Accepted for publication in *Psychoneuroendocrinology*

Association between changes in heart rate variability during the anticipation of a stressful situation and the stress-induced cortisol response

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Abstract

Vagal activity -reflecting the activation of stress regulatory mechanisms and prefrontal cortex activation- is thought to play an inhibitory role in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis. However, most studies investigating the association between stress-induced changes in heart rate variability (HRV, an index of cardiac vagal tone) and cortisol have shown a non-significant relationship. It has been proposed that physiological changes observed during anticipation of a stressor allow individuals to make behavioral, cognitive, and physiological adjustments that are necessary to deal with the upcoming actual stressor. In this study, we investigated whether changes in HRV during the anticipation of a stressful event is associated with inter-individual differences in the cortisol response to stress. In a large sample of 171 healthy adults (96 men and 75 women; mean age=29.98, *SD*=11.07), we investigated whether the cortisol response to a laboratory-based stress task was related to anticipation-induced or stress task-induced changes in HRV. As expected, regression analyses showed that a larger decrease in HRV during the anticipation of a stress task was related to higher stress task-induced cortisol increase, but not cortisol recovery. In line with prior research, the stress task-induced change in HRV was not significantly related with cortisol increase or recovery. Our results show for the first time that anticipatory HRV (reflecting differences in stress regulation and prefrontal activity before the encounter with the stressor) is important to understand the stress-induced cortisol increase.

1. Introduction

An excessive and/or prolonged cortisol response to stress has been related to physical and psychological disorders (McEwen, 2008). Understanding the factors that contribute to differences in the stress-induced cortisol response is crucial to prevent and treat stress-related disorders. The vagus nerve, the main component of the parasympathetic division of the autonomic nervous system, is assumed to play an inhibitory role in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis (Thayer and Sternberg, 2006). Considering its link with the HPA axis, vagal activity may provide relevant information to understand the inter-individual differences in stress-induced cortisol response to stressors.

High vagal activity is considered a marker of successful emotion regulation and stress adaptability (Park et al., 2014; Thayer et al., 2012; Vanderhasselt et al., 2015). Recent meta-analyses demonstrated that high vagal function is associated with prefrontal cortex activity (Makovac et al., 2017; Thayer et al., 2012), a brain region that has inhibitory connections with the amygdala (Baeken et al., 2010). Under stress, the amygdala is activated and initiates the HPA axis response to stress (Herman et al., 2005); however, an increase in prefrontal cortex activity would inhibit the activation of the amygdala and reduce the HPA axis response to stress (Baeken et al., 2014, 2010). Thus, given that the activity of the vagus nerve reflects the inhibitory control of the prefrontal cortex on the amygdala, better emotion regulation and stress adaptability are expected in individuals showing higher vagal activity (Thayer et al., 2012; Vanderhasselt et al., 2015). Moreover, it has been proposed that there are bidirectional connections between vagal nuclei in the medulla oblongata and the hypothalamus, supporting the idea of a connection between the two systems (Benarroch, 1997; Marca et al., 2011; Palkovits, 1999). Taken together, given that vagal activity reflects stress/emotion regulation and have bidirectional connections with the HPA axis, an association with cortisol in stressful events could be expected.

Vagal activity can be indexed by heart rate variability (HRV; variation in inter-beat intervals; Task Force, 1996). Under stressful situations, a decrease in HRV and an increase in cortisol levels is observed

(e.g., Marca et al., 2011; Zandara et al., 2017, 2016), and resting HRV has been related to cortisol increase and recovery during stress, and during cognitive challenge (Gunnar et al., 1995; Johnsen et al., 2012; Smeets, 2010; Weber et al., 2010). Although these results suggest a relationship between HRV and cortisol under stress, most of the studies that investigated the association between changes in HRV and cortisol in stressful situations have found non-significant results (Altemus et al., 2001; Bosch et al., 2009; Cacioppo et al., 1995; Gunnar et al., 1995; Heilman et al., 2008; Looser et al., 2010; Marca et al., 2011; but see Doussard-Roosevelt et al., 2003). For instance, Looser et al. (2010) showed an association between cortisol values and change in HRV during high levels of stress, but they did not observe a significant relationship between changes in HRV and changes in cortisol. Moreover, La Marca et al. (2011) showed a negative relationship between stress-induced changes in HRV and cortisol, but the association was not statistically significant. Therefore, although a link between vagal activity and the HPA axis is assumed (Thayer and Sternberg, 2006), most studies have shown weak and non-significant relationships between changes in both HRV and cortisol levels in response to stress (Altemus et al., 2001; Bosch et al., 2009; Cacioppo et al., 1995; Gunnar et al., 1995; Heilman et al., 2008; La Marca et al., 2011; Looser et al., 2010).

It is worth noticing that the physiological response to stress does not occur only when confronted with the stressful situation (Engert et al., 2013). Instead, before the actual encounter with the stressor, the anticipation of a threat to well-being or disruption of homeostasis may also trigger the HRV response (e.g., Zandara et al., 2017). This rapid response suggests that differences in stress regulation might be observed before the actual encounter with the stressful event and that the regulatory role of the vagal tone in the HPA axis response to stress might start during the anticipation of the stressor. Importantly, the physiological changes observed during anticipation are considered an adaptive response that allows individuals to make behavioral, cognitive, and physiological adjustments that are necessary to deal with the upcoming actual stressor (Schulkin, 2011; Schulkin et al., 1994; Turan, 2015; Turan et al., 2015), and previous studies have shown that stress anticipation is crucial to understand the differences in the physiological response to stress (Engert et al., 2013; Gaab et al., 2005, 2003). For

instance, the anticipatory stress appraisal (i.e., the evaluation of the stressor as a threat or challenge and the evaluation of the own abilities to deal with the stressor) is associated with lower cortisol response to a stress task (Gaab et al., 2005; 2003). Moreover, in their Neurocognitive Framework for Regulation Expectation, De Raedt and Hooley, (2016) have proposed that proactively anticipating a stressful event is associated with sustained prefrontal activation, which would decrease amygdala activation and improve stress regulation. Together, it is possible that changes in HRV during stress anticipation reflect the ability of the individuals to anticipate the stressful event successfully. Following this idea, one could expect that larger decreases in HRV during anticipation (reflecting poorer stress/emotion regulation associated with reduced prefrontal activity) might be associated with higher HPA axis responses to stress (reflected in higher increases in cortisol).

To test this idea, we investigated the relationship between changes in HRV provoked by the anticipation of a stressful task and the changes in cortisol levels in response to a laboratory-based stress task in 171 healthy adults. We expected a larger decrease in HRV during the anticipation of the stress task to be related to higher stress-induced cortisol increase. To compare our results with previous studies, we also investigated the relationship between changes in HRV due to the stress task and cortisol indexes. In accordance with previous studies, we expected no significant relationship in these analyses.

2. Methods

2.1. Participants

The sample of this study was recruited for the Pittsburgh Cold Study 3, a prospective viral challenge study with data collected from 2007-2011 among 213 healthy volunteers ages 18-55 from the Pittsburgh, Pennsylvania metropolitan area. The data were collected by the Laboratory for the Study of Stress, Immunity, and Disease at Carnegie Mellon University under the directorship of Sheldon Cohen, Ph.D.; and were accessed via the Common Cold Project website (www.commoncoldproject.com; grant

number NCCIH AT006694). The exclusion criteria for the whole project were: regular medication regimen (including but not limited to use of antidepressants, sleeping pills, or tranquilizers), previous nasal/otologic surgery, psychiatric hospitalization within the last 5 years, history of chronic illness or any psychiatric disorder treated within one year or before study enrollment, abnormal clinical profile (discovered via urinalysis, complete blood count, or analysis of blood chemistry), human immunodeficiency virus seropositivity, current pregnancy or lactating, use of steroids or immunosuppressants within three months of the trial, participation in another study involving psychological questionnaires and/or investigational products within the last 30 days or plans to participate in such research while enrolled in the current study, cold or flu-like illness within 30 days prior the infection with a virus as part of the project, living with someone who has chronic obstructive pulmonary disease or an immunodeficiency, previous hospitalization as a consequence of a flu-like illness and allergies to eggs or eggs products.

The final sample included in this study was 171 (96 men and 75 women). Table 1 shows the characteristics of the study sample.

2.2. Procedure

Participants provided informed consent and received 1000$ for their participation in the whole protocol. The study was approved by the Carnegie Mellon University and University of Pittsburgh institutional review boards. The protocol of the whole project lasted between 14 and 16 weeks. Ten to 12 weeks after the beginning of the study, the participants were infected with a virus to investigate susceptibility to the common cold. In the current study, we focus on the data available for the physiological (cortisol and HRV) and psychological (negative and positive affect) response to a stress task that was collected before the virus inoculation.

The protocol of the project is described in detail at the Common Cold Project website (www.commoncoldproject.com), and here we describe only the protocol for the stress task. For the induction of stress response, participants attended an individual session that started between 3:00pm and 9:00pm

and took place at the University of Pittsburgh. They were asked to abstain from alcohol for 48 hours, from exercise and nonprescription medications for 24 hours, from eating and drinking (except water) for 2 hours, and from smoking for 1 hour prior to the session. Upon arrival to the laboratory, participants were interviewed to determine whether they had followed these instructions and anthropomorphic measures were taken. At the beginning of the experimental session, participants were asked to sit quietly for 20 min (Habituation phase). Importantly, in a recent meta-analysis, Goodman et al. (2017) concluded that a habituation period of at least 15min could be used to avoid elevated pre-stress baseline cortisol levels. Immediately after the habituation phase, participants performed a modified version of the Trier Social Stress Test (TSST, Kirschbaum, et al., 1993), a well-validated laboratory-based stress task. The participants were informed that they had to deliver a 5min videotaped speech defending themselves against an alleged transgression (shoplifting or traffic violation counterbalanced across the two occasions of testing) and a 5min mental arithmetic task. Importantly for the current study, after receiving the instruction for the TSST, the protocol included an anticipatory phase in which the participants had 5min to prepare the stress task. Immediately after the anticipatory phase, they performed the speech and the arithmetic task (Stress phase). After the TSST, the participants were asked to rest quietly for 50min (Recovery phase).

Salivary samples were collected immediately before the introduction to the TSST (Baseline), immediately after the TSST (+15min), and every 10min after the TSST (+25min, +35min, +45min, +55min, and +65min) to measure cortisol levels. HRV was recorded continuously from the beginning of the session until 15min after the TSST. Measures of negative and positive affect were taken immediately before the introduction to the TSST (Baseline), 10min after the end of the TSST (+25min) and at the end of the recovery period (+65min).

2.3. Questionnaires

Stress Reactivity Questionnaire (SRQ). The SRQ is based on the State Adjective Questionnaire (Usala and Hertzog, 1989) and was used to assess (using a 4-points scale; 0 = Not at all, 4=A lot) positive and negative emotions. To measure positive affect, the participants had to rate how happy, cheerful,

full of pep, lively, calm and at ease, they felt during the baseline phase, the TSST and at the end of the recovery phase. Positive affect was computed as the mean of the participants´ responses to these questions for each period. To measure negative affect, they had to indicate how hostile, angry, on edge, tense, sad and unhappy they felt in the same periods. Negative affect was computed as the mean of the participants´ responses to these questions for each period.

2.4. Heart rate variability

HRV was recorded continuously throughout the baseline phase (20min), the anticipatory phase (5min), the stress task (10min) and the first 15min of the recovery phase using three electrodes (one at the right mid-clavicular line directly below the clavicle; and one each at the left and right lower margins of the rib cage in line with the midpoints of the respective left and right clavicles). A respiratory band was used to measure respiration rate. Following the Task Force recommendations (Schipke et al., 1999), the inter-beat interval sequences were extracted using a sampling frequency of 250hz and using an automated inter-beat interval extraction algorithm (Mindware Version 2.51, Mindware Technology, LTD., Gahanna, OH). Then, the extracted inter-beat were examined for artifacts and corrected manually. As an index of HRV, we used the widely utilized root mean square successive difference (RMSSD). The RMSSD is considered an index of vagal tone that reflects parasympathetic outflow and successful emotion regulation, it is relatively free of respiratory influences, and it has been shown that the TSST provokes decreases in this index (Fagundes et al., 2011; Laborde et al., 2017; Sghir et al., 2012; Thayer et al., 2012). The percentage of successive normal sinus RR intervals more than 50ms (pNN50) is also an index of HRV that reflects vagal activity (Laborde et al., 2017). The conclusions of the study are similar if pNN50 is used for the analyses.

2.5. Cortisol

Samples were collected using Salivette® (Sarstedt, Rommelsdorft, Germany) and analyzed at the laboratory of Dr. Clemens Kirschbaum, Dresden, Germany. Cortisol levels were determined by time-resolved fluorescence immunoassay with a cortisol-biotin conjugate as a tracer. Intra- and inter-assay variability were lower than 12%.

2.6. Data management and statistical analyses

Cortisol and HRV values did not show normal distributions, and they were log transformed. For HRV, the last 10min of the habituation phase and the first 10min of the recovery phase were separated into 5min epochs and averaged to compute the HRV levels at baseline and recovery, respectively. HRV levels during the speech and the arithmetic tasks were averaged to compute the HRV during the stress task.

We used ANOVA for repeated measures to investigate the physiological and psychological stress response, using Time as a within-subject factor (for positive and negative affect: Baseline, +25min and +65min; for HRV: Baseline, anticipation, stress task and recovery; for cortisol: Baseline, +15min, +25min, +35min, +45min, +55min and +65min). Greenhouse–Geisser was used because the requirement of sphericity was violated in these analyses. Post-hoc comparisons were performed using Bonferroni adjustments for *p* values.

The relationships between cortisol indexes (dependent variables) and HRV indexes (predictor variables) were analyzed in separate hierarchical regression analyses. For cortisol levels, three indexes were computed and used as dependent variables in the regression analyses: (I) The area under the curve with respect to the increase (AUCi) was calculated, using the seven cortisol samples (see Pruessner et al., 2003 for the specific formula), as a measure of dynamic of the cortisol change after the stress task, (II) the cortisol reactivity was computed as the change in cortisol from baseline to the maximum cortisol levels after the stress task, and (III) cortisol recovery was computed as the decrease in cortisol from the maximum cortisol levels after the stress task to the last cortisol sample. For HRV, two indexes were calculated and used as predictors in separate regression analyses: (I) $HRV_{Anticipation}$ was calculated as the change in HRV from baseline to the anticipatory phase (i.e., the 5min period that the participants had to prepare the stress task), (II) HRV_{Stress} was calculated as the change in HRV from baseline to the stress task. Lower values in HRV indexes indicate a larger decrease in HRV and thus,

poorer stress regulation. In the first step of the regression analyses, we included one of the HRV indexes ($HRV_{Anticipation}$ or HRV_{Stress}) as a predictor variable, and one of the cortisol indexes (cortisol AUCi, cortisol reactivity or cortisol recovery) as the dependent variable. Because baseline levels of HRV and cortisol were correlated with HRV and cortisol indexes (p<0.004), respectively, in the first step of all the regression analyses we included HRV and cortisol levels at baseline as covariates. In the second step of all the analyses, we included the following covariates to control for possible confounders: Age, Sex (Men=0, Women=1), body mass index (kg/cm^2), subjective socioeconomic status (measured using the nine-rung 'social ladder', cf., Adler et al., 2000), time of the beginning of the session, smoking (No=0, Yes=1), total of drinks consumed per week, use of hormonal contraceptives (0=No, 1=Yes), and being postmenopausal women (No=0, Yes=1). The inclusion of these covariates was based on previous research showing that these variables may affect HRV and/or cortisol response to stress (e.g., Allen et al., 2014; Gruenewald et al., 2006; Kudielka et al., 2009; Pulopulos et al., 2015; Pulopulos et al., 2018; Quintana et al., 2013; Sjoberg and Saint, 2011; Villada et al., 2017). Tolerance values indicate that there were no collinearity issues for the variables included in the model. We investigated the relationship between HRV ($HRV_{Anticipation}$ or HRV_{Stress}) and these three cortisol indexes (AUCi, cortisol reactivity and cortisol recovery) because they reflect different characteristics of the cortisol response: (I) the AUCi reflects the changes over time, (II) the cortisol reactivity reflects the magnitude of the cortisol response provoked by the stressor, and (III) the cortisol recovery reflects the ability of the HPA axis to recover and return to baseline levels after the confrontation with the stressor (Fekedulegn et al., 2007; Linden et al., 1997). These indexes have been associated with different risk factors for stress-related disorders (e.g., perceived stress, serotonin transporter genes, and rumination)(Gotlib et al., 2008; Roy et al., 2001; Vrshek-Schallhorn et al., 2017). We also computed the area under the curve with respect to the ground (AUCg), using the seven cortisol samples (see Pruessner et al., 2003 for the specific formula). The AUCg is as a measure of the total cortisol secretion. Therefore, given that we are interested in stress-induced changes in cortisol levels (i.e., AUCi, cortisol reactivity and cortisol recovery), the AUCg is reported for informative purposes, but it was not included in the regression analyses. Outliers were

defined as values ±3 SD and were winsorized by replacing their values by values equal to the mean ±3 SD (two outliers were detected for $HRV_{Anticipation}$, one for HRV_{Stress}, three for cortisol AUCi and cortisol reactivity, and five for cortisol recovery).

Two hundred and thirteen subjects participated in the study. Forty-two participants were removed from all the analyses because cortisol or HRV indexes could not be calculated due to missing data. Thus, the final sample of this study was 171 (96 men and 75 women). Moreover, 12 participants had missing data for HRV during the stress task, seven participants for HRV during the recovery phase, three participants had missing data for positive affect, and one participant had missing data for negative affect. These participants were excluded from the analyses that included these variables. Thus, for the repeated measures ANOVAs the sample was 152 for HRV, 168 for positive affect, and 170 for negative affect. For regression analyses, the sample size was 171 for analyses with $HRV_{Anticipation}$, and 159 for HRV_{Stress}[1]. The proportion of missing data was less than 5% (260 values out of 7,621), and the participants with missing data were not different from the participants without missing data in the variables of interest for this study (p>0.183).

Analyses were performed using SPSS 24.0 (IBM SPSS Statistics 24.0) and all p-values reported are two-tailed.

3. Results

3.1. Stress response

The ANOVAs for repeated measures showed an effect of Time for negative affect ($F(1.739, 293.895)=84.703$, $p<0.001$), positive affect ($F(1.895, 316.445)=88.29$, $p<0.001$), HRV ($F(2.446, 369.356)=76.309$, $p<0.001$) and cortisol levels ($F(4.196,713.355)=11.44$, $p<0.001$).

[1] The sample for HRV_{Stress} (n=159) was smaller than for $HRV_{Anticipation}$ (n=171). To control that the difference in results for these two indexes is not due to differences in sample size, we repeated the analyses for $HRV_{Anticipation}$ including only participants without missing data for HRV_{Stress}. The statistical conclusion of the study are the same if only participants with data for HRV_{Stress} are included in the analyses.

The tests of simple effects showed an increase in negative affect and a decrease in positive affect from baseline to immediately after the stress task (p<0.001). Forty minutes after the stress task, the negative affect returned to baseline levels (p=0.243), but the positive affect was still lower than baseline (p<0.001)(see Table 1).

For HRV, the tests of simple effects showed that HRV decreased from baseline to the anticipatory phase (p<0.001). Importantly, no difference in HRV between the anticipatory phase and the stress task was observed (p>0.999). After the stress task, an increase in HRV was observed from the stress task to the recovery phase (p<0.001)(Figure 1).

Regarding cortisol levels, the tests of simple effects showed that the increase in cortisol levels after the stress task was not statistically significant (p>0.999). Cortisol levels in the last two salivary samples were below baseline levels (p<0.012) (Figure 2).

3.2. Relationship between HRV and cortisol indexes

Table 2 shows the results of the regression analyses for the relationship between $HRV_{Anticipation}$ and cortisol indexes (i.e., cortisol AUCi, cortisol reactivity and cortisol recovery). In Step 1 of the regression analyses, we included pre-stress cortisol and HRV levels as covariates. The analyses showed that a larger decrease in HRV during anticipation ($HRV_{Anticipation}$) was related to higher cortisol AUCi (β=-0.163, p=0.031) and higher cortisol reactivity (β=-0.150, p=0.040). In Step 2, we controlled for pre-stress cortisol and HRV levels, Age, Sex, BMI, subjective socioeconomic status, time of the beginning of the session, smoking, total of drinks consumed per week, postmenopausal status, and use of hormonal contraceptives. The analyses showed that $HRV_{Anticipation}$ was significantly related to cortisol AUCi (p=0.022) and cortisol reactivity (p=0.024). Table 3 shows the results of the regression analyses for the relationship between HRV_{Stress} and cortisol indexes. HRV_{Stress} was significantly related to cortisol AUCi and cortisol reactivity when the analyses are controlled for pre-stress cortisol and HRV levels (p<0.046); however, these relationships became marginally significant after controlling for all the covariates (p>0.051). $HRV_{Anticipation}$ and HRV_{Stress} were not significantly related to cortisol recovery (p>0.283).

3.3. Analyses with cortisol responders

Importantly, although the participants showed a change in positive and negative affect, and in HRV, the stress task provoked only a moderated cortisol response and not all the participants showed an increase in cortisol levels after the stress task. Thus, we decided to investigate whether $HRV_{Anticipation}$ is still related to cortisol response when the stress task triggers an HPA axis response to stress. To do so, we repeated the regression analyses including only those participants showing a stress-induced cortisol increase. For the classification of cortisol responders, we used the 1.5nmol/L baseline-to-peak increase criterion (Miller et al., 2013). A different criterion used in previous studies to differentiate between responders and non-responders is the 2.5nmol/L baseline-to-peak increase criterion (e.g., Schoofs and Wolf, 2009; Wust et al., 2005). We decided to use the 1.5nmol/L baseline-to-peak increase criterion because Miller et al. (2013) demonstrated that, in comparison to the 2.5nmol/L criterion, the 1.5nmol/L criterion shows less false-positive and less false-negative classifications. Using the 1.5nmol/L criterion, 70 participants (men=51, women=19; for HRV_{Stress} 64 participants: men=45, women=19) were considered as cortisol responders (AUCi: mean=182.33, SD=258.79; Cortisol reactivity: mean=6.41, SD=6.37; Cortisol recovery: mean=5.77, SD=5.23; AUCg: mean=632.63, SD=342.79; $HRV_{Anticipation}$: mean=-10.73, SD=17.21; HRV_{Stress}: mean=-11.99, SD=19.42). The ANOVA for repeated measures, with Time as a within-subject factor (Baseline, +15min, +25min, +35min, +45min, +55min and +65min), confirmed that the stress task provoked a significant change in cortisol levels (Time: $F(3.279,226.268)=10.650$, $p<0.001$). Post hoc comparison showed that cortisol levels 15min, 25min and 35min after the stress task were higher than baseline ($p<0.001$). Cortisol levels 45min, 55min, and 65min after the stress task were not significantly different from baseline ($p>0.124$). Regarding the psychological response to stress, we investigated the differences between Responders and Non-responders in positive and negative mood using a mixed measures ANOVA with Group (responders vs. non-responders) as a between-subject factor, and Time (pre-TSST, immediately after the TSST and 40min after the TSST) as a within-subject factor. The results showed a significant effect of Time (Posi-

tive mood: F(1.89,313.886)=87.15, p<0.001; Positive mood: F(1.74,292.287)=83.90, p<0.001). However, the factor Group and the interaction between Group and Time were not statistically significant (p>0.24), indicating that the stress task provoked a similar psychological response in both groups.

To investigate the relationship between HRV and cortisol indexes, we repeated the regression analyses including only cortisol responders. In Step 1 of the regression analyses, we included pre-stress HRV and cortisol values as covariates. In Step 2, we included Age, Sex, BMI, subjective socioeconomic status, time of the beginning of the session, smoking, total of drinks consumed per week, postmenopausal status, and use of hormonal contraceptives as covariates. For $HRV_{Anticipation}$, as observed for the whole sample, regression analyses with cortisol responders showed that a larger decrease in HRV from baseline to anticipation ($HRV_{Anticipation}$) was related to higher cortisol reactivity (Step 1: R^2=0.389, β=-0.231, p=0.022; Step 2: ΔR^2=0.091,β=-0.202, p=0.048). For cortisol AUCi, the relationship was statistically significant after controlling for pre-stress cortisol and HRV levels, and marginally significant after controlling for all the covariates (Step 1: R^2=0.299, β=-0.225, p=0.037; Step 2: ΔR^2=0.046, β=-0.217, p=0.058). The relationship between $HRV_{Anticipation}$ and cortisol recovery was not statistically significant (Step 1: R^2=0.683, β=0.015, p=0.831; Step 2: ΔR^2=0.072, β=0.042, p=0.542).

For HRV_{Stress}, regression analyses with cortisol responders showed that a larger decrease in HRV from baseline to the stress task (HRV_{Stress}) was not significantly related to cortisol reactivity after controlling for pre-stress cortisol and HRV levels, and the relationship was marginally significant after controlling for all the covariates (Step 1: R^2=0.374, β=-0.171, p=0.111; Step 2: ΔR^2=0.133, β=-0.199, p=0.071). The relationship between HRV_{Stress} and AUCi was marginally significant (Step 1: R^2=0.295, β=-0.204, p=0.073; Step 2: ΔR^2=0.062, β=-0.235, p=0.061). HRV_{Stress} was not significantly related to cortisol recovery (Step 1: R^2=0.684, β=0.040, p=0.595; Step 2: ΔR^2=0.074, β=0.059, p=0.439).

3.4. Relationship between cortisol indexes and changes in HRV from anticipation phase to the stress task.

The ANOVA for repeated measures showed that, at the group level, HRV decreased from baseline to the anticipation phase, but the stress task did not provoke a significant change in HRV compared to the anticipation phase. Additionally, $HRV_{Anticipation}$ and HRV_{Stress} were highly correlated (for the whole sample r=0.396, p<0.001; for cortisol responders r=0.384, p=0.002). Thus, it is possible that the marginally significant results observed for HRV_{Stress} are due to inter-individual differences in $HRV_{Anticipation}$. To investigate whether changes in HRV specifically provoked for the stress task may be associated with differences in cortisol response, we repeated the regression analyses including the change in HRV from the anticipation phase to the stress task as the predictor variable. The results showed that changes in HRV specifically due to the stress task are not related to cortisol indexes (n=159; Cortisol AUCi: Step 1: R^2=0.094, β=0.045, p=0.560; Step 2: ΔR^2=0.085, β=0.064, p=0.414; Cortisol reactivity, Step 1: R^2=0.165, β=0.034, p=0.650; Step 2: ΔR^2=0.145, β=0.050, p=0.488; Cortisol recovery, Step 1: R^2=0.760, β=-0.035, p=0.379; Step 2: ΔR^2=0.032, β=-0.034, p=0.389). The same results are observed if only cortisol responders are included in the analyses (n=64; Cortisol AUCi, Step 1: R^2=0.260, β=0.059, p=0.603; Step 2: ΔR^2=0.053, β=0.045, p=0.718; Cortisol reactivity, Step 1: R^2=0.358, β=0.105, p=0.320; Step 2: ΔR^2=0.121, β=0.074, p=0.499; Cortisol recovery, Step 1: R^2=0.683, β=0.018, p=0.813; Step 2: ΔR^2=0.072, β=0.006, p=0.936). Together, these results indicate that the change in HRV specifically due to the stress task (i.e., change in HRV from anticipation to the stress task) is not related to the cortisol indexes.

4. Discussion

High HRV, as a measure of the vagal tone, is considered a marker of successful emotion regulation and stress adaptability (Park et al., 2014; Thayer et al., 2012; Vanderhasselt et al., 2015). The aim of this study was to investigate whether changes in HRV during the anticipation of a stress task were related to the cortisol response (i.e., cortisol change during the session, cortisol increase and cortisol recovery) to a laboratory-based stress task in 171 healthy adults. Results showed that a larger decrease

in HRV during anticipation of a stressful event (i.e., $HRV_{Anticipation}$) was related to higher cortisol reactivity (i.e., cortisol increase) after the stress task. Higher $HRV_{Anticipation}$ was also related to higher cortisol AUCi (i.e., the dynamic of the stress-induced cortisol increase and decrease), but this relationship became marginally significant when the analyses were performed including only cortisol responders and controlling for all the covariates. The anticipation-induced change in HRV was not related to cortisol recovery. These results indicate that changes in HRV due to the anticipation of a stress task is associated with the stress-induced cortisol increase, but not the cortisol recovery. The change in HRV from baseline to the stress task (i.e., HRV_{Stress}) showed a significant association with cortisol reactivity and cortisol AUCi for the whole sample when the analyses were controlled for pre-stress HRV and cortisol levels. However, these relationships did not remain significant when the analyses were controlled for all the covariates and when the analyses were performed with participants who showed a cortisol response to stress. Most importantly, the changes in HRV from anticipation phase to the stress task were not related to the cortisol indexes, indicating that the change in HRV specifically related to the stress task is not associated with the differences in the cortisol response to stress.

At the group level, HRV decreased from baseline to the anticipation phase, indicating that participants are anticipating the stressful situation and that stress/emotion started already during anticipation. Importantly, inter-individual differences in phasic HRV during anticipation would reflect individual differences in anticipatory stress/emotion regulation. In accordance with our hypothesis, lower anticipation-induced HRV decrease was related to lower stress-induced cortisol increase. Regarding the stress task-induced changes in HRV, we observed that changes in HRV from baseline to the stress task were not significantly related to cortisol in those individuals who showed a cortisol response to stress. Moreover, changes in HRV specifically due to the stress task (i.e., change in HRV from anticipation to the stress task) were not significantly related to cortisol indexes. These results are in line with previous studies showing weak and non-significant associations between changes in HRV and cortisol in stressful situations (Altemus et al., 2001; Bosch et al., 2009; Cacioppo et al., 1995; Gunnar et al., 1995; Heilman et al., 2008; La Marca et al., 2011; Looser et al., 2010, but see Doussard-Roosevelt et al., 2003).

Together, these results indicate that individual differences in the regulatory effort during the anticipation of a stressful event are important to understanding the individual differences in the stress-induced cortisol response. These results are also in line with previous research showing that the anticipatory phase is a key determinant of the cortisol response to stress (Engert et al., 2013; Gaab et al., 2003). Importantly, the anticipation of a stressful event is considered an adaptive response that allows individuals to initiate psychological and biological changes to facilitate the confrontation with the upcoming demand (Schulkin, 2011; Schulkin et al., 1994; Turan, 2015). Along this line, in the Neurocognitive Framework for Regulation Expectation, De Raedt and Hooley (2016) have recently proposed that individuals who proactively anticipate a stressful situation would show better stress regulation during anticipation, and that this would reduce the effort needed to successfully regulate stress during the actual confrontation with the stressor. This implies that higher HRV during anticipation would be observed in individuals who successfully initiate mechanisms to regulate stress and to prepare themselves for the confrontation with the stressor, facilitating the actual confrontation with the stressor. This would be reflected in a lower activation of the HPA axis and a reduced cortisol response to stress.

Our results show that differences in stress regulation during anticipation are related to differences in cortisol increase, but not cortisol recovery. One possible explanation for this result is that the glucocorticoids negative feedback inhibition of the HPA axis, a feedback mechanism that inhibits the secretion of cortisol when high levels of this hormone are detected (Sapolsky et al., 2000), may be more influential to reduce cortisol secretion after the end of the stressor than the process of anticipatory stress regulation. However, it is also possible that the stress response observed in the participants of the current study would contribute to a lack of relationship between HRV and cortisol recovery. Although the stress task was effective in provoking a psychological and HRV change, the participants showed a moderated cortisol response and a cortisol increase was not observed in all the participants. It is possible that higher cortisol response is needed to observe a relationship between anticipatory HRV and cortisol recovery. For cortisol increase, when the analyses are performed with the complete sample, and adjusted for baseline cortisol and HRV levels, the anticipatory HRV response accounted

for 16.5% of the variance in cortisol reactivity. However, when the analyses are performed only with those participants considered as cortisol responders (cortisol increase after stress >1.5nmol/L), the anticipatory HRV response accounted for 38.9% of the variance in cortisol reactivity. These results indicate that the relationship between HRV and cortisol is stronger under conditions that elicit higher HPA axis responses. Thus, further studies are needed to investigate whether the anticipatory HRV response is related to cortisol recovery using a stress protocol that triggers a higher HPA axis response.

Despite the novel findings, some limitations should be considered. The protocol included a short anticipatory phase (5min). This time is enough to trigger and investigate anticipatory HRV responses; however, as indicated by Engert et al. (2013), a longer anticipatory phase is needed to observe differences in HPA axis activity during anticipation. Thus, we cannot know whether anticipation-induced changes in HRV are related to the changes in cortisol levels during anticipation and/or in response to the stress task. Future research using a longer anticipatory phase and measuring cortisol levels immediately before the speech task is needed to investigate whether anticipatory HRV response may explain differences in cortisol response before and after the encounter with the stressor. Another limitation of the study is that the participants were not tested at the same time of the day (i.e., the sessions started between 3pm and 9pm). Moreover, we did not control for the phase of the menstrual cycle in 52 premenopausal women not taking hormonal contraceptives. However, it is important to note that similar results are observed if we perform the analyses including only participants tested between 3pm and 6pm (when most of the participants were tested, n=122), or if we exclude premenopausal women not taking hormonal contraceptives (results not shown). Future research investigating the relationship between HRV and cortisol should test the participants in similar hours and should control for the menstrual cycle of women not taking hormonal contraceptives. Finally, the protocol did not include a non-stressful control task with a similar mental workload and global physical activity. Therefore, we cannot investigate whether the change in cortisol and HRV and the associations observed are specifically due to the stressful components of the tasks.

Future research

Our results support the relevance of the regulatory processes that occur during stress anticipation to understand the differences in the cortisol response to stress (Engert et al., 2013; Gaab et al., 2003; Schulkin, 2011). However, more research is needed to determine the psychological and neurocognitive mechanism behind a successful stress anticipation. For instance, Gaab et al. (2003), using the Primary Appraisal Secondary Appraisal scale (PASA), showed that anticipatory cognitive appraisal is an important determinant of the stress-induced cortisol response (Gaab et al., 2003). Moreover, De Raedt and Hooley (2016) proposed that proactive cognitive control related to prefrontal cortex activity is necessary for a successful anticipatory stress regulation. Along this line, higher HRV has been associated with better performance on cognitive control-related tasks such as working memory, inhibitory control, and sustained attention (for a review see Thayer et al., 2009) and increased prefrontal cortex activity (Makovac et al., 2017). Further studies investigating whether HRV during anticipation may be explained by anticipatory appraisal (for instance using the PASA) and/or cognitive process (e.g., cognitive control) may add relevant evidence to understand the differences in stress anticipation and its role in the process of stress regulation.

Conclusion

To conclude, inter-individual differences in stress regulation during the anticipation of a stressful situation, indexed by changes in HRV, is associated with differences in the stress-induced cortisol increase. This study demonstrates the importance of the stress regulation during anticipation to understand differences in cortisol response to stress.

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Legends

Legend Fig 1. Heart rate variability (RMSSD) during the session. Means and standard errors.

Legend Fig 2. Cortisol (nmol/L) levels during the session. Means and standard errors.

Contributors

MMP performed the data analyses and wrote the manuscript. MAV and RDR provided critical feedback on the data analyses and edited the manuscript.

Acknowledgements

CCP data are made publically available through a grant from the National Center for Complementary and Integrative Health (NCCIH AT006694). The conduct of the study was supported by grants from the National Institute of Allergy and Infectious Diseases (R01 AI066367). Secondary funding was provided by National Cancer Institute Cancer Center Support Grant (P30CA047904) and by the National Institutes of Health; grant awarded to the University of Pittsburgh Clinical and Translational Science Institute (UL1 RR024153 and UL1 TR000005). Supplemental support was provided by John D. and Catherine T. MacArthur Foundation Research Network on Socioeconomic Status & Health. The authors want to thank the study staff and participants. M.M.P. is a postdoctoral research fellow of the Special Research Fund (BOF; 01P18916) at Gent University.

Role of the funding source

Grants and funding sources had no further role in the analysis and interpretation of the data, in the writing of the report, or in the decision to submit the paper for publication.

Table 1. Characteristics of the sample, positive and negative affect during the session and, HRV and cortisol indexes

	Mean/n	SD
Age (years)	29.98	11.07
Sex and hormonal status	Men=96 Women=75 Premenopausal: -Free cycling =52 -Hormonal contraceptives=13 Postmenopausal=10	
Subjective Socioeconomic Status	6.01	1.64
Body Mass Index (kg/cm^2)	27.01	6.24
Smoking	No=118 Yes=53	
Alcohol (drinks consumed per week)	3.54	7.26
Time beginning session	16:58	01:07
Mean respiration rate	12.91	2.97
Positive Affect baseline	1.96	0.82
Positive Affect 10min Post-stress	1.34	0.90
Positive Affect 45min Post-stress	1.79	0.89
Negative Affect baseline	0.29	0.43
Negative Affect 10min Post-stress	0.88	0.78
Negative Affect 45min Post-stress	0.37	0.50
$HRV_{Anticipation}$	-9.13	22.42
HRV_{Stress}	-8.80	19.68
Cortisol reactivity	2.31	5.50
Cortisol recovery	3.57	4.09
Cortisol AUCi	7.65	250.76
Cortisol AUCg	465.31	308.79

Note: n=171 for all the variables except for HRV_{Stress} (n=159), Positive affect baseline (n=170), Positive affect 10min post-stress (n=169), and Negative affect 10min post-stress (n=170). $HRV_{Anticipation}$ was calculated as the change in HRV from baseline to the anticipatory phase. HRV_{Stress} was calculated as the change in HRV from baseline to the stress task. Cortisol reactivity was computed as the change in cortisol from baseline to the maximum cortisol levels after the stress task was computed as the change in cortisol from baseline to the maximum cortisol levels after the stress task. Cortisol recovery was computed as the decrease in cortisol from the maximum cortisol levels after the stress task to the last cortisol sample. Cortisol AUCi and AUCg were calculated, using the seven cortisol samples, as a measure of dynamic of the cortisol change after the stress task and total cortisol secretion, respectively (see Pruessner et al., 2003 for the specific formulas).

Table 2. Regression analyses for the relationship between $HRV_{Anticipation}$ and cortisol indexes (n=171).

		Cortisol AUCi		*Cortisol reactivity*		*Cortisol Recovery*	
		β	*p*	β	*P*	β	*p*
Step 1	$HRV_{Anticipation}$	-0.163	0.031	-0.150	0.040	-0.002	0.965
	Pre-stress cortisol	-0.294	<0.001	-0.380	<0.001	0.869	<0.001
	Pre-stress HRV	-0.052	0.491	-0.026	0.726	-0.010	0.799
Step 2	$HRV_{Anticipation}$	-0.176	0.022	-0.160	0.024	0.001	0.985
	Pre-stress cortisol	-0.371	<0.001	-0.498	<0.001	0.815	<0.001
	Pre-stress HRV	-0.103	0.204	-0.098	0.195	-0.034	0.409
	Age	-0.233	0.017	-0.238	0.009	-0.159	0.002
	Sex (Men=0, Women=1)	-0.186	0.033	-0.278	0.001	-0.108	0.016
	PM (No=0, Yes=1)	0.069	0.449	0.063	0.457	0.035	0.458
	Use of HC (No=0, Yes=1)	-0.070	0.385	-0.052	0.487	0.002	0.954
	Subj. Socieconomic Status	0.101	0.175	0.099	0.156	0.023	0.548
	Body Mass Index	0.058	0.472	0.062	0.411	0.108	0.011
	Smoking (No=0, Yes=1)	-0.036	0.639	-0.077	0.286	-0.016	0.676
	Alcohol	-0.148	0.054	-0.011	0.882	0.002	0.965
	Time session	-0.093	0.213	-0.195	0.006	-0.090	0.020

Note: Cortisol AUCi: R^2=0.110 for Step 1, ΔR^2=0.085 for step 2; Cortisol Reactivity: R^2=0.165 for Step 1, ΔR^2=0.136 for step 2; Cortisol Recovery: R^2=0.757 for Step 1, ΔR^2=0.031 for step 2. HC=Hormonal contraceptive; PM=Postmenopausal woman.

Table 3. Regression analyses for the relationship between HRV_{Stress} and cortisol indexes (n=159).

		Cortisol AUCi		*Cortisol reactivity*		*Cortisol Recovery*	
		B	*p*	β	*p*	β	*p*
Step 1	HRV_{Stress}	-0.160	0.046	-0.158	0.039	-0.045	0.283
	Pre-stress cortisol	-0.311	<0.001	-0.409	<0.001	0.868	<0.001
	Pre-stress HRV	-0.039	0.621	-0.009	0.904	-0.015	0.714
Step 2	HRV_{Stress}	-0.152	0.063	-0.147	0.051	-0.041	0.325
	Pre-stress cortisol	-0.381	<0.001	-0.525	<0.001	0.814	<0.001
	Pre-stress HRV	-0.078	0.359	-0.069	0.376	-0.040	0.360
	Age	-0.232	0.024	-0.230	0.014	-0.162	0.002
	Sex (Men=0, Women=1)	-0.147	0.102	-0.242	0.004	-0.109	0.017
	PM (No=0, Yes=1)	0.064	0.507	0.057	0.516	0.045	0.361
	Use of HC(No=0, Yes=1)	-0.103	0.220	-0.090	0.240	-0.002	0.968
	Subj. Socieconomic Status	0.106	0.181	0.107	0.139	0.025	0.524
	Body Mass Index	0.073	0.386	0.068	0.372	0.104	0.016
	Smoking (Yes=0, No=1)	-0.015	0.851	-0.074	0.326	-0.014	0.732
	Alcohol	-0.131	0.100	0.007	0.918	-0.002	0.967
	Time session	-0.102	0.195	-0.223	0.002	-0.095	0.019

Note: Cortisol AUCi: R^2=0.115 for Step 1, ΔR^2=0.079 for step 2; Cortisol Reactivity: R^2=0.187 for Step 1, ΔR^2=0.139 for step 2; Cortisol Recovery: R^2=0.761 for Step 1, ΔR^2=0.032 for step 2. HC=Hormonal contraceptive; PM=Postmenopausal woman

Figure 1

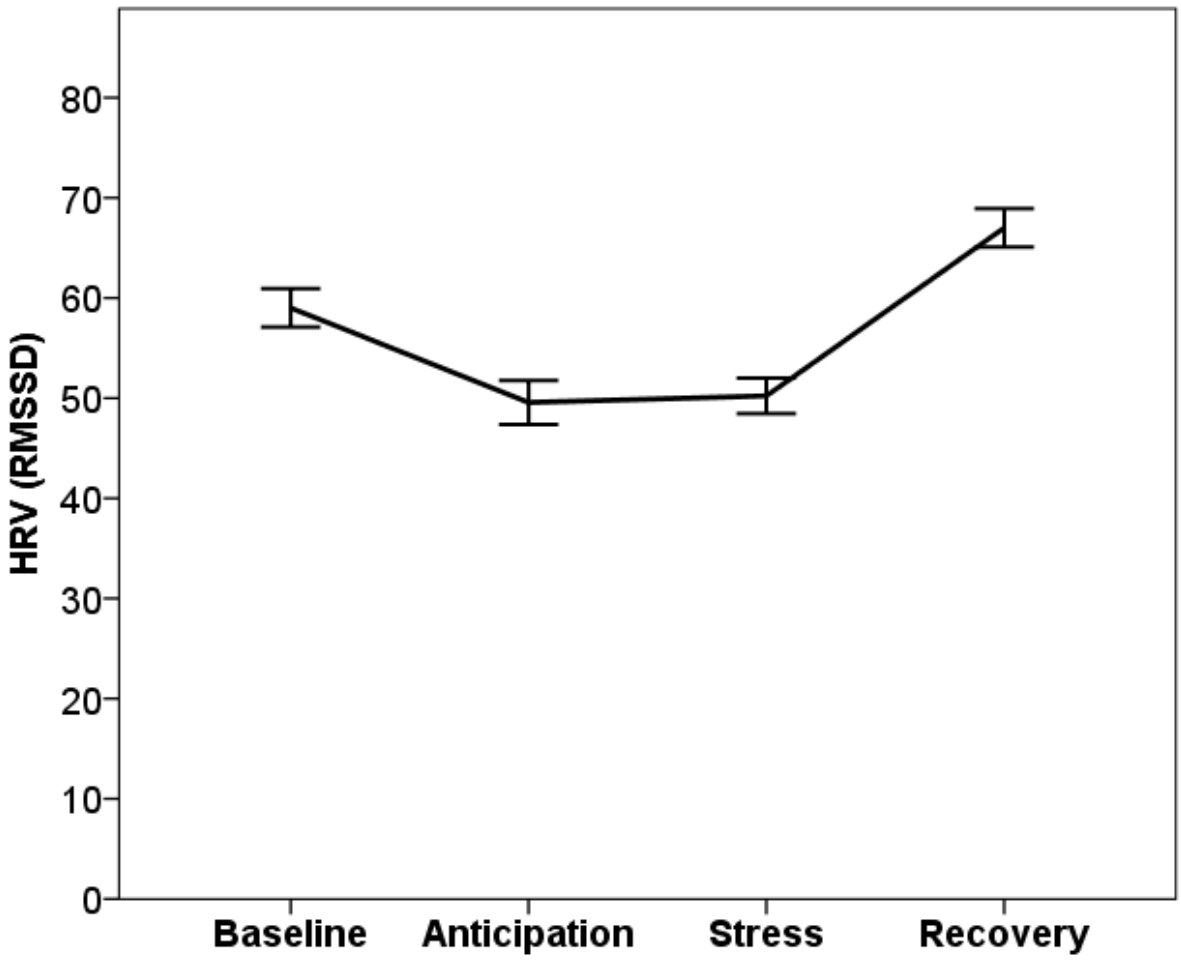
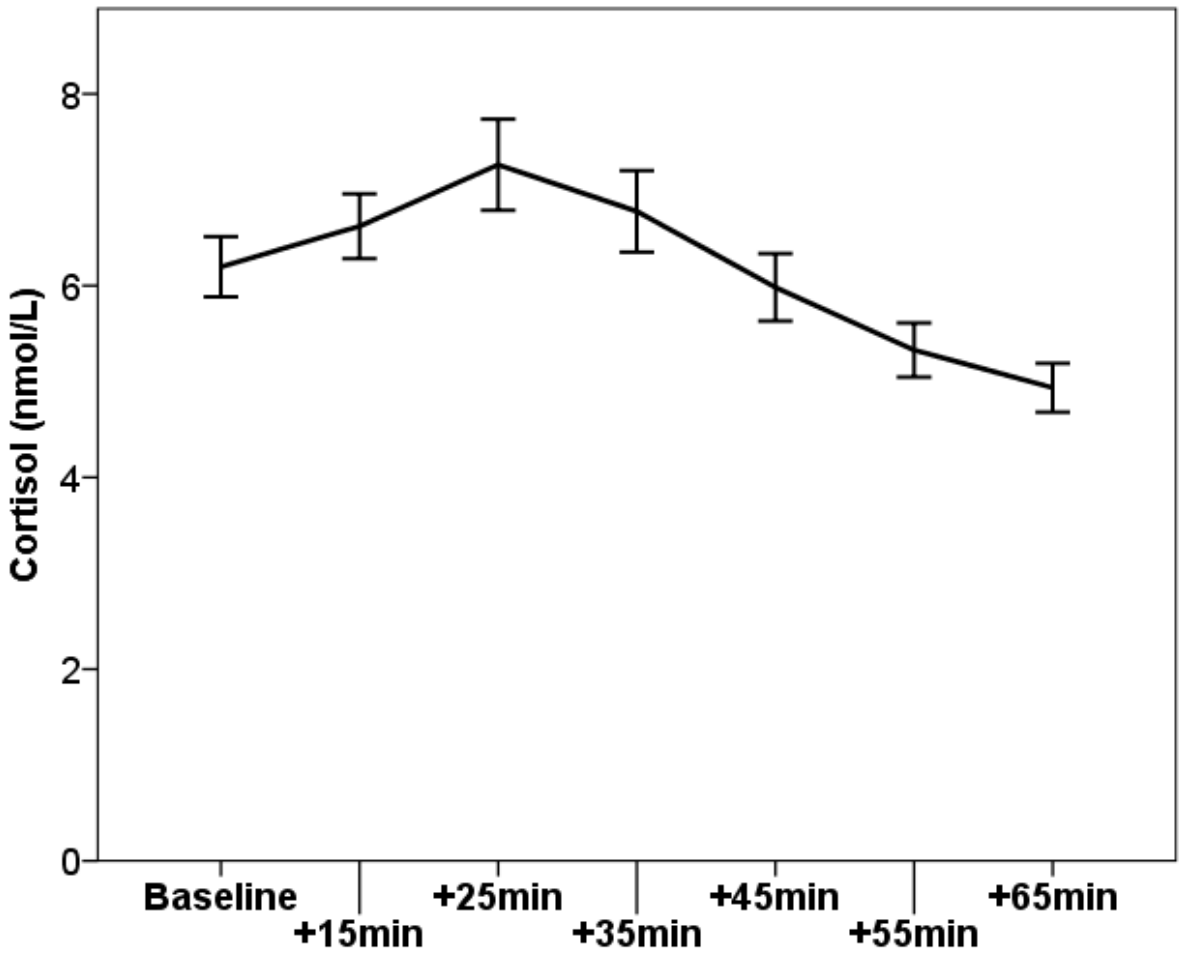

HRV (RMSSD)
80
70
60
50
40
30
20
10
0
Baseline
Anticipation
Stress
Recovery

Figure 2


Cortisol (nmol/L)
0
2
4
6
8
Baseline
+15min
+25min
+35min
+45min
+55min
+65min