



The influence of coping strategies and behavior on the physiological response to social stress in women: The role of age and menstrual cycle phase



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HIGHLIGHTS

- Age and hormonal status influence the psychophysiological stress response.
- Women in the luteal phase showed the highest psychophysiological reactivity to social stress.
- Post-menopausal women displayed a buffered stress response.
- Coping strategies influence the stress regulatory system depending on age and hormonal status.

ARTICLE INFO

Article history:

Received 3 July 2016

Received in revised form 8 December 2016

Accepted 8 December 2016

Available online 15 December 2016

Keywords:

Social stress

Coping styles

HPA axis

Heart rate

Menstrual cycle phase

Menopause

ABSTRACT

There is information indicating that the variations induced by the menstrual cycle may influence the capacity of young women to respond to stress. The physiological response to stress changes across the stages of the lifespan; however, in spite of the great increase in life expectancy, the way women react after menopause, a period characterized by a dramatic decline in sex hormones, has not been sufficiently studied. The main objective of the study was to examine the capacity to respond to and recover from an acute social stressor in post-menopausal women compared to young women. The second objective was to investigate the consequences of behavior on the self-regulatory systems. We measured behavior, cortisol, and heart rate during a speaking task in front of a committee in sixty-seven women: 36 post-menopausal and 31 pre-menopausal (follicular group $n = 14$; luteal group $n = 17$). No differences in heart rate reactivity between three groups were found. Post-menopausal women showed less cortisol reactivity to stress; they also displayed a higher percentage of Gestures during the speaking task, reflecting a clearer pattern of active coping compared to the young women. In post-menopausal women, behaviors that reflect active coping strategies were related to better autonomic regulation. By contrast, in pre-menopausal women, cortisol changes seemed to be modulated by passive and reactive behaviors such as Submission and Assertion. These results emphasize the importance of considering age and Hormonal Status in coping processes, including reactivity and recovery from stressful situations.

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1. Introduction

The main stress response systems involve physiological responses of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA). Both systems have been studied exhaustively, although differences in the regulation of the physiological stress response

due to changes in reproductive hormones are not yet clear. In women, hormonal variations induced by the menstrual cycle and menopause may modulate the changes observed with aging [1].

Regarding the effects of changes in reproductive hormones due to menopause, few studies have focused on age differences in women considering the physiological stress response, and contradictory results have been reported. After menopause, the physiological stress response to social stress does not seem to differ from that of younger women in terms of free salivary cortisol [2,3] or heart rate [2]. However, other studies have found greater plasma cortisol increases in response to a

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mental challenge in older women [4] and diminished heart rate reactivity to social stress with age [1,4,5]. Although no age differences have been found in salivary cortisol in response to social stress in women [6,7], as women age, there appears to be a worse HPA axis functioning, which can lead to a prolonged recovery period after acute stress [8]. These findings agree with the concept of an HPA negative feedback dysregulation in the older population [9] that is more prominent in women [10]. This diminished capacity to recover could be associated with lower levels of estrogen in women, which may be associated with an increased risk due to the protective properties of these sex steroids [11].

So far, most of the research focused on young women shows greater HPA axis reactivity (salivary cortisol) in response to social stress in the luteal phase than in other menstrual cycle phases or compared to women taking oral contraceptives [12,1,13,14]. Moreover, no effects of the menstrual cycle phase have been found on heart rate and blood pressure reactivity [15,13] or heart rate variability [16] changes in response to social stress. As a result, HPA axis activity may be more sensitive to changes in sex hormones than the autonomic nervous system. In addition, women's Hormonal Status seems to have an impact on psychological perceptions, although the results have been mixed. Whereas some studies have found that women in the luteal phase show higher negative feelings in response to social stress [17,18], other studies show higher scores on anxiety and anger-hostility in women in the early follicular phase compared to women in the middle luteal phase [14]. This worsening in mood in both groups suggests that women in the (early) follicular phase could be suffering from the discomfort of menstruation symptoms, whereas women in the luteal phase could be experiencing premenstrual syndrome symptoms. Taken together, these findings highlight the relevance of the menstrual cycle phase in the physiological stress response, as well as its interactions with psychological states.

Behavior could be considered an output of the stress response processes that is closely related to coping strategies, which in turn will interact with the stress response. In a previous study from our laboratory [19], focused on behavior and the psychophysiological stress response, we found that women in their early follicular phase were more sensitive to stress, displaying slightly more Flight, Submission, and Displacement behaviors and a significantly larger percentage of assertive behaviors (low-aggressiveness), compared to OC users. In this study, we found that passive coping styles (e.g. Flight) were related to negative psychological states (anxiety and mood), and submissive behaviors were related to lower basal cortisol levels. Previous research on this topic found some relationships between behaviors displayed during a speaking task and physiological changes, suggesting that submissive behaviors were related to lower autonomic activation [20]. In women, displacement behaviors during social stress have been found to reduce heart rate on recovery, whereas the patterns included in the Submission category produced the opposite effect [16]. However, behavior as a modulator of the self-regulation systems has not yet been studied in the older population.

In addition, the role of coping styles should also be taken into account because they contribute to a more comprehensive understanding of the psychological and neuroendocrine responses to social stress. As Salvador et al. [19] pointed out, active coping has been associated with an optimal activation of the autonomic nervous system (ANS) and cortisol release, whereas passive coping is characterized by an inefficient autonomic and cortisol response [21]. Hence, a different psychobiological stress response pattern could be associated with distinct ways of coping with social stress, emphasizing the importance of individual differences.

Considering the aforementioned studies, our purpose was to examine coping styles and behavior in post-menopausal women dealing with an acute social stressor and compare them to pre-menopausal women, taking into consideration different phases of the menstrual cycle (follicular and luteal). To do so, we aimed to analyze the psychophysiological stress response, measured by salivary cortisol and heart rate, and the

mood experienced. The second objective was to investigate the role of age and Hormonal Status in pre-menopausal women in the behavioral patterns displayed during the speaking task of the Trier Social Stress Test (TSST). And finally, we aimed to explore how certain social behaviors and coping styles measured by self-reports may influence the neuroendocrine capacity to react to and recover from social stress differently depending on age.

Based on previous studies, we expected to find a greater cortisol response in young women, especially in women in the luteal phase [12,13,3], and lower HPA regulation in post-menopausal women [10], that is, less capacity to recover after the stressor. We do not expect to find heart rate differences in response to social stress between the two groups of young women [13,15], but we do expect a lower heart rate response and recovery capacity in post-menopausal women due to the impact of age on the autonomic response [1,4]. We hypothesized that acute physiological stress changes would be modulated by the behavioral response patterns exhibited during the speech task of the TSST. More specifically, we anticipated that, in young women, the intensity of the cardiac and cortisol changes (AUCi) would be associated with the number of submissive and escape behaviors, which are commonly associated with a passive coping style [20,21]. Moreover, given that post-menopausal women have not been studied from an ethological perspective, we aimed to explore the relationships between behavior, coping styles (as detected via the evaluation of self-reports), and physiological changes (AUCi and recovery indices) related to stress exposure in post-menopausal women compared to young women.

2. Methods

2.1. Participants

The sample was composed of 67 women: 36 post-menopausal women between 56 and 73 years old (means \pm sem: age = 63.86 ± 0.69 ; Body Mass Index (BMI) = 25.27 ± 0.53) and 31 young women between 18 and 23 years old (mean \pm sem: age = 19.07 ± 0.27 ; BMI = 21.35 ± 0.72) divided into two groups: 17 in the luteal phase (4th to 8th day before the onset of the new menstrual cycle) and 13 in the follicular phase (5th to 8th day after the onset of the new menstrual cycle). For women in the luteal phase, the mean age was 19.11 ± 0.44 , and the mean BMI was: 22.14 ± 0.97 . For women in the follicular phase, the mean age was 18.23 ± 0.17 , and the mean BMI was 20.32 ± 1.04 . The menstrual cycle phase was calculated using two estimation procedures [12,22]. 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 [23]. 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 in the morning. To analyze the BBT, the "smoothed curve" method (SMC) was used, as described by McCarthy and Rockette [24,25]. All the post-menopausal women had had their last menstrual period >2 years before the testing time, and none of these women had received estrogen replacement therapy. Most of the young participants (94%) were college students from different fields (psychology, medicine, occupational sciences, among others). The post-menopausal participants belonged to a study program at the University of Valencia for people over 55 years of age, and none of the participants received academic or economic compensation for their participation.

The exclusion criteria were: smoking >5 cigarettes a day, alcohol or other drug abuse, visual or hearing problems, presence of cardiovascular, endocrine, neurological or psychiatric disease, presence of a stressful life event during the past year (the presence or not of any important event in the past year that they considered stressful and that changed their significantly: e.g. widowhood, winning the lottery), and use of medication related to cognitive, emotional or endocrine function, such

as glucocorticoids, β -blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, or psychotropic substances. In addition, the young women had to be nulliparous, with no gynecological problems, no diagnosis severe premenstrual syndrome or dysmenorrhea symptoms, and regular menstrual cycles (24–36 days). Participants meeting the criteria were contacted by telephone and asked to attend experimental sessions that took place in a laboratory at the Faculty of Psychology (University of Valencia, Spain). 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 to only drink water, and not eat or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, 2 h prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the University of Valencia Ethics Research Committee. All the participants received verbal and written information about the study and signed an informed consent form.

2.2. Study protocol

Participants arrived at the laboratory, and the experimenter checked whether they had followed the instructions given previously. All experimental sessions were run between 3 pm and 6 pm, when basal cortisol levels are low and stable. Then, participants' weight and height were measured.

To produce a stress response, participants were subjected to the Trier Social Stress Test (TSST). The stress task consisted of 5 min of free speech and a 5 min arithmetic task (see for more details [26]), which were performed in front of a committee composed of a man and a woman. Additionally, a video camera and a microphone were clearly visible, and the speech task was video recorded. During the speech, participants had to convince the committee that they were the best applicants for a vacant position by focusing on their personal characteristics (for young women: a job directly related to their studies; for older women: becoming a representative of the students at their university). The participants remained standing at a distance of 1.5 m from the committee. Furthermore, it was announced that the participant's performance was to be recorded on a video-cassette-recorder in order to later analyze the interview and nonverbal behavior. If the participant finished her speech in <5 min, the members of the committee asked standardized questions.

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. Five minutes after this phase started, baseline measures were obtained for cortisol (–20 min pre-stress), anxiety (Stai-S) and mood (PANAS). After the habituation phase, the introduction phase started (duration 3 min). In this phase, participants were informed about the procedure for the stress task. They received the instructions in front of the committee in the same room where the task took place. Next, the participants had 10 min to prepare for the task in the first room. During this period, they provided a saliva sample (–5 min pre-stress).

Following the preparation phase, the stress test was carried out. Subjects had 20 min to recover after the 10-min stress task. During this period, they again answered the two questionnaires (Stai-S and PANAS) and provided the second saliva sample (+15 min post-stress). The participants provided the last saliva sample 25 min later (+40 min post-stress). In the last part of the session, all the participants completed the coping strategies questionnaire [27] (see Fig. 1).

2.3. Biochemical analyses

2.3.1. Cortisol

Participants provided four saliva samples of 3 mL each in plastic vials. They took approximately 5 min to fill the vial. The samples were frozen at –80 °C until the analyses were performed. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Coat-A-Count C (DPC, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Assay sensitivity was 0.5 ng/mL. The findings are expressed in nanomolar units (nmol/L). For each participant, all the samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%.

2.4. Cardiac measurements

2.4.1. Heart rate

HR was continuously recorded using a Polar®RS800cx watch (Polar CIC, USA), which consists of a chest belt and a Polar watch. The transmitter is located on the chest belt, which is placed on the solar plexus and transmits heart rate information to the receiver (Polar watch). The Polar watch records R–R intervals with a sampling frequency of 1000 Hz, providing a time resolution of 1 ms for each R–R interval. The data collected by the Polar watch were downloaded and stored in the Polar ProTrainer5™ program in the computer, and they were

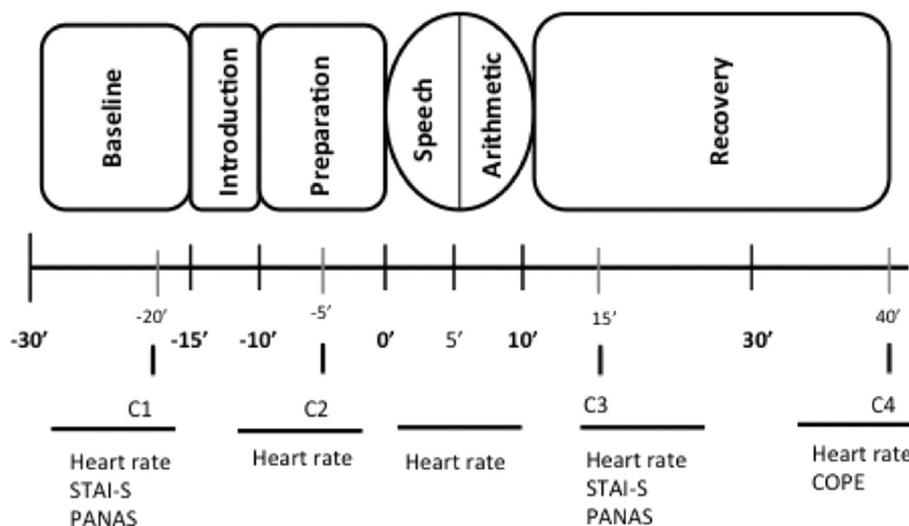


Fig. 1. Timeline of the experimental condition. Sequential salivary cortisol sampling (C1 to C4). The heart rate values were calculated using the central 5 min of each period. State anxiety inventory form S (STAI-S), positive and negative affect (PANAS). Coping strategies (COPE).

analyzed using Kubios Analysis (Biomedical Signal Analysis Group, University of Kuopio, Finland). The recording periods when participants were walking from one room to another were removed, and only the 5 central minutes of each phase (namely from –20 to –15 min for baseline, from –6 to –1 min for preparation, from 0 to +5 for speech, from +5 to +10 for arithmetic, and from +15 to +20 for recovery) were used to calculate the participants' average heart rate values. After eliminating the artifacts, the HR mean was computed for each phase. HR artifacts and HR analysis were performed with Kubios software (Biomedical Signal Analysis Group, University of Kuopio, Finland).

2.5. Psychological assessment

2.5.1. Coping strategies

The dispositional version of the COPE Inventory is a theoretically driven self-report questionnaire that addresses different ways of coping [27]. Subjects must indicate what they *generally* do and feel when experiencing stress. Items are rated on a 4-point scale, ranging from 1 (*I don't usually do this at all*) to 4 (*I usually do this a lot*). We employed the Spanish version of the long form, which consists of 60 items from 15 subscales (such as Planning, Seeking Instrumental Support, Suppression of Competing Activities, Restraint Coping, and Venting of Emotions, among others). With a second-order factor analysis, they can be grouped in four basic coping domains: active coping, cognitive and emotional coping measures (active coping strategies), and Avoidance (passive coping strategies) [27]. The Spanish version of the scale had Cronbach's alphas ranging from 0.78 to 0.92 [28].

2.5.2. Anxiety

To assess state anxiety, the Spanish version of the State Anxiety Inventory was used (STAI form E) [29]. It consists of 20 phrases (e.g. '*I feel at ease*', '*I feel upset*'), with a 4-point Likert scale ranging from 0 (not at all) to 3 (extremely) to evaluate how participants felt at the moment they gave their answers. The Spanish version of the scale had Cronbach's alphas ranging from 0.90 to 0.93 [30].

2.5.3. Mood

Mood was evaluated by the Spanish version [31] of the PANAS (Positive and Negative Affect Schedule) [32]. This 20-item questionnaire assesses mood divided into two dimensions: positive affect (PA: *interested, excited, strong, enthusiastic*, etc.) and negative affect (NA: *distressed, upset, guilty, scared*, etc.), with 10 items measuring each state. Participants were asked to complete the questionnaire based on how they felt at that particular moment. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). Sandin et al. (1999) [31] reported a high internal consistency for the Spanish version, with Cronbach's alphas for PA ranging from 0.87 to 0.89, and for NA from 0.89 to 0.91.

2.6. Ethological analysis

2.6.1. ECSI

Participants' behavior during the TSST speech task was quantified by means of the Ethological Coding System for Interviews (ECSI) [33]. The interview was videotaped with a camera adjusted so that the subject's face and trunk were in full view. Subsequently, behavioral assessment was carried out according to Troisi and colleagues [16,33,20]. This version of the ECSI includes 32 different patterns, mostly facial expressions and hand movements. The ECSI was specifically designed to measure non-verbal behavior during stress interviews by combining behavior patterns described in published human ethograms [33]. The 32 behavioral patterns were then grouped in seven behavioral categories, each reflecting a different aspect of the subject's emotional and social attitude [35], namely: (1) Eye contact; (2) Affiliation; (3) Submission; (4) Flight; (5) Assertion; (6) Gesture; (7) Displacement. The score for a given

behavioral category was expressed as the sum of the percentages of all the behavioral patterns belonging to it [33].

2.7. Statistical analyses

One-way ANOVAs were used to analyze differences between groups on the demographic/anthropometric variables, coping strategies, heart rate reactivity (AUCi) and behavioral patterns. We employed Age (post-menopausal women vs. Pre-menopausal women) or Hormonal Status (Post-Menopausal women, Luteal women, and Follicular women) as between-subject factors. Mixed ANOVAs for repeated measures were used to assess the effects of stress on mood, anxiety, heart rate, and cortisol. We added Period (mood and anxiety: pre and post task; Cortisol: Baseline, Preparation, Speech and Recovery; HR: Baseline, Preparation, Speech, Arithmetic and Recovery) as a within-subject factor.

Pearson's correlations were calculated to assess whether the physiological changes were related to the behavioral patterns. To do this, we calculated the cortisol and stress-induced HR changes over time quantified as the area under the curve with respect to the increase (AUC_i) [34]. In addition, in order to obtain a physiological index of recovery, we calculated cortisol and heart rate Recovery indices as the difference between the mean speech values and the recovery phase values. This index indicates the decline in cortisol and HR from stress to the recovery phase, where lower values indicate a greater capacity to recover.

Stepwise regression analyses were conducted to examine whether coping styles (COPE) predict changes (AUCi and recovery indices) in cortisol and heart rate in each group. In stepwise regression, the independent variables are entered into the regression one at a time until they have all been added, with the provision that each meets the specified criterion. For this study, the criterion used was a significance level of $p < 0.100$ [35]. To avoid the problem of multicollinearity of independent variables, in the stepwise approach, all the variables are reexamined after the addition of the other variables, in order to verify that each is still a significant and independent predictor.

Five participants were excluded from the analyses: 1 post-menopausal and 2 luteal women for the cortisol data and 1 post-menopausal woman for the HR because their levels differed by >3 S.D. from the sample mean, and 1 woman in the follicular phase due to technical problems in the analysis of the videotape and missing data in the physiological measures.

We used Greenhouse-Geisser when the requirement of sphericity in the ANOVA for repeated measures was violated, and the results were reported along with unadjusted degrees of freedom, adjusted p -values, and partial eta squared. Post hoc planned comparisons were performed using the Bonferroni adjustments for the p -values. All the p -values reported are two-tailed, and the level of significance was set at <0.05 . When not otherwise specified, results shown are means \pm standard error of means (SEM). We used SPSS 22.0 to perform the statistical analyses.

3. Results

We analyzed the stress response in each age group (menopausal women vs. pre-menopausal women). Post-menopausal women showed lower levels of cortisol and heart rate than young women in all the periods analyzed (all $p < 0.029$). ANOVA of repeated measures indicated that only older women did not show significant increases from Baseline to the Speech Period ($p > 0.1$). However, all the groups showed a significant cardiac response, that is, higher HR in the Speech Period compared to Baseline (all $p < 0.05$). Moreover, post-menopausal women were characterized by lower scores on anxiety and negative mood, and they scored higher on positive mood than young women (all $p < 0.05$). Finally, post-menopausal women displayed larger percentages of Gestures, whereas young women displayed larger

percentages of Submissive and Displacement behaviors (all $p \leq 0.006$), reflecting a more passive coping style compared to menopausal women.

To obtain a better overview of the results, we performed the analyses focusing on the Hormonal Status of the women (luteal vs. follicular vs. post-menopausal women). Figures show the results by age and Hormonal Status group.

3.1. Cortisol

ANOVAs for repeated measures showed significant effects of Period, $F(3,180) = 29.371, p \leq 0.001, \eta^2_p = 0.329$, Hormonal Status, $F(2,60) = 14.869, p \leq 0.001, \eta^2_p = 0.331$, and the Hormonal Status \times Period interaction, $F(6,180) = 8.427, p \leq 0.001, \eta^2_p = 0.219$. Post-hoc analyses revealed that post-menopausal women show, in general, the lowest cortisol levels; these differences were significant compared to the two groups of young women at Baseline (both $p \leq 0.021$) and in the Preparation Period (both $p \leq 0.015$); no differences in these phases were found between the young groups (all $p > 0.1$). After the stress period, the luteal group showed the highest cortisol response, compared to the follicular and post-menopausal groups ($p = 0.007$; $p \leq 0.001$, respectively); and in the recovery period, the post-menopausal group showed the lowest levels of cortisol, but these differences were significant only in comparison to the luteal group ($p = 0.004$) (see Fig. 1). When comparisons were made within each group, although the three groups showed increases >2.5 nmol/L, which reflect a cortisol secretory episode [36,37], only the luteal group showed statistically significant increases from Baseline to the Speech Period ($p \leq 0.001$); the luteal and post-menopausal groups did not return to their baseline levels, but the difference only reached significance in the luteal group ($p = 0.002$; $p = 0.09$, for the post-menopausal group). (for post-menopausal group ($N = 35$) at baseline: 3.31 ± 0.49 , preparation: 2.97 ± 0.65 , post task: 6.05 ± 1.45 , recovery: 5.32 ± 1.02 ; for Pre-menopausal group ($N = 28$) at baseline: 6.67 ± 0.565 , preparation: 6.87 ± 0.73 , post task: 15.87 ± 1.63 , recovery: 9.26 ± 1.14 ; for follicular group ($N = 13$) at baseline: 6.7 ± 0.83 , preparation: 7.32 ± 1.08 , post task: 10.72 ± 2.23 , recovery: 6.77 ± 1.63 ; for luteal group ($N = 15$) at baseline: 6.65 ± 0.77 , preparation: 6.47 ± 1.01 , post task: 20.34 ± 2.07 , recovery: 11.41 ± 1.52) (Fig. 2).

3.2. Heart rate (HR)

ANOVA for repeated measures revealed significant effects of the Period, $F(4,248) = 110.87, p \leq 0.001, \eta^2_p = 0.641$, Hormonal Status,

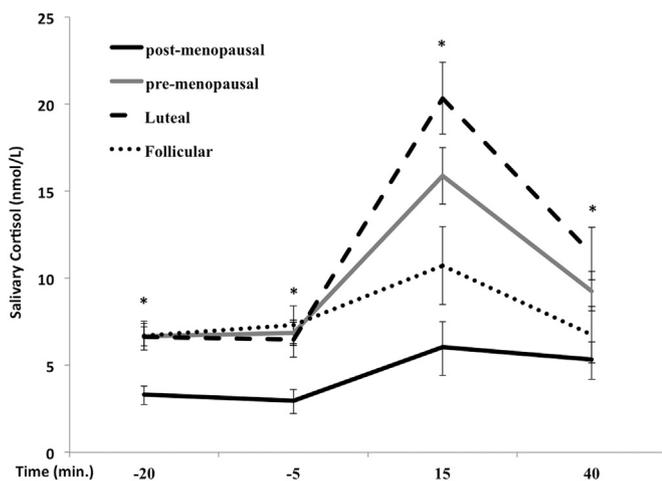


Fig. 2. Mean values \pm SEM of salivary cortisol response (nmol/L) by age and hormonal status group in each period analyzed. Post-menopausal women showed lower levels of cortisol compared to luteal group in all periods (*all $p \leq 0.021$). The luteal group showed the highest cortisol levels after the stress period (+15 min.) compared to Follicular and Post-menopausal groups (*both $p \leq 0.007$).

$F(2,62) = 8.994, p \leq 0.001, \eta^2_p = 0.225$, and the Hormonal Status \times Period interaction, $F(8,248) = 4.147, p = 0.002, \eta^2_p = 0.118$. Post-menopausal women showed lower HR values than the two groups of young women at Baseline (both $p \leq 0.05$) and in the Preparation period (with Luteal: $p = 0.003$; with Follicular: $p = 0.065$). In the Speech and Arithmetic periods, differences were significant only between the post-menopausal and luteal groups (both $p \leq 0.004$). No differences were found among the three groups in the Recovery period (all $p > 0.1$). No significant differences were observed between the young groups in any period analyzed (all $p \geq 0.41$). Post-hoc comparisons within each group revealed that all the groups increased their HR significantly from baseline to stress (speech and arithmetic) (all $p \leq 0.002$) and recovered to their baseline levels (all $p \geq 0.1$). (for post-menopausal group ($N = 35$) at baseline: 76.94 ± 1.93 , preparation: 86.15 ± 2.25 , speech: 97.07 ± 2.89 , arithmetic: 89.65 ± 2.65 , recovery: 79.49 ± 1.98 ; for pre-menopausal group ($N = 28$) at baseline: 90.7 ± 2.07 , preparation: 98.27 ± 2.42 , speech: 111.43 ± 3.12 , arithmetic: 108.1 ± 2.88 , recovery: 85.97 ± 2.12 ; for follicular group ($N = 13$) at baseline: 90.54 ± 3.17 , preparation: 96.46 ± 3.69 , speech: 107.92 ± 4.74 , arithmetic: 103.23 ± 4.34 , recovery: 84.85 ± 3.24 ; for luteal group ($N = 15$) at baseline: 90.82 ± 2.77 , preparation: 99.65 ± 3.23 , speech: 114.12 ± 4.15 , arithmetic: 111.82 ± 3.8 , recovery: 86.82 ± 2.84) (see Fig. 3). In addition, no significant differences in HR reactivity (AUCi) were observed among the three groups, $F(2,64) = 0.914, p = 0.406$.

3.3. State anxiety

A repeated-measures ANOVA of the STAI scores revealed main effects of Period, $F(1, 63) = 64.551, p \leq 0.001, \eta^2_p = 0.506$, Hormonal Status $F(2, 63) = 12.732, p \leq 0.001, \eta^2_p = 0.288$, and marginally, the Period \times Hormonal Status interaction, $F(2, 63) = 2.964, p = 0.059, \eta^2_p = 0.086$. The three groups increased their subjective anxiety scores after the stress (all $p \leq 0.001$). The post-menopausal group scored lower on anxiety before and after stress than the luteal group (all $p \leq 0.002$). No other significant differences were found between groups (all $p > 0.07$). (For post-menopausal ($N = 36$) group before task: 12.28 ± 1.01 , after task: 18.42 ± 1.50 ; for luteal group ($N = 17$) before task: 18.65 ± 1.47 , after task: 30.71 ± 2.19 ; for follicular group ($N = 13$) before task: 16.23 ± 1.69 , after task: 25.15 ± 2.50) (see Fig. 4).

3.4. Mood

For positive mood, the ANOVA revealed significant effects of Period, $F(1, 63) = 37.498, p \leq 0.001, \eta^2_p = 0.2373$, Hormonal Status, $F(2, 63) = 9.984, p \leq 0.001, \eta^2_p = 0.241$, and the Period \times Hormonal Status interaction, $F(2, 63) = 5.400, p = 0.007, \eta^2_p = 0.156$. All the groups decreased their positive mood after the stress task (all $p \leq 0.018$). The post-menopausal group showed higher positive mood scores than luteal women before the task ($p = 0.013$). After the stress task, post-menopausal women scored significantly higher on positive mood only compared to luteal women ($p \leq 0.001$) and, as a trend, follicular women (all $p = 0.054$). (For post-menopausal group ($N = 36$) before task: 31.167 ± 0.90 , after task: 29.46 ± 0.99 ; for luteal group ($N = 17$) before task: 26.47 ± 1.31 , after task: 20.71 ± 1.44 ; for follicular group ($N = 13$) before task: 27.69 ± 1.50 , after task: 24.77 ± 1.66) (see Fig. 5).

For negative mood, significant effects of Period, $F(1, 63) = 33.420, p \leq 0.001, \eta^2_p = 0.4347$ Hormonal Status, $F(2, 63) = 8.683, p \leq 0.001, \eta^2_p = 0.216$ and the Period \times Hormonal Status interaction were found, $F(2, 63) = 3.507, p = 0.036, \eta^2_p = 0.10$. All the groups increased their negative mood after the stress task, but these increases were only significant for the luteal and post-menopausal groups (both $p = 0.001$). The follicular group did not reach significance ($p = 0.096$). Before stress, post-menopausal women scored lower on negative mood than the young groups (both $p = 0.032$). In addition, negative mood scores after stress were lower in the post-menopausal group than in the luteal group ($p = 0.032$). No other differences were found between groups

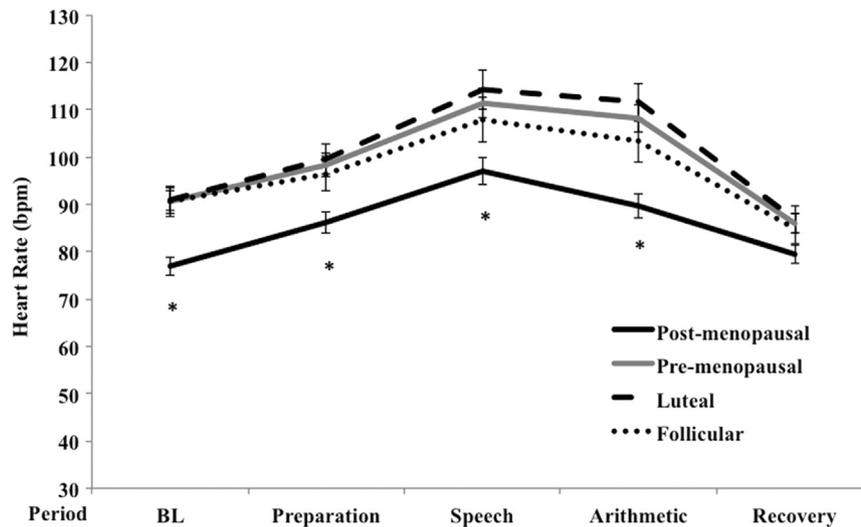


Fig. 3. Means \pm SEM of heart rate response by age and hormonal status group in each period analyzed. Post-menopausal women showed the lowest levels of heart rate at each phase analyzed compared to luteal group (*all $p \leq 0.05$).

(all $p \geq 0.21$). (for post-menopausal group ($N = 36$) before task: 12.47 ± 0.75 , after task: 15.51 ± 1.02 ; for luteal group ($N = 17$) before task: 15.94 ± 1.09 , after task: 22.71 ± 1.48 ; for follicular group ($N = 13$) before task: 16.69 ± 1.24 , after task: 19.15 ± 1.69) (Fig. 6).

3.5. Ethological data (ECSI)

Table 1 summarizes the behavioral response exhibited by the three groups of women during the speech task, with each behavioral category quantified as cumulative percentages. Statistically significant differences between groups were found on: Submission, $F(2,65) = 9.005$, $p \leq 0.001$; Gesture $F(2,65) = 4.760$, $p = 0.012$; and Displacement $F(2,65) = 8.634$, $p = 0.001$. Data revealed that post-menopausal women, compared to both groups of young women, showed lower percentages of Submissive behaviors (Luteal: $p = 0.039$, Follicular: $p = 0.001$) and Displacement behaviors (Luteal: $p = 0.012$, Follicular: $p = 0.002$). Finally, post-menopausal women showed significantly higher percentages of Gestures during the speaking task than follicular

women ($p = 0.014$). No other differences were found between groups (all $p > 0.1$).

3.6. Relationships between behavioral categories and physiological changes

Given that each of the three groups has a different hormonal status, correlation analyses were performed by group in order to provide new insights on neuroendocrine and behavioral stress responsivity associations.

The number of submissive behaviors displayed during the speaking task was positively related to the Cortisol AUCi, and negatively related to the Cortisol Recovery index ($r = 0.632$, $p = 0.011$; $r = -0.607$, $p = 0.016$, respectively) in the luteal group. However, in the follicular group, the expression of behavioral patterns included in the Assertion category (low-aggressiveness) was negatively related to the Cortisol Recovery index ($r = -0.615$, $p = 0.025$). No relationships between behavior and HR indexes were found (all $p > 0.1$).

In the post-menopausal group, the number of Affiliative behaviors displayed during the speaking task was negatively related to the HR

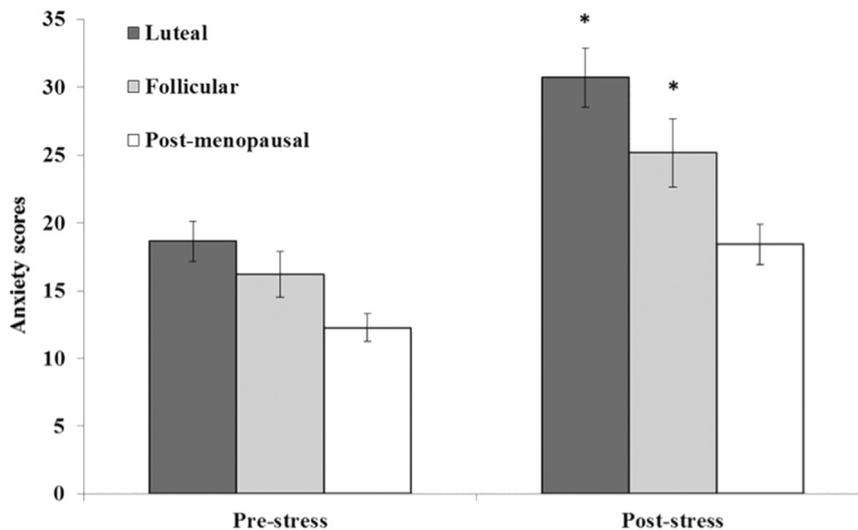


Fig. 4. Means \pm SEM of anxiety scores (STAI-S) by hormonal status group (* $p < 0.05$).

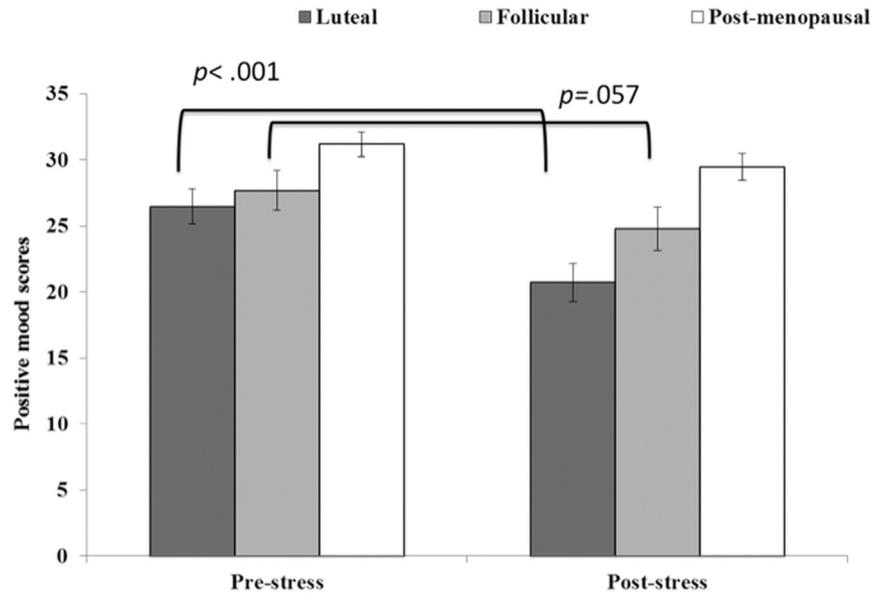


Fig. 5. Means \pm SEM of positive affect scores (PANAS) by hormonal status group.

Recovery index ($r = -0.355, p = 0.036$) and, as a trend, to the total amount of Eye Contact ($r = -0.302, p = 0.065$). The more these patterns of nonverbal behavior were used during the acute psychosocial challenge, the lower the HR recovery index was; that is, there was a better cardiac recovery after menopause.

3.7. Coping styles (COPE)

The analyses revealed significant differences between groups on two of the four basic coping styles, Active Coping: $F(2,65) = 13.399, p \leq 0.001$, and Emotional Coping $F(2,65) = 6.344, p = 0.003$. Post-menopausal women showed higher scores on Active coping than both groups of young women (both $p \leq 0.001$), and lower scores on Emotional coping, but these differences were statistically significant only compared to the luteal group ($p = 0.005$) and, as a trend, the follicular group ($p = 0.072$). As a tendency, group differences were found in the Avoidance factor: $F(2,65) = 2.853, p = 0.065$, post-menopausal women showed higher scores than luteal women ($p = 0.061$), but similar scores to the follicular group ($p = 1$). No differences were found in Cognitive coping between groups: $F(2,65) = 1.09, p = 0.342$, and no

differences were found between the young groups in any COPE factor analyzed (all $p \geq 0.456$).

3.8. Relationships between coping strategies (COPE) and physiological changes (AUCi and recovery indices of cortisol and HR)

To test whether there were any associations between coping styles and changes in stress reactivity (reactivity and recovery of Cortisol and Heart rate), stepwise multiple regression analyses were conducted. In the first step of the regression analyses, we included BMI as a covariate. In the second step, the four coping styles from the COPE questionnaire were introduced (behavioral coping, cognitive coping, emotional coping, and avoidance) as possible predictors in four separate models, the first predicting the cortisol AUCi, the second predicting the heart rate AUCi, the third predicting the cortisol Recovery index, and the fourth predicting the heart rate Recovery index. We performed separate analyses for each Hormone Group.

In the Luteal and follicular groups, the four coping strategies did not predict physiological increases (Cortisol and HR AUCi) or cardiac and HPA axis recovery capacity (all $p > 0.1$). When stepwise regression

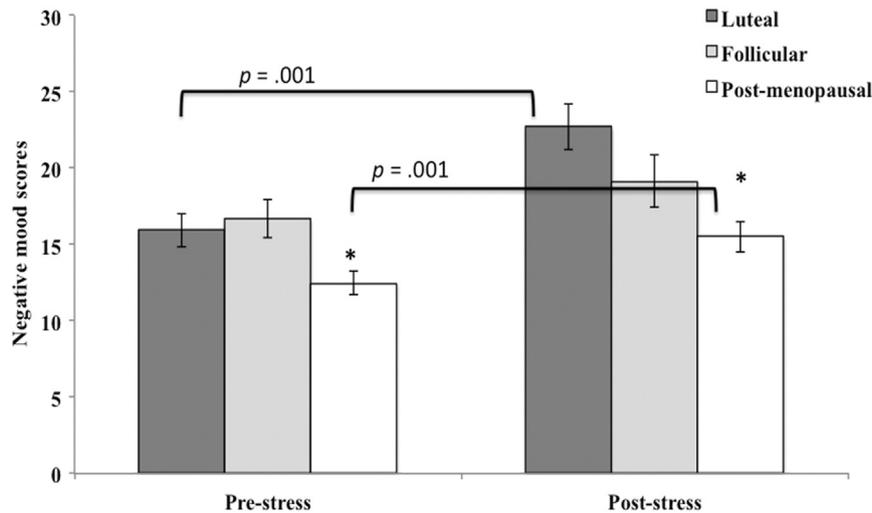


Fig. 6. Means \pm SEM of negative affect scores (PANAS) by hormonal status group (* $p < 0.05$).

Table 1

Total sum of the behavioral pattern values for each behavioral category (% mean \pm SEM) displayed during speech (ECSI). In bold are the categories with significant differences between groups (*all $p < 0.05$). No significant differences between young groups were found. *Each significant difference shown in the table is between Post-menopausal women and the two groups of young women.

Ethological data	Luteal (n = 17)	Follicular (n = 13)	Post-menopausal (n = 36)
Eye contact	97,94 \pm 1,21	92,69 \pm 3,28	97,5 \pm 0,98
Affiliation	44,12 \pm 6,79	42,31 \pm 9,33	56,53 \pm 6,36
Submission	12,35 \pm 2,39	17,31 \pm 3,56	5,42 \pm 1,20*
Flight	129,11 \pm 3,84	131,54 \pm 4,36	124,31 \pm 4,36
Assertion	10,29 \pm 2,89	6,54 \pm 3,01	15,14 \pm 2,84
Gesture	25 \pm 6,81	11,15 \pm 4,35	41,81 \pm 6,30*
Displacement	63,23 \pm 7,04	73,46 \pm 12,31	33,05 \pm 5,43*

analyses were performed for the young groups as a whole, none of the four coping styles predicted the physiological changes (all $p > 0.1$).

However, higher scores on the Avoidance factor predicted poorer recovery of Cortisol levels in post-menopausal women: $F(1,32) = 9.769$, $p = 0.004$, $\beta = 0.485$, accounted for 23.3% of its variance. A significant predictive model was also found for the HR AUCi, as higher scores on Active coping and lower scores on Avoidance factors predicted greater increases in heart rate ($\beta = 0.704$, $p \leq 0.001$; $\beta = -0.392$, $p = 0.011$). Together, they accounted for 12.8% of its variance, $F(1,31) = 7.259$, $p = 0.011$. These associations reveal the positive influence of active coping, rather than avoidance strategies, on the physiological regulatory functions in situations of stress. No other associations were found among the coping styles, Cortisol AUCi, and heart rate Recovery index (all $p > 0.1$).

4. Discussion

The current study examined the psychophysiological and behavioral stress response in women while performing the speaking task of the Trier Social Stress Test (TSST) [26]. This study provides new information about how behavior and coping styles measured by self-report may affect the stress regulatory system in different ways depending on age and hormonal status.

Our results confirmed that the speaking task of the TSST provokes a significant stress response in heart rate, anxiety, and mood in all women. By contrast, the cortisol stress response did not reach significance in post-menopausal and follicular women. Although overall the three groups showed increases above 2.5 nmol/L, which is considered to be a significant stress response [26,36,37], when checking the number of responders in each group (2.5 nmol/L as a cut off), we observed that almost all the women in the luteal phase were responders (14 responders, 3 non-responders). However, only about 50% of follicular women and even fewer post-menopausal women increased their cortisol levels >2.5 nmol/L (in follicular: 6 responders, 7 non-responders; in post-menopausal: 13 responders, 22 non-responders). Examining the cortisol profile more in-depth, we observed a different response pattern across the three groups. As expected, women in the luteal phase showed higher levels of cortisol after the stressor than women in the follicular phase and post-menopausal women. The blunted cortisol response in post-menopausal and follicular women compared to luteal women agrees with previous findings focused on the effect of sex hormones on the stress response in young women [12,26,38] and compared to post-menopausal women [4,39]. As documented in a recent study by Gordon and Girdler [15], these results are in accordance with the quantity of estradiol and progesterone in each group, where progesterone seems to increase HPA stress responsivity. Therefore, the assumed lower levels of estradiol and progesterone in post-menopausal women and women in the follicular phase coincide with the lower cortisol response in these groups. However, focusing on the recovery phase, we found that only women in the follicular phase returned to baseline

levels after the stress period. The fact that women in the luteal phase did not return to their baseline levels after stress could be due to their higher increases; hence, they would need more time (more than 40 min after the stressor onset) to recover. However, in post-menopausal women, this poor recovery could be due to HPA axis negative feedback dysregulation, due to their loss of reproductive hormones [9,10]. In addition, we have to take into account that this group is composed of mixed responders and non-responders. Further research on this point is needed.

Regarding cardiac activity, based on previous literature that failed to find associations between the phases of the menstrual cycle phase and the heart rate response [13,15,16,41], we hypothesized that there would be no significant differences between the young groups in response to stress. Our results confirmed this hypothesis. Moreover, we observed that the three groups (young and post-menopausal) recovered until reaching basal levels. These findings coincide with the aforementioned literature; however, other authors suggest a higher adrenocortical response in the luteal phase [1,17]. In addition, we found that post-menopausal women showed the lowest heart rate values during the stressor, but this difference was only significant compared to women in the luteal phase. In general, post-menopausal women showed lower cardiac activity during all the phases, except the recovery phase, but no significant age or hormonal status differences in response to stress (AUCi of heart rate) were found.

Research focused on age-related differences has shown contradictory results. Whereas some studies have found no significant age-related differences in heart rate reactivity to social stressors [2,42,5], others have reported lower heart rate responses in older individuals than in younger people [43,4]. The different stress paradigms used in each study (TSST, speaking tasks or cognitive tasks) could explain these discrepancies in the literature, in addition to the fact that some of these studies did not consider the menstrual cycle phase at all [42], or they only tested women in the follicular [24] or luteal phase [5,6].

Furthermore, with age, people's perceptions of stressful social situations could be attenuated due to their greater range of experiences and repertoire of coping resources [44]. For example, older people have been found to report fewer negative emotions than younger people [45–47], and to perceive less frustration than younger adults when facing an acute laboratory challenge [48]. Following this line of reasoning, the psychological response (perceived anxiety and mood) to social stressors would be lower in older people than in younger people. Our results confirmed that older women generally showed less change in anxiety and mood when facing an acute social stressor. In addition, we found a menstrual cycle phase effect. Thus, women in the luteal phase showed higher anxiety and mood changes than post-menopausal women, whereas the scores of women in the follicular phase were similar to those of post-menopausal women. These results, combined with the aforementioned physiological response, agree with previous studies that also found higher sensitivity to stress in women in the luteal phase, with greater increases in negative affect [18], but no differences in cortisol response. However, they are inconsistent with other findings that suggest higher negative psychological symptoms during the follicular phase [14,49]. This discrepancy could be explained by changes in the circulating progesterone in each phase of the cycle and individual differences in the sensitivity to this hormone. Another interpretation of these mixed results might be the higher psychological reactivity in women in the (early) follicular phase due to the discomfort of menstruation symptoms, whereas these symptoms disappear in the mid-follicular phase. This issue highlights the relevance of the time-point in the cycle phase when psychological changes are measured.

Therefore, we can confirm that the hormonal status influences both the psychological and biological stress response, with post-menopausal women showing a buffered stress response, and women in the luteal phase being more reactive to stress. However, the contribution of age to these differences cannot be determined because age and hormonal status are inseparable in this comparison.

Likewise, the behavioral strategies displayed during the stressor were different depending on age and hormonal status, with young women exhibiting a higher percentage of behaviors that reflect passive and reactive coping styles (Submission and Displacement), whereas post-menopausal women displayed higher percentages of behaviors that reflect an active coping style (Gestures), although the difference was only significant in comparison to the follicular group.

Based on previous studies in young women, we expected that the neuroendocrine response to stress would be positively influenced by Displacement behaviors and negatively by submissive behavioral patterns in young women [16,19]. However, in this study, we failed to find these associations, perhaps due to the differences in the groups' compositions. For example, we previously [19] measured women in the earliest follicular phase and women taking oral contraceptives, whereas in the present study the women were in the mid-follicular (5th to 8th day after the onset of the new menstrual cycle) and mid-luteal phase (4th to 8th day before the onset of the new menstrual cycle). Nonetheless, our results revealed that in young women (follicular and luteal groups), the behaviors that reflect passive coping (Submission and Assertion) are associated with an improved recovery of the HPA axis, whereas in post-menopausal women, affiliative behaviors seem to have a cardiac regulatory function. These latter results agree with the tend-and-befriend theory [50], which explains the adaptive function of affiliative behaviors as a physiological regulation tool in women.

On the other hand, the COPE scores provide information about the way an individual usually copes with stressful situations. The main objective of using this questionnaire was to explore the possible differences in coping strategies and the influence of the coping styles on the physiological stress response. Some authors have suggested that older adults are more flexible in their problem-solving strategies than younger adults [51,52]. At first, our results showed age-related differences, with the group of post-menopausal women scoring higher on the Active coping subscale than both groups of young women; and we also found that women in their luteal phase scored higher on Emotional coping styles (they use more strategies related to focusing on and venting emotions, such as letting out their emotions) than the post-menopausal and follicular groups. Second, with the regressions performed, we observed that, only in post-menopausal women, avoidance strategies were associated with a greater cortisol recovery index, whereas higher scores on Active coping and lower scores on Avoidance predicted greater heart rate reactivity. However, we failed to find any associations between the coping styles and neuroendocrine adjustment in young women. Previous research in adult women (41 to 69 years old) reported that an adaptive coping style predicted greater cortisol [53] and faster cardiac recovery in a group of women with a broad age range (18 to 79 years old) [54] who were undergoing stressful cognitive tasks. By contrast, avoidance coping styles have been associated with greater blood pressure reactivity in adult men and women [55].

In conclusion, in the present study, important differences in the psychophysiological responses and coping behavior of the three groups of women have been found. We believe that these results also provide new relationships involving age and reproductive status in behavioral and physiological stress responsivity, as well as the influence of coping strategies on the neuroendocrine self-regulatory processes (cortisol and heart rate). In addition, the consideration of the stress response as the interaction between psychological perceptions and physiological systems highlights the need to better understand the stress response in the adult population. Specifically, it increases the interest in studying women in the post-menopausal period, as this group is defined by dramatic decreases in reproductive hormones [15], along with a social background that has the potential to be one of the most important protective factors in the study of the social stress response. In our study, in addition to being women after menopause, they are also a select group with an active and healthy lifestyle (e.g., non-smokers, with no age-related illnesses, non-stressed, and university students). Therefore, future studies should be carried out in women with other circumstances, such

as excessive family responsibilities or age-related illnesses, in order to elucidate how they are able to react to and recover from social stress. Moreover, it is important to note that the participants' regular physical activity was not controlled. Future studies should pay attention to this issue, given that physical activity can influence the stress response and the ability to recover from acute stress. The low reactivity in the postmenopausal group could be due to their more sedentary lifestyle, compared to the younger groups. Another limitation of the study is that the estrogen levels were not measured. This information could shed light on the psychological and biological differences among the three groups, specifically between postmenopausal women and women in the follicular phase.

Finally, the associations found in this study must be interpreted with caution, due to the number of correlations performed, increasing the possibility of a type I error. However, the choice of multiple comparison corrections also increases the possibility of a type II error, thus hampering potentially important findings. Undoubtedly, future studies with larger samples are needed to further validate these results. Moreover, further examination of oral contraceptive users is required, due to the social importance of the effect of contraceptives on the psychophysiological stress response.

In sum, these results provide new insights into the importance of considering age and the menstrual cycle phase and their interaction with coping processes, as well as the reactivity and recovery capacity, in situations of social stress. To the best of our knowledge, this is the first study to analyze and compare behavior, coping styles, and the psychophysiological stress response in groups of young women in different phases of the menstrual cycle and in post-menopausal women.

Conflicts of interest

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

Acknowledgments

The authors wish to thank Prof Dr. Matías Pulópulos, Dr. María C. Fuentes and Sara Puig for their support in the research process, and Ms. Cindy DePoy for the revision of the English text.

This research was supported by the Spanish Education and Science Ministry: PSI2010/21343, PSI2013-4688, FPU AP2010-1830: MMP, FPI/BES-2008-004224: VH, FPU AP2009-4713: CV, FPU12/04597: SPP. This research was partially supported by Generalitat Valenciana: ACOMP/2015/227, PROMETEO 2011/048, PROMETEOII 2015/20.

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