular measures

Significant effects of the time x condition interaction for sAA (F (5,350) = 2.79; p = 0.01; η^2_p = 0.03), HR (F (4,280) = 14.37; $p < 0.001$; η^2_p = 0.17) and RMSSD of HRV (F (4, 280) = 2.976; p = 0.02; η^2_p = 0.04) were found**.** The participants exposed to the TSST condition

showed higher sAA levels than those exposed to the control condition at all the sampling times ($p \leq 0.025$), except at baseline (t-5) and t+0 (for both $p>0.05$). For HR, significant differences between the two conditions were found in the preparation ($p< 0.001$), speech ($p=0.001$), and arithmetic task ($p=0.004$), with higher HR in the TSST condition than in the control condition. For RMSSD, there were no significant differences between conditions in the baseline and recovery periods ($p>0.10$ in both cases), but a significant difference between conditions was found during the recovery period ($p=0.03$) with lower RMSSD levels found in the stress condition than in the control condition.

The time x group interaction was significant for LF/HF (F (8, 280) = 4.583; $p \leq 0.001$; $\eta^2_p = 0.11$), as men showed higher LF/HF than luteal women on the arithmetic task ($p\leq 0.001$). No differences were found between men and follicular women ($p=0.12$) or between the two groups of women (p= 0.10). In addition, results revealed significant increases from preparation to the speech in follicular women ($p=0.04$), whereas no differences were found between these phases in luteal women (all $p>0.10$). Men showed significant increases from habituation to speech ($p=0.004$), habituation to arithmetic ($p\leq 0.001$), speech to recovery ($p=0.002$), and arithmetic to recovery ($p\leq 0.001$).

The condition x group interaction was significant only for LF/HF (F (2, 70) = 4.270; $p = 0.018$; $\eta^2_p = 0.10$). In the TSST condition, men showed a higher LF/HF ratio than the two groups of women (both $p< 0.02$). There were no significant differences between the two groups of women ($p>0.99$) (see Figure 2).

[INSERT FIGURE 2 HERE]

In addition, only men showed higher LF/HF levels in the stress condition than in the control condition ($p\leq 0.001$). In the control condition, no significant differences between groups were found (all $p>0.10$) (see Figure 3).

[INSERT FIGURE 3 HERE]

With regard to the AUCi for the sAA, the condition x group interaction was significant (F (2, 70) = 4.82; p = 0.01; η^2_p = 0.12). In the TSST condition, men and luteal women showed higher sAA output than their counterparts in the control condition (p=0.03 and p=0.009, respectively). No condition differences were found in follicular women (p=0.16). In the control condition, no significant differences between groups were found (all p> 0.99), whereas in the TSST condition, follicular women showed a significantly less pronounced stress-induced sAA output, compared to men (p=0.03) and luteal women (p=0.002). There were no significant differences between luteal women and men (p=0.87) (see Figure 4).

[INSERT FIGURE 4 HERE]

For the AUCi for the HR, a significant effect of the condition was found, showing higher cardiac reactivity in the TSST condition than in the control condition (F (1, 70) = 12.68; p= 0.001; η^2_p = 0.15), but there were no other significant differences (all p>0.10).

4. DISCUSSION

Our aim was to study the effects of sex and the menstrual cycle phase on sAA and cardiac responses to psychosocial stress. As expected, the participants exposed to the TSST showed significant increases in sAA and HR (sympathetic response) and a decrease in the RMSSD (parasympathetic response). Thus, our results confirm previous findings showing an

increase in sAA (Nater et al., 2005, 2006) and HR (Nater et al., 2005) and lower parasympathetic activity in response to the TSST (Villada et al., 2018).

A ~~negative~~ less pronounced sAA reactivity to the TSST in follicular women was found, compared to men and luteal women, but no differences were found between men and luteal women. In addition, the LF/HF response to the TSST increased only in men, compared to both groups of women in the TSST condition and men in the control condition.

The sAA response was different between groups in the experimental condition. Women in the follicular phase showed a less pronounced sAA reactivity (AUCi) to the TSST compared to men and women in the luteal phase. Physiological differences in estrogen levels probably contribute to the differences observed in the stress responsiveness of women in the luteal phase because estrogen concentrations are high during this phase (Kajantie & Phillips, 2006). However, estrogen levels are also known to be high during the mid-to-late follicular phase compared to the early follicular phase (Gordon & Girdler, 2014). The luteal and follicular phases differ most in progesterone levels, with women in the luteal phase presenting the highest levels of this hormone (Gordon & Girdler, 2014; Kajantie & Phillips, 2006). The effects of progesterone on the physiological response to stress are unclear. Some studies show a sedative effect (de Wit, Schmitt, Purdy, & Hauger, 2001; Soderpalm, Lindsey, Purdy, Hauger, & de Wit, 2004), whereas others find inconsistent effects (Childs et al., 2010). In our study, luteal women (high levels of progesterone) showed an increased sAA response compared to follicular women, and so it is possible that progesterone levels have an enhancing effect on the physiological response to stress. The result found in luteal women is consistent with other study showing that progesterone increases in response to acute stress (Childs et al., 2010), and that its effects are bimodal and paradoxical depending on the released dose of this hormone and the mount of associated allopregnanolone. This metabolite of progesterone has biphasic effects, with low doses increasing an adverse anxiogenic effect,

and high doses decreasing this effect and having more calming properties. The exact mechanism of this phenomenon is not known, but it is often referred to as a biphasic or bimodal effect (Andreen et al., 2009). Along these lines, one study found an association between greater stress-induced increases in plasma allopregnanolone in luteal women compared to follicular women, directly linking the activation of the sympathoadrenal system to greater availability of progesterone, a precursor of allopregnanolone in luteal women (Girdler et al., 2006). These higher levels of allopregnollone during the luteal phase has also been related to higher sensitivity to stress, measured through HR and noradrenaline, and lower phasic activity in the amygdala and in the mPFC (Ossewarde et al., 2010). Findings from another study showed that women in the follicular phase exhibited blunted noradrenaline responses to the TSST (Childs et al., 2010). However, a recent study found a higher sAA response to social stress in women in the follicular phase compared to women in the luteal phase (Hlavacova et al., 2017).

In our study, considering the sAA response as an indirect indicator of catecholaminergic activity, we suggest that the blunted sAA reactivity to the TSST found in follicular women could be explained by the blunted catecholaminergic activity in this group. These results provide new information about the effects of the menstrual cycle on sAA reactivity to stress because most of the studies have only included men and women in the luteal phase (Smeets, 2010; Strahler et al., 2010; Thoma et al., 2012), only women who use oral contraceptives (Almela et al., 2011), or only women in the early follicular phase (Hidalgo et al., 2014).

The results highlight the blunted sAA reactivity to stress found in women in the follicular phase, compared to men and women in the luteal phase. These cycle-related differences may provide clues about sex differences in the prevalence of stress-related disorders. For example, blunted sAA reactivity was recently associated with atopic diseases

(i.e. allergic rhinitis, asthma) in adult men and women (Hlavacova et al., 2017), and in children with asthma between 8-18 years old (Wolf et al., 2008). Thus, a repeated blunted adrenocortical response to acute stress in women could contribute to heightened susceptibility to some autoimmune diseases. In this regard, the low reactivity associated with the menstrual cycle phase could influence the manifestation and treatment of some disorders associated with women. In women, an increase in anxiety symptoms has been found to coincide with the peak in gonadal hormones (Kessler et al., 2005), and sex differences have been observed in depression (Ge et al., 2001). Therefore, this aspect should be taken into account in clinical practice because these hormonal changes during the menstrual cycle could correspond to changes in the presentation of psychiatric illnesses, thus affecting treatment success. Moreover, women in the follicular phase seem to show more psychological complaints than women in other phases of the menstrual cycle (Guillermo et al., 2010). Personality traits and stress coping styles are factors that could be measured in future studies. Understanding how the mechanisms involved in the stress response interact with each other will have important benefits for future interventions and help patients to improve their strategies for coping with challenging life events better than classical pharmacological approaches. From these results, some questions arise to investigate in future studies. The underlying mechanisms through which progesterone may moderate sex differences require further study. In addition, the impact of other sex-related hormones or factors that we did not measure should be considered, such as estrogen and progesterone levels to confirm the menstrual cycle phase evaluated, as well as allopregnollone.

Furthermore, men exhibited a higher LF/HF response to the TSST than the two groups of women, whereas the HR and RMSSD response to the TSST did not differ according to sex or menstrual cycle. These data suggest that sympathetic nervous system activity in response to the TSST appears to be more dominant in men than in women (both luteal and follicular).

These results partly match other studies using the TSST that did not find sex differences in the HR response (Kelly et al., 2008; Kirschbaum et al., 1999; Smeets, 2010), and they contrast with results obtained by other authors (Lustyk, Olson, Gerrish, Holder, & Widman, 2010) who found that women in the luteal phase were significantly more HR reactive than women in the follicular phase. However, these latest results did not consider men and, therefore, are not completely comparable to ours. Previous studies have shown that, in young adults, there is a predominance of sympathetic regulation in men and a dominant parasympathetic influence in women (Evans et al., 2001). Furthermore, stress tasks in women have generally reported a higher HF component, whereas men exhibited a higher LF/HF and LF component (Sato & Miyake, 2004). The reason for these differences is not clear. One could speculate that sex differences could be due to the type of stressor used, but Ordaz and Luna (2012) stated that any sex differences reflect the sex-specific sensitivity of the physiological domain rather than the type of stressor that elicited the response. Along these lines, one possible explanation for our findings would be that female reproductive hormones such as estrogen or progesterone might inhibit sympathetic activation. In some settings, estrogen has had an attenuating effect on sympathetic activity (Ryan et al., 1994), and during the luteal phase, higher estradiol concentrations were associated with lower cardiac output responses to a speech task (Sita & Miller, 1996). In addition, some differences have been found between women in the late follicular and early follicular phases, with higher HR in the late follicular group characterized by higher levels of estradiol (Pollard et al., 2007).

A limitation of this study could be that the design used was between-subjects instead of intra-subject. Despite criticism of the between-subjects design, we think this design could be one of the strong points of the present study. The inclusion of a control condition and strictly random assignment of the subjects to the experimental and control groups guaranteed that both groups were equivalent in the important dimensions. Therefore, we can assume that

the differences found in the two groups were solely due to the experimental manipulation, thus guaranteeing the internal validity of the study. Another limitation is the fact that the sex hormones were not measured. Although we used a rigorous method to estimate the menstrual cycle phase and check the ovulatory phase, further studies should include the concentrations of the women's sex hormones in each menstrual cycle phase.

Importantly, despite these possible limitations, the current study addresses several gaps in the literature on the menstrual cycle and stress reactivity. The simultaneous measurement of both autonomic and neuroendocrine reactivity in the same individuals advanced our current understanding of the autonomic mechanisms contributing to stress reactivity in women across the reproductive cycle. In addition, we confirmed that the sAA response to acute stress is sensitive to hormonal variations associated with the menstrual cycle phases evaluated. It is important to note that the stressful task used, the TSST, was able to induce a significant stress response. Therefore, we can confirm that it is a valid method to make possible sex differences emerge in the autonomic reactivity to acute stress in healthy young men and women. In addition, the TSST also increased our understanding of how women's hormonal status can modulate the autonomic reactivity to acute stress, which might be useful in future research.

Overall, these findings make an important contribution to the literature by clarifying the impact of sex and the menstrual cycle phase on ANS indicators of psychosocial stress reactivity. Moreover, they support the importance of understanding the underlying mechanisms that influence this association. Because men and women tend to react differently to stress, psychologically and biologically, these reactivity differences need to be considered in order to better understand some gender-related disorders and how they can be prevented and/or treated.

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Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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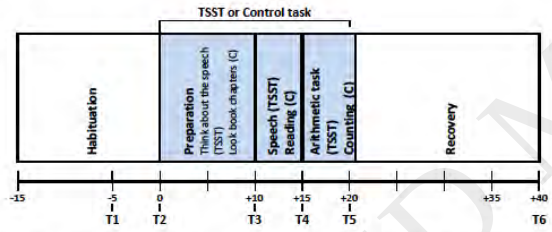
Figure captions

Fig. 1. Timeline of the TSST and control (C) conditions. Sequential sAA sampling (from T1 to T6). The Inventory of Situations and Responses of Anxiety (ISRA) was applied during the recovery period

Fig. 2. Estimated means for each moment of the TSST condition in the three groups for sAA (a), HR (b), RMSSD (c) and LF/HF (d) measures. Error bars represent standard errors of the mean (SEM).

Fig. 3. Means of LF/HF ratio in the TSST and Control conditions in the three groups analyzed. Error bars represent standard eror of the mean (SEM)

Fig. 4. Means of sAA AUCi in the TSST and control conditions in the three groups analyzed. Error bars represent standard error of the mean (SEM)



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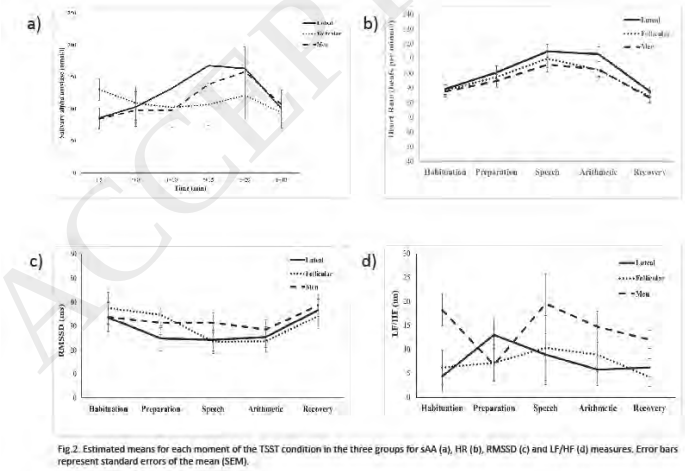

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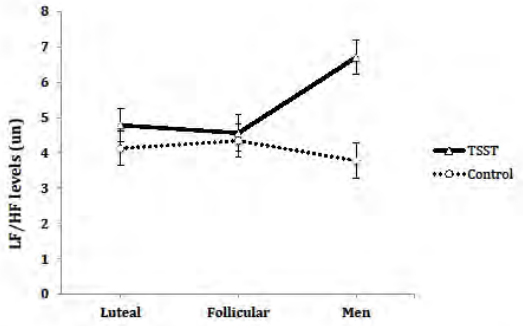

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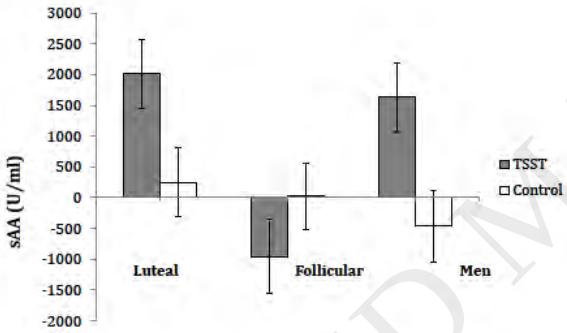

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Table captions

Table 1: One-way ANOVA for descriptive characteristics of age, body mass index (BMI), and scores on the subscales and total ISRA score. The values represent means and standard error of the mean (S.E.M.)

N=76	Groups	Mean	S.E.M	Ranges	ANOVA
Age (years)	Luteal	19.00	0.31	18-23	F= 1.50, p=0.22
	Follicular	18.80	0.27	18-24	
	Men	19.56	0.36	18-25	
BMI (kg/m^2)	Luteal	21.94	0.63	18.65-29.85	F= 5.56, p=0.006
	Follicular	20.81	0.57	16.14-28.96	
	Men	23.69	0.62	18.05-30.39	
ISRA Cognitive (direct scores)	Luteal	90.15	5.82	35-141	F= 2.55, p=0.08
	Follicular	84.48	6.64	23-169	
	Men	70.20	6.83	27-154	

ISRA Physiology (direct scores)	Luteal Follicular Men	56.55 50.20 39.22	6.52 6.93 6.19	12.50-128 10-160.50 6-111	F= 1.79, p=0.17
ISRA Motor (direct scores)	Luteal Follicular Men	56.84 57.44 48.12	7.33 6.71 6.24	7-142 5-141 6-108	F= 0.58, p=0.56
ISRA Total (direct scores)	Luteal	204.86	17.58	77.00-375.50	F= 2.03, p=0.13
	Follicular	192.52	18.45	48.00-434.50	
	Men	155.54	17.90	45.50-371.50	

Table 1: One-way ANOVA for descriptive characteristics of age, body mass index (BMI), and scores on the subscales and total ISRA score. The values represent means and standard error of the mean (S.E.M.)