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The effects of an anticipated challenge on diurnal cortisol secretion

ABSTRACT

In healthy, non-challenged individuals, the secretion of cortisol typically follows a diurnal profile characterised by a peak in the period following waking (cortisol awakening response) and a gradual decline throughout the day. In addition, cortisol secretion is increased in response to acutely stressful stimuli, particularly stressors involving social evaluation. The current study is the first to assess the impact of an anticipated acute laboratory stressor upon the typical diurnal pattern of HPA activation and relationship to acute cortisol secretion.—A sample of 23 healthy young adults provided salivary cortisol samples at four time points (immediately upon awakening, 30 minute post awakening, 1200h and before bed) on two consecutive days. On the second day, participants attended the laboratory and undertook an anticipated acute socially evaluative stressor immediately following provision of their 1200h saliva sample. Heart rate, blood pressure and mood were recorded immediately before and after the stressor and at 10 and 20 minutes post stressor along with additional salivary cortisol samples. Typical patterns of cortisol secretion were observed on both days and exposure to the laboratory stressor was associated with the expected increases in cortisol, heart rate, blood pressure and negative mood. However, significant differences in diurnal cortisol secretion were observed between the two days with greater secretion, in particular, during the period following awakening, evident on the day of the anticipated laboratory stressor. Furthermore, secretion of cortisol during the period following awakening was positively related to secretion during the acute reactivity periods.—This is the first study to integrate a laboratory stressor into a typical day and assess its impact on indices of diurnal cortisol secretion in an ambulatory setting. The current findings support the notion that the cortisol awakening response is associated with anticipation of the upcoming day and the subsequent demands required of the individual.

Keywords: Stress, Cortisol, CAR, Anticipation

INTRODUCTION

Cortisol, secreted by the hypothalamic-pituitary-adrenal (HPA) axis follows a marked diurnal profile characterised by a rapid increase in the 30-45 minutes following awakening (the Cortisol Awakening Response: CAR) and a diurnal decline to a nadir around midnight (Saxbe, 2008). Aspects of this profile are associated with a range of psychosocial factors. Higher levels of perceived and accumulated psychosocial stress (Abercrombie et al., 2004, Bauer et al., 2005, Lovell et al., 2011) are associated with a flattening of the diurnal decline, characterised by diurnal hypersecretion and higher levels of evening cortisol. Similarly, higher levels of evening cortisol are observed in individuals with greater trait anxiety (Van den Bergh et al., 2008) and more symptoms of depression (Van den Bergh et al., 2009). Reactivity of cortisol to acute challenges is also predictive of diurnal cortisol secretion (Kidd et al., 2014) demonstrating that the ability to mount an appropriate response to an acute challenge relates to basal functioning of the HPA axis.

The CAR is a related but distinct aspect of the diurnal cortisol profile (Wilhelm et al., 2007). Blunted CARs, characterised by reduced responses following awakening, have been observed in chronic fatigue syndrome (Roberts et al., 2004), post-traumatic stress disorder (Rohleder et al., 2004), burnout (de Vente et al., 2003), exhaustion (Mommersteeg et al., 2006), and depression (Stetler & Miller 2005).

Conversely, increased CARs predict first onset of anxiety disorders (Adam et al., 2014) and have been observed in individuals experiencing proximal stress such as work overload and worry (Schulz et al., 1998, Schlotz et al., 2004), and job stress (Steptoe et al., 2004).

The CAR is determined more by situational than trait-like factors (Hellhammer et al., 2007) and as such reflects proximal circumstances. It has recently been posited that the CAR is an adaptive response to maximise day-to-day functioning (Clow et al., 2010) and plays a crucial role in preparing for forthcoming demands (Fries et al., 2009). In support, increased CARs have been observed in circumstances that require increased demand. Newly qualified doctors demonstrated greater CARs at the beginning of a

clinical placement, characterised by a lack of control, compared to the end of a placement (Brant et al., 2009). Increased CARs have also been observed on workdays, characterised by feelings of stress, compared to less stressful weekend days, in civil servants (Kunz-Ebrecht et al., 2004), in seafarers during onshore training (Liberzon et al., 2008) and in teachers following an observed demonstration lesson compared to a regular working day (Wolfram et al., 2013). Increased CARs have also been observed on the day of temporary stressors. Increased cortisol has been observed on mornings of dancing; motorcycling and tennis competitions compared to control days (Rohleder et al., 2007, Filaire et al., 2007, 2009) and levels remained elevated across the day of competition. Levels of cortisol during the CAR period have also been associated with affect. In a longitudinal study, indices of the CAR were positively associated with self-reported anticipation of the forthcoming day and state tension and stress recorded 45 minutes following awakening (Stalder et al 2010).

To empirically assess the effects of an anticipated challenge on diurnal indices of cortisol it is necessary to develop protocols that allow for the manipulation of forthcoming demand. The current study assessed the effects of anticipated challenge in relation to diurnal cortisol secretion and assessed the association between acute cortisol reactivity and basal functioning. It was predicted that anticipation of a challenging stressor would differentiate between the two days in terms of diurnal cortisol secretion and that acute cortisol reactivity would be representative of basal secretion.

METHOD

Participants

Healthy young participants were recruited from an undergraduate population and interested participants were screened on the basis of the following exclusion criteria: self-reported current or previous anxiety or stress-related disorder, hypertension, pregnancy, current medication apart from over-the-counter analgesia and the contraceptive pill. A total of 27 participants were recruited; however, N=4 failed to provide

saliva samples. A final sample of 23 participants (female = 17, male = 6, $M_{\text{age}} = 20.21$ years, $S.D = 4.23$) provided complete data and were included in analyses.

Materials & Apparatus

Perceived stress was measured using the 10 item Perceived Stress Scale (PSS) (Cohen et al., 1983), which, using responses ranging from ‘never’ to ‘very often’ provides a measure of perceived stress in the preceding month. Brief psychological distress was measured using the short form Profile of Mood States (POMS-SF) (Shacham 1983), which comprises 37 items with response ranging from ‘not at all’ to ‘extremely’. Scores from the POMS-SF are used to derive a total score for ‘mood disturbance’, as well as subscores for the domains of ‘tension’, ‘depressed’, ‘anger’, ‘vigour’, ‘fatigue’ and ‘concentration’. Paper diaries were used to record information regarding the provision of saliva samples including waking time, timing of samples as well as self-reports of the prior nights’ sleep and menstrual cycle stage. Heart rate and blood pressure measurements were taken using an upper arm inflatable cuff (Omron M2, Omron Worldwide, UK)

Procedure

All procedures were approved by the institutional ethics review board. Participants attended a baseline session to provide written consent and to complete the PSS and the POMS-SF. Participants were informed that the study would involve testing over two consecutive days: day one would involve the provision of saliva samples in their own homes and day two would involve an additional testing session in the laboratory.

All participants were given training regarding the appropriate collection and storage of saliva samples including a demonstration of how to provide saliva using salivettes (Sarstedt Ltd). In addition, the importance of the timing of samples and abstinence from behaviours known to affect the concentrations of cortisol in saliva were emphasised. Specifically, participants were asked to refrain from consumption

of food, caffeinated or alcoholic beverages, nicotine, brushing of teeth, the use of mouthwashes or antacids and exercise for 1 hour prior to provision of each sample (Kudielka et al., 2003).

Details of the day two testing session were then provided; specifically participants were informed that they would be required to attend the laboratory in the afternoon to take part in a stress task that would involve the completion of challenging speech and mental arithmetic tasks whilst being socially evaluated by a panel. Participants were provided with labelled salivettes and written instructions regarding the saliva collection protocol and the testing days were agreed between the researcher and the participant.

On two consecutive typical days, participants collected saliva by chewing on the cotton roll of a salivette for 1-2 min at four time points: immediately upon awakening, 30 minutes post awakening, at 1200 and immediately before bed. On day one all samples were provided in participants' homes. On day two, participants provided their awakening, 30 minutes post awakening and pre-bed samples at home and their 1200 sample was provided during a testing session in the laboratory. Samples collected in homes were refrigerated by participants until they were returned to the researcher. All samples were then frozen (-20 °c) and subsequently assayed in house using the enzyme-linked immunosorbent assay method (Salimetrics-Europe, Cambridge UK, intra and inter assay coefficients < 10%). To maximise adherence to the saliva collection protocol and as a means of assessing the timing of samples, participants were instructed to record the precise time at which they provided each of their saliva samples using a paper diary (Lovell et al., 2009)

On the test day participants attended the laboratory prior to the provision of their 1200 saliva sample, completed the PSS and were reminded that they were to take part in a stress task involving challenging tasks whilst being socially evaluated. A stress protocol based on the Trier Social Stress Test (Kirschbaum et al. 1995) was then administered. The chair of a three person panel instructed participants that they would have 10 minutes to prepare for a mock job interview. Following a 10 minute preparation period, participants presented to the panel and were asked to explain why they were the best candidate for the

chosen job for a period of 5 minutes; if speech faltered, they were prompted by the chair to continue. No other verbal interaction occurred. At the end of a 5 minute period, the participant was stopped and informed that they would be assessed for their mental arithmetic abilities. Participants were instructed to subtract aloud from 1017 in multiples of 13 for 5 minutes; if an incorrect response was given the Chair informed them that their response was incorrect and they must begin the task again. The POMS-SF was completed and heart rate and blood pressure recorded immediately before and after the stressor and 10 and 20 minutes following stressor cessation. Saliva samples were obtained immediately before (12:00), and 10 and 20 minutes following the stressor. Following provision of the final samples, participants were debriefed and remunerated £10.

Data Analysis

The efficacy of the stressor was assessed using a series of one-way ANOVAs with four sampling points (pre-stress, immediately post-stress, +10 min, +20 min) for POMS-SF items, heart rate, systolic blood pressure and diastolic blood pressure, and three sampling points (pre-stress, +10 min and +20 min) for salivary cortisol. Post-hoc analyses were conducted to assess post-stress changes (immediately post-stress, +10 min and +20 min) from pre-stress and those comparisons that remained significant following Bonferroni corrections for multiple comparisons are reported. Differences in perceived stress and POMS-SF items between the baseline and test day were assessed using paired samples t-tests.

The diurnal secretion of cortisol was assessed using 2-way repeated measures ANOVAs with day (day 1, test day) and time (awakening, +30, noon, bed) and individual time points were compared across days using paired samples t-tests. Total cortisol secretion was assessed by area under the curve with respect to ground (AUC_G). AUC_G was calculated for each participant on each day using the cortisol level (nmol/l) at each sampling point and the time (minutes) between each sample (Pruessner et al., 2003) for diurnal secretion (awakening, awakening +30, noon and bed) and for total cortisol secretion during the CAR

period (awakening and awakening +30). Additionally, area under the curve with respect to increase (AUC_I) from waking (Pruessner et al., 2003) and mean increase (awakening +30 values minus values at awakening) were also calculated during the CAR period.

Diurnal / CAR AUC was not calculated for participants who did not provide sufficient information regarding the timing of their saliva samples (n=2). AUC_G was also calculated on the test day (pre stress, 10 and 20 minutes post stress) to assess cortisol secretion in response to acute stress. Differences between day 1 and test day were compared using paired samples t-tests and relationships between diurnal / CAR indices and acute cortisol reactivity were assessed using Pearson correlations.

JUST ACCEPTED

RESULTS

Given the unequal number of males (n=6) and females (n=15) in the final sample, potential sex differences in cortisol indices were assessed. Males demonstrated significantly greater levels of cortisol at 1200 on Day 1 ($t_{(5,52)} = 2.79, p = 0.034$) but no other significant differences were observed.

Stressor manipulation

Psychological, cardiovascular and cortisol measures of acute reactivity and recovery are presented in Table 1.

Self-report stress and mood

There was a significant effect on feelings of tension ($F_{(3,20)} = 6.4, p = 0.003, \eta^2 = 0.49$); anger ($F_{(3,20)} = 4.66, p = 0.013, \eta^2 = 0.41$); concentration ($F_{(3,20)} = 4.01, p = 0.022, \eta^2 = 0.38$) and total mood disturbance ($F_{(3,20)} = 3.44, p = 0.037, \eta^2 = 0.34$); however, post-stress values were not significantly different from pre-stress values following correction for multiple comparisons. The stressor led to significant reductions in feelings of vigour ($F_{(3,20)} = 3.97, p = 0.023, \eta^2 = 0.37$); levels immediately ($p = 0.018$); 10 min ($p = 0.009$) and 20 min ($p = 0.006$) post-stress were significantly lower than pre-stress. In contrast, levels of fatigue reduced ($F_{(3,20)} = 6.46, p = 0.003, \eta^2 = 0.49$) with significant reductions from pre-stress to 10 min ($p = 0.018$) and 20 min ($p = 0.003$) post stress. There were no significant effects on feelings of depression ($F_{(3,20)} = 0.75, p = 0.54, \eta^2 = 0.10$).

Cortisol Reactivity

The stressor paradigm induced changes in cortisol which approached significance, $F_{(2, 21)} = 3.44, p = 0.051, \eta^2 = 0.25$). Bonferroni adjusted post-hoc comparisons revealed that following initial increases from pre to post stress, cortisol significantly reduced from 10 min to 20 min post-stress ($p = 0.04$).

Cardiovascular Reactivity

The stressor had a significant effect on levels of SBP ($F_{(3, 20)} = 28.80, p < 0.001, \eta^2 = 0.81$); levels immediately post-stress were greater than pre-stress ($p < 0.001$). The stressor exerted a similar effect on DBP ($F_{(3, 20)} = 11.57, p < 0.001, \eta^2 = 0.63$), with significantly greater DBP immediately post, ($p < 0.001$); 10 min ($p < 0.001$) and 20 min ($p = 0.009$) post stress relative to pre-stress. No significant changes were observed in HR.

INSERT TABLE 1 ABOUT HERE

Basal stress

Measures of psychological distress (baseline and test day) and cortisol indices (day 1 and test day) are presented in Table 2.

Self-report stress and mood

Levels of perceived stress ($t_{(22)} = 3.46, p = 0.002$), tension ($t_{(22)} = 2.34, p = 0.025$) and concentration ($t_{(22)} = 2.78, p = 0.01$) were significantly greater on the test day relative to baseline. There was also a trend towards greater levels of anxiety on the stress day compared to the baseline ($t_{(22)} = 1.81, p = 0.08$). There were no significant differences between the baseline and stress days for depression, anger, vigour, fatigue or total mood disturbance.

Cortisol Indices

There were no significant differences in self-reported time of awakening between day one and test day ($p = 0.35$). There was a significant main effect of time on diurnal cortisol ($F_{(3, 20)} = 37.22, p < 0.001, \eta^2 = 0.86$); t-tests revealed significant differences between all time-points ($p < 0.001 : p = 0.039$) representing the typical diurnal profile of cortisol characterised by a peak from awakening to 30 minutes post-awakening and a subsequent decline from the +30 minute sample to the afternoon and pre-bed samples. There was also a main effect of day ($F_{(1, 20)} = 14.03, p = 0.001, \eta^2 = 0.41$) representing significantly greater levels of cortisol secretion on the test day relative to day one. This was supported by greater secretion of cortisol as indexed by AUC_G on the test day compared to day 1 ($t_{(20)} -2.39, p = 0.027$). Furthermore, CAR indices of $CAR AUC_G$ ($t_{(20)} -3.26, p = 0.004$), CAR mean output ($t_{(20)} -3.26, p = 0.004$) and peak levels ($t_{(20)} -2.99, p = 0.007$) were greater on the test day compared with day 1 as were the mean increase during the CAR period ($t_{(20)} -1.77, p = 0.09$) and the $CAR AUC_1$ ($t_{(20)} -1.77, p = 0.09$) although not significantly so. Diurnal cortisol profiles on day one and the test day are presented in Figure 1.

INSERT TABLE 2 ABOUT HERE

INSERT FIGURE 1 ABOUT HERE

Significant relationships were observed between indices of basal function and acute cortisol reactivity. Greater secretion of cortisol across the diurnal period was related to greater pre-stress levels of cortisol (r

= .91) and higher individual peak response ($r = .59$) and greater secretion of cortisol ($r = .72$) across the stress period. Levels of cortisol upon awakening were higher in those that demonstrated the greatest levels of cortisol immediately pre-stress ($r = .57$) and the greatest secretion of cortisol during the acute stress period ($r = .44$). Finally, greater secretion of cortisol during the CAR period was related to greater pre-stress levels ($r = .59$), greater individual peak response ($r = .57$) and greater cortisol secretion ($r = .61$) during the stressor period. Correlation coefficients are reported in Table 3.

INSERT TABLE 3 ABOUT HERE

DISCUSSION

Using a two day testing protocol this is the first study to assess the effects of an anticipated laboratory stressor on indices of diurnal cortisol secretion. Typical patterns of cortisol secretion, characterised by an increase in the 30 minutes following awakening and a diurnal decline towards a nadir before bedtime, were observed on both days; however, the second day was characterised by greater diurnal secretion of cortisol, in particular during the CAR period. The two sampling days were the same with the exception of participation in a laboratory stressor on the test day and as such, the greater secretion of cortisol can be attributed to this atypical but anticipated challenging event. Specifically, the greater secretion of cortisol on the test day is in the main, driven by greater secretion of cortisol during the CAR period. This supports previous observations of an anticipatory effect such that cortisol levels increase when faced with novel challenging procedures (Lovallo et al., 2010) and identifies the influence of state factors on the CAR (Hellhammer et al., 2007).

The current design has enabled us to directly compare the CAR on a typical day (on which trait factors alone impacted upon the CAR) with a day in which both trait and state factors influenced the CAR.

Given that the participants in our study had been explicitly told that the test day would involve exposure

to a laboratory stressor involving social evaluation and cognitive challenge, we suggest that our data reflect diurnal cortisol variation in response to anticipation of forthcoming demands. Specifically, the greater levels following awakening observed on the test day reflect the proposed adaptive nature of the CAR (Clow et al., 2010) and suggest that the CAR may play a role in preparation for forthcoming daily challenges. Further research investigating the influence of stress anticipation on the CAR, and the role of the CAR in preparing the individual for forthcoming demands is therefore warranted.

While this is the first study to report that the CAR is modulated by anticipation of a manipulated laboratory stressor, similar increases have been associated with periods of increased demand (Brant et al., 2009; Kunz-Ebrecht et al., 2004; Liberzon et al., 2008) and single anticipated challenges (Rohleder et al., 2007; Filaire et al. 2007, 2009). Furthermore, higher levels of cortisol in the CAR period have been associated with increased reports of state tension, stress and anticipation of forthcoming tension (Stalder et al., 2010). In support, self-reported levels of perceived stress, tension and anxiety in the current study were also greater on the morning of the anticipated stressor. These findings are concomitant with the notion that the CAR serves to maximise day to day functioning (Clow et al., 2010) and plays a role in the preparation of forthcoming challenges (Fries et al., 2009). The current study adds further support to this notion by replicating the observations from naturalistic studies through the explicit manipulation of forthcoming demand.

The current study design also enabled the combination of a controlled acute event (the laboratory stressor) with otherwise typical activity in an ambulatory setting over a two day period. This design provided the opportunity to assess basal functioning, as well as reactivity to and recovery from an acutely challenging event. During recruitment participants were instructed to select two consecutive, typical days for participation. As such, the diurnal profile obtained on day 1 provided an indication of each individual's typical CAR and diurnal cortisol profile when acutely stressful events were not anticipated or experienced, whereas the cortisol samples collected on the test day enabled the influence of acute psychosocial stress exposure on the CAR, diurnal profile and cortisol reactivity to all be investigated.

Comparing observations on the day of an acute stressor with a resting control day is a recommended approach to the assessment of individual differences in cortisol responding (Lovallo et al., 2010) and serves as a more appropriate reference point when assessing the effects of acute stress on cortisol responses (Wolfram et al., 2013). Furthermore, given the state-like influences on diurnal secretion, particularly during the CAR period, two-day protocols allow for the assessment of acute stress on the underlying diurnal cycle. In this instance, we have been able to observe the effects of anticipating an acutely stressful event on basal functioning of the HPA axis in a more controlled manner than afforded by an observation of a pre-existing event and with more flexibility and ecological validity than afforded by an entirely laboratory based protocol.

The current protocol also allowed for the investigation of potential relationships between acute cortisol reactivity and indices of basal HPA function. The association between acute and diurnal secretion supports recent observations from a large cross-sectional sample (Kidd et al., 2014) and reinforces the use of laboratory stressor techniques as valid analogues of everyday function. Additionally, the current study observed the previously unreported association between cortisol secretion during the acute stressor and cortisol awakening periods suggesting that the CAR may play a priming role and influence subsequent function across the day (Clow et al., 2014).

The current study should however be considered in light of its limitations. First, the sample size is small; however, it was sufficient to detect meaningful differences in the predicted indices of diurnal secretion. Second, the reliability of diurnal cortisol measurement is reliant on good adherence to the sampling protocol, including the accuracy of the timing of samples. In line with recommendations (Okun et al., 2010; Adam & Kumari, 2009; Saxbe, 2008) steps were therefore taken to maximise protocol adherence. Participants were given verbal and written instructions and the importance of sample timing was emphasised. Individuals are generally accurate in the reporting of their own wake up times (Kraemer et al., 2006; DeSantis et al., 2009) and it is often easier for participants to integrate saliva sampling into more standardised morning routines (Golden et al., 2014). As such, participants were requested to

accurately record the times at which they provided their samples in relation to waking and two participants were subsequently excluded on the basis of timing discrepancies and suspected non-adherence. The remaining participants reported good adherence to the sampling protocol. Although evidence suggests that the use of self-reported sample timings are effective in ensuring protocol adherence, and moreover are preferred by participants (Kraemer et al., 2006), other techniques, for example, Medical Event Monitoring (MEMS) caps (Smyth et al., 2013) and actigraphy (Clow et al., 2014) would provide additional markers of adherence with regards to sampling accuracy and sleep and awakening times respectively.

Third, the current study used only two samples to index the CAR. Although the protocol was devised to reduce participant burden and ease adherence to protocol, a greater number of samples during the post-awakening period (e.g., 15, 45 and 60 minutes post-awakening) would provide more robust indices of the CAR (Stalder et al., 2009). These initial findings therefore warrant a more thorough assessment of the CAR in relation to manipulated forthcoming demand; however, protocols with increased sampling should be mindful of not over-burdening participants to minimise impact on recruitment or retention (Wetherell & Montgomery 2014).

Finally, although ambulatory studies provide the opportunity for real life assessment, they lack the level of control afforded in laboratory studies. Participants were instructed to identify two typical consecutive days for study protocol and were notified of the second day stressor manipulation from the commencement of participation. Participants did not report any atypical events; however, in the absence of objective observation of participants across the study period, changes in the CAR cannot be solely attributed to the anticipation of the laboratory stressor. This naturalistic sampling also prevents the counterbalancing of conditions that would typically be employed in an experimental manipulation. That is, random allocation of participants to experience the stressor on either day 1 or day 2 would avoid potential systematic differences that may occur. Although we cannot rule out any such effect, there were no differences in sampling times or non-stressor procedures between the two days. Furthermore, the

current design offers a good representation of how people typically function in relation to forthcoming events in the everyday life.

Conclusions

This study has observed for the first time, differences in cortisol secretion across the diurnal period in relation to a manipulated stressful event. Moreover, this difference was in the main, driven by changes during the period immediately following awakening. That increased levels of cortisol were evident during the CAR period on the day of a forthcoming challenging laboratory stressor provides empirical evidence for the notion proposed by Clow et al., (2010) that the CAR is an adaptive mechanism that aids maximal day to day functioning. That is, an increased CAR serves as a preparatory mechanism that provides an individual with sufficient resources to cope with an anticipated demanding event, in this case an anticipated cognitively challenging stressor. Furthermore, secretion during the CAR period was concomitant with secretion during the acute stress phase, demonstrating that the CAR may influence subsequent functioning across the day (Clow et al., 2014).

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DECLARATIONS

The authors report no conflicts of interest

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Fig 1. Diurnal cortisol profiles on Day 1 and Test Day (S.E)

**p < 0.01 (CAR peak, CAR AUC_G) *p < 0.05 (Diurnal AUC_G)

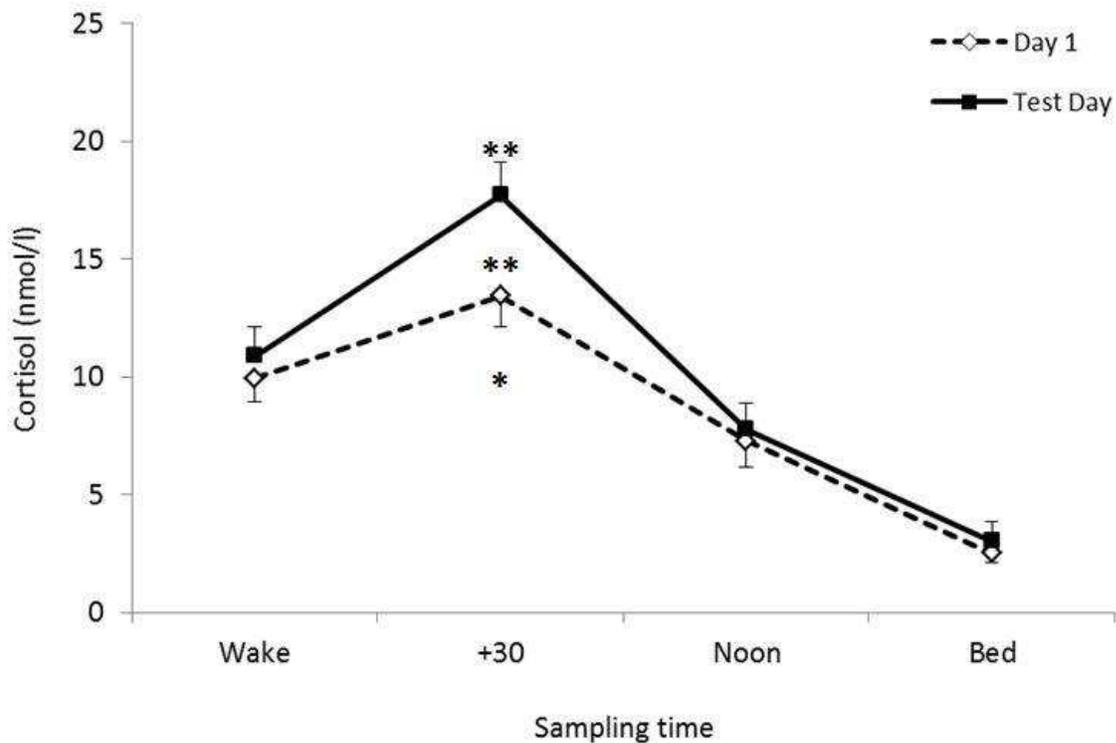


Table 1 Mean (s.e) psychological, cardiovascular and cortisol measures of reactivity and recovery

| | Pre-stress | Post-stress | +10 | +20 |
|-------------------------------------|---------------|--------------------|-------------------|-------------------|
| Tension | 12.57 (.91) | 14.83 (1.06) | 11.52 (.73) | 10.96 (.65) |
| Depression | 12.26 (.92) | 13.26 (1.03) | 12.61 (.94) | 12.00 (1.07) |
| Anger | 12.26 (1.13) | 12.30 (1.10) | 10.96 (.94) | 10.48 (.84) |
| Vigour | 16.96 (.86) | 15.39 (.82)* | 15.00 (.85)** | 14.74 (.86)** |
| Fatigue | 9.70 (.63) | 9.48 (.82) | 8.13 (.54)* | 7.74 (.49)** |
| Concentration | 10.44 (.63) | 11.70 (.86) | 10.13 (.63) | 9.48 (.62) |
| Total disturbance | 40.83 (3.36) | 46.17 (4.00) | 38.35 (2.99) | 35.91 (2.99) |
| Heart rate (bpm) | 71.74 (1.93) | 75.74 (2.94) | 73.04 (2.31) | 73.09 (2.26) |
| Systolic blood pressure (mm hg) | 115.13 (2.33) | 128.04 (2.90)** | 117.09 (2.58) | 114.48 (2.44) |
| Diastolic blood pressure (mm hg) | 67.30 (1.19) | 75.70 (1.82)** | 72.22 (1.38)** | 70.57 (1.50)** |
| Salivary cortisol (nmol/l) | 7.77 (1.04) | | 9.63 (1.74) | 8.50 (1.41) |
| Acute Peak | | | | 10.23 (1.88) |
| Acute Reactivity | | | | 2.08 (1.43) |
| Acute AUC _G | | | | 276.49 (43.82) |

Denotes significant difference from pre-stress: ** $p < 0.01$ * $p < 0.05$

NB. +10 min = 10 min following cessation of the task (20 min following onset of social evaluative task / 30 min following instructions)

Table 2 Mean (s.e) psychological and cortisol measures at baseline, day 1 and test day

| | Baseline | Day 1 (pre-test) | Test Day |
|-------------------------------|-------------|------------------|-----------------|
| Perceived Stress ** | 14.57 (.99) | | 17.39 (.96) |
| Anxiety | 6.35 (.72) | | 7.34 (.78) |
| Depression | 1.87 (.48) | | 2.13 (.40) |
| Tension * | 10.21 (.74) | | 12.57 (.91) |
| Depression | 11.26 (.89) | | 12.26 (.92) |
| Anger | 11.30 (.77) | | 12.26 (1.13) |
| Vigour | 17.47 (.75) | | 16.96 (.86) |
| Fatigue | 9.65 (.70) | | 9.70 (.63) |
| Concentration * | 9.09 (.71) | | 10.44 (.63) |
| Total disturbance | 34.09 (.30) | | 40.83 (3.36) |
| Awakening time | | 08:43 (0:20) | 08:28 (0:14) |
| Salivary cortisol (nmol/l) | | 10.47 (1.08) | 11.51 (1.25) |
| Awakening +30 ** | | 13.92 (1.34) | 18.13 (1.52) |
| Noon | | 7.31 (1.15) | 8.15 (1.07) |
| Bedtime | | 2.60 (.38) | 3.21 (.86) |
| Diurnal AUC _G * | | 5058.72 (491.0) | 6144.27 (596.4) |
| CAR AUC _G ** | | 365.91 (33.3) | 444.64 (34.6) |
| CAR AUC _I | | 51.58 (16.7) | 99.31 (23.8) |
| CAR Mean Increase | | 3.44 (1.12) | 6.62 (1.59) |
| CAR Mean output ** | | 12.20 (1.1) | 14.82 (1.2) |

** p < 0.01 * p < 0.05

Table 3 Correlation coefficients for basal and acute reactivity indices

| | Acute reactivity Indices | | | |
|-----------------------------|--------------------------|--------|------------|------------------|
| | Pre-Stress | Peak | Reactivity | AUC _G |
| Test Wake | .57 ** | .36 | .02 | .44 * |
| CAR AUC _G | .59 ** | .57 ** | .30 | .61 ** |
| CAR AUC _I | -0.7 | .26 | .40 | .20 |
| Diurnal AUC _G | .91 ** | .59 ** | .06 | .72 ** |

* $p < 0.05$ ** $p < 0.01$

JUST ACCEPTED