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Examination stress results in altered cardiovascular responses to acute challenge and lower cortisol ☆

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Summary
The present study examined how cardiovascular and salivary cortisol responses varied in response to an acute challenge in medical students under exam stress versus those not under exam stress. One hundred and twenty-nine medical students were randomly assigned to undertake a CO_2 inhalation test either prior to an examination period (exam group) or during a regular academic period (non-exam group). Heart rate (HR) and blood pressure (BP) were measured for 5 min before and 5 min after the task, and salivary cortisol samples were collected 1 min before and 10 and 30 min after the CO_2 inhalation test. Participants also completed a questionnaire measuring self-reported perceived stress.
The exam group exhibited significantly higher HR reactivity following the CO_2 inhalation test and slower systolic blood pressure (SBP) recovery compared with the non-exam group. The exam group also reported higher perceived stress and higher stress scores were related to higher HR reactivity following CO_2 inhalation. Female students across both groups exhibited significantly lower SBP reactivity compared with male students. Salivary cortisol levels were consistently lower in the exam group.
These findings indicate that ongoing natural stress alters cortisol secretion and cardiovascular responses in the face of an acute stress challenge.

1. Introduction

It is now well documented that both chronic and acute stressors can have adverse effects on a range of psychological and physiological outcomes. It is widely agreed that activation of the hypothalamic pituitary adrenal axis (HPA)

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and the autonomic nervous system (ANS) during periods of transient or acute stress may be adaptive (Segerstrom and Miller, 2004); and that chronic activation can result in maladaptive outcomes such as impaired immunity (Kiecolt-Glaser et al., 1995; Vedhara et al., 1999), increased vulnerability to new diseases (Reiche et al., 2005), and more rapid progression of existing conditions (Leserman et al., 2000). These findings have been observed in numerous highly stressed populations, such as students undergoing academic assessment and spousal caregivers, yet it remains unclear how episodes of chronic stress impact on physiological and neuroendocrine responses during other acutely stressful challenges.

Recent studies exploring how responses to experimental laboratory stressors are affected by other naturally occurring stressful experiences, suggest that responsiveness is altered by the presence/absence of 'background stress' (Fleming et al., 1987; Matthews et al., 1997; Gump and Matthews, 1999). For example, notable variations in cardiovascular response following a reaction time task were reported in children 2 months following the 9/11 terrorist attacks in 2001 (Gump et al., 2005). Similarly, cardiovascular responses to four acute laboratory stress tasks were heightened in children and adolescents reporting ongoing and frequent background stressors (Matthews et al., 1997), and a prospective study of the same children identified that BP reactivity and cardiac output during the laboratory stressors predicted subsequent rises in resting BP levels 3 years later (Matthews et al., 2003). In contrast, middle-aged participants reporting higher chronic stress have been shown to exhibit lower systolic blood pressure (SBP), and lower cortisol, epinephrine and norepinephrine secretion following laboratory tasks (Matthews et al., 2001). Males exhibited higher diastolic blood pressure (DBP) responses during the tasks and higher SBP, DBP and epinephrine responses during recovery, which is consistent with previously reported gender disparity in cardiovascular and neuroendocrine responses to acute stress tasks (Stoney et al., 1988; Girdler et al., 1990; Kirschbaum et al., 1992; Dixon et al., 2004).

Although research using laboratory stress tasks provides useful enquiry, results are equivocal due to limitations of the laboratory tasks, such as participant habituation to the task, uncertain degree of challenge, and the inability to measure concurrent activation of several biological pathways. Several recent investigations have evaluated the usefulness of CO_2 inhalation as a momentarily intense laboratory stressor (Argyropoulos et al., 2002; Kaye et al., 2004; Wetherell et al., 2006). Historically, CO_2 inhalation has been used in psychiatry to precipitate panic attacks and enable diagnosis of panic disorder (Griez et al., 1990; Verburg et al., 1998). Inhalation of a single breath of CO_2 and oxygen induces acute hypercapnia which is mildly anxiogenic in healthy subjects and provides a well tolerated and non-invasive method of activating neuroendocrine, cardiovascular and psychological responses to stress. Stable patterns of cardiovascular reactivity following 35% CO_2 inhalation have been observed over a 4–6 week follow-up period, with a bradycardiac response that supports simultaneous activation of the sympathetic (SNS) (increased BP) and parasympathetic (PNS) (decreased heart rate (HR)) nervous systems (Kaye et al., 2004; Wetherell et al., 2006). Additionally, these investigations have reported activation of both HPA and sympathetic–adrenal–medullary (SAM) systems following 35% CO_2 inhalation, evidenced by increased plasma and salivary cortisol and noradrenaline secretion (Argyropoulos et al., 2002; Kaye et al., 2004; Wetherell et al., 2006).

As CO_2 inhalation offers an advantageous method for eliciting and measuring an acute stress response, including simultaneous activation of key stress response systems, we have included the CO_2 inhalation test in the present study. In addition, the temporal proximity of academic examinations has been included as a background stressor, as to date only a modest number of studies have examined how background stress affects responses to acute laboratory stressors. Academic examinations provide a real-life threat for many students and although predictable and short-lived, the unfavourable impact on students has been well supported in the stress literature. Students report elevated anxiety and other negative emotional states at exam time (Spangler et al., 2002), and exhibit altered neuroendocrine, immune status and health outcomes (Amario et al., 1996; Marucha et al., 1998; Glaser et al., 1999a; Sarid et al., 2001; Chiu et al., 2003). Cardiovascular responses measured during intense academic periods have shown challenge-specific responses that differentiate them from laboratory challenges (Spangler, 1997), maximum BP, HR and plasma cortisol values measured during examinations (Fortuyn et al., 2004), and persistent physiological changes 12–14 days following an examination period (Vassend et al., 1987).

This research sought to determine how individuals burdened by impending academic assessment would respond to provocation from an acute stressor, and whether physiological reactivity to the challenge differentiates them from those under less stress or those reporting lower levels of perceived stress. We hypothesised that students tested shortly before an examination period would report higher psychological stress and would exhibit higher baseline and peak salivary cortisol levels, higher cardiovascular reactivity and poorer recovery following a CO_2 inhalation test compared with students tested during a less stressful academic period. We expected that comparison of physiological responses to a laboratory challenge in stressed and non-stressed participants might enable the identification of physiological mechanisms that may lead to poorer health outcomes in stressed individuals.

2. Methods

2.1. Participants

A total of 129 undergraduate medical students participated in this study, with 73 enroled in year 2 and 56 in year 3 at The University of Auckland, New Zealand. The students were aged between 17 and 34 years of age with a mean age of 21 years (SD = 3.47), and consisted of 81 females and 48 males. Just over half the sample (51%) was European, 34.9% were Asian, and 14.1% were from other ethnic groups.

2.2. Experimental procedure

The study was granted ethics approval by the University of Auckland Human Participants Ethics Committee in August

2004. Participant recruitment began in October 2004 for year 3 students and March 2005 for year 2 students. Written informed consent was gained on the day of the CO_2 inhalation test. Each participant was paid NZ$50.00 to participate in the study.

Participants were randomly assigned to one of two groups; the non-exam group carried out the experimental procedures during the first 4 weeks of the semester, and the exam group carried out the procedures at the end of the semester, 2–4 weeks before the mid year examinations. Randomisation was carried out independently of the principal investigators. Equal numbers of the words "exam" and "non-exam" were placed inside envelopes and ordered according to random number tables with equal numbers of males and females in each group. The study procedures consisted of a CO_2 inhalation test, salivary cortisol collection and completion of demographic and psychological questionnaires.

2.3. CO_2 inhalation test

Each participant underwent a physiological stress challenge by inhaling one single breath of 35% CO_2 and 65% oxygen. A single inhalation of 35% CO_2 has been found to reliably stimulate both the HPA and ANS and is safe and well tolerated by research participants (Van Beek and Griez, 2000; Wetherell et al., 2006). Participants visited the laboratory on one occasion when they sat in a reclining chair and an automated BP cuff and sensor was placed on the left arm. Physiological measurements (HR, SBP and DBP) were recorded through a powerlab and tonometer. A Douglas bag filled with 35% CO_2 and 65% oxygen (supplied by BOC Limited, Auckland) was attached to a three-way tap and mouthpiece. A pneumotachometer was attached to the three-way tap to measure and record each participant's vital capacity breath. A viral filter was attached to the mouthpiece which was placed in the participant's mouth and the nostrils were occluded with a nose clip. Participants practised the CO_2 procedure by inhaling a vital capacity breath with the three-way tap open to air, and the practise breath was visually displayed on a computer screen. Once familiar with the procedure, participants rested quietly and HR and BP measurements were then recorded continuously for 5 min. Participants then exhaled fully and inhaled a single vital capacity breath of the 35% CO_2 mixture and held it for 4 s. Participants were encouraged to observe the computer screen in order to match the vital capacity breath practised earlier. Following inhalation, the tap to the CO_2 mixture was closed, the mouthpiece and nose clip were removed, and participants were instructed to breathe normally. HR and BP measurements were recorded continuously (at 1 min intervals) throughout the CO_2 inhalation test, from 5 min before CO_2 inhalation to 5 min after inhalation. The CO_2 inhalation test procedure is shown in Fig. 1. In line with previous investigations examining 35% CO_2 inhalation as an acute experimental stressor in healthy volunteers (Argyropoulos et al., 2002; Kaye et al., 2004; Wetherell et al., 2006), none of the participants experienced distress following CO_2 inhalation and no adverse events were recorded during the procedure.

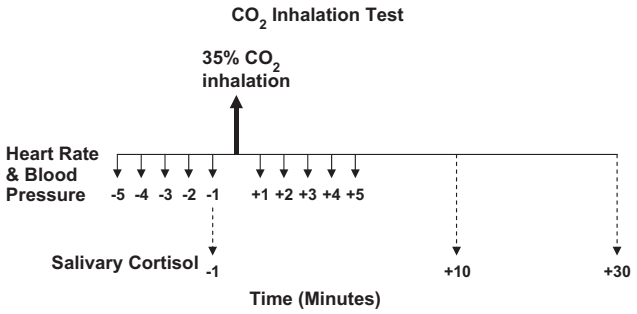

Fig. 1 The CO_2 inhalation test.

2.4. Salivary cortisol

Participants were instructed not to eat for half an hour before attending the CO_2 inhalation test. Three salivary cortisol samples were collected from participants using salivettes (Sarstedt Ltd, Aktiengesellschaft & Co, Germany, D-51588), at 1 min before CO_2 inhalation, and at 10 min and 30 min after CO_2 inhalation. As in previous investigations, these intervals have been used to capture peak salivary cortisol secretion following CO_2 inhalation (Argyropoulos et al., 2002; Wetherell et al., 2006), as delays in peak salivary cortisol secretion of 30 min following acute challenge have been observed compared to plasma cortisol secretion (Aardal-Eriksson et al., 1998). Participants were instructed to chew on a cotton swab for 30 s at each time point. The saturated swab was then returned to the salivette and samples were frozen and stored until assayed for salivary cortisol concentration. Salivary cortisol assays were performed commercially (LabPlus, Auckland City Hospital, New Zealand) using cortisol electrochemiluminescence immunoassay (ECLIA) kits (Roche Diagnostics, Switzerland) (Fig. 2).

2.5. Psychological measures

All participants completed a questionnaire following the CO_2 inhalation test. The questionnaire included participant demographics, such as age, gender and ethnic group. The 14-item Perceived Stress Scale (PSS) was included in the questionnaire to assess how stressful participants' perceived their lives to have been over the past month (Cohen et al., 1983). Participants rated items such as "How often have you felt nervous and stressed" from 0 to 4 as "never, almost never, sometimes, fairly often or very often". The PSS has been used widely in the stress literature, with reports of significant relationships between perceived stress and poorer immune outcomes (Glaser et al., 1999b; Burns et al., 2002). Internal consistency for the measure is high, with Cronbach's alpha previously assessed at 0.75–0.86. Test re-test reliability over 2 days has been assessed at 0.85 and the PSS has been found to predict future physical symptoms, with correlations ranging from 0.52 to 0.70 (Cohen et al., 1983).

2.6. Data analysis

In order to analyse changes in HR, SBP, DBP and salivary cortisol in relation to the CO_2 inhalation test, a robust regression analysis was carried out using the Huber–White

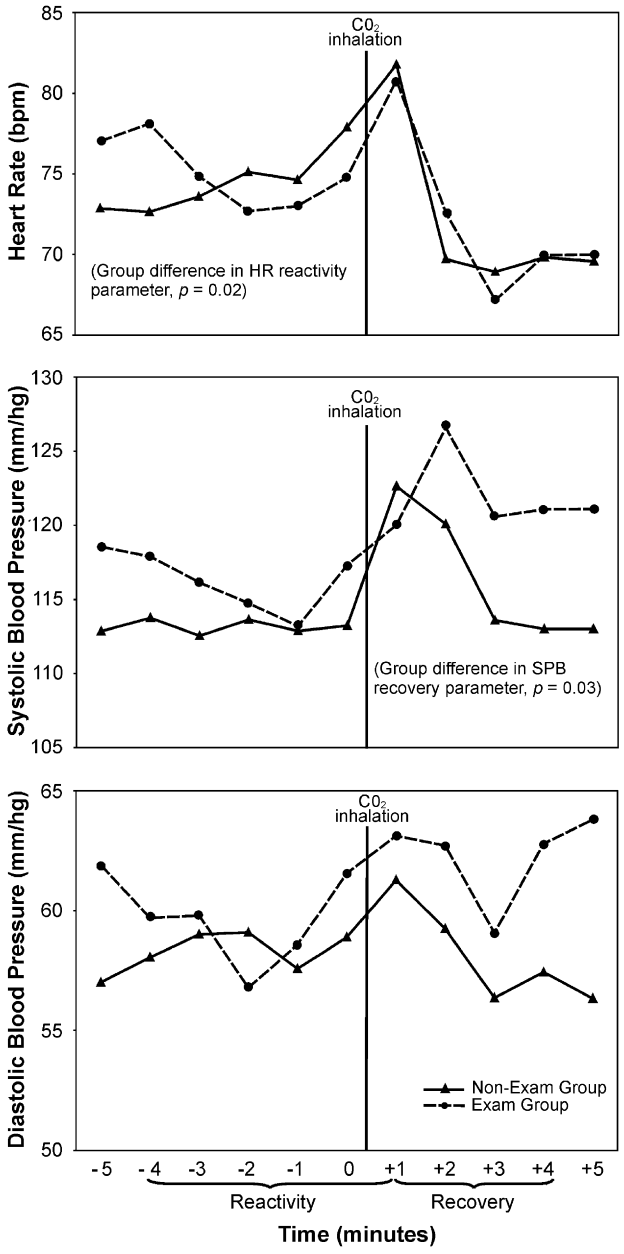

Fig. 2 Group differences in HR, SBP and DBP 5 min before and 5 min following CO_2 inhalation.

sandwich estimator in Stata 8.0. Each model contained regression parameters for the intercept, reactivity and recovery. The intercept was a reference value against which all other parameters were assessed and represented the mean values for HR, SBP and DBP at 1 min post-CO_2, except for cortisol where the intercept represented the mean value at 10 min post-CO_2. The reactivity parameter represented the change in HR, SBP and DBP between 4 min pre-CO_2 and 1 min post-CO_2, and between 1 min pre-CO_2 and 10 min post-CO_2 for cortisol. The recovery parameter represented the change in HR, SBP and DBP from 1 min post-CO_2 to 4 min post-CO_2, and between 10 min post-CO_2 and 30 min post-CO_2.

Interaction variables were created to explore how the reactivity and recovery parameters varied according to examination status (temporal proximity to examinations), gender and self-reported perceived stress. Thus, models were constructed to examine reactivity and recovery with no covariates, with each covariate separately, and with all covariates in a single model.

As the CO_2 inhalation test was administered to participants throughout the day (between 08:00 and 17:00), the potential influence of diurnal cortisol rhythms was controlled in the data analysis, with cortisol values residualised on time and time squared to control for the effects of the time of testing.

3. Results

Initially, HR was assessed to ascertain whether it was altered by the CO_2 inhalation test. HR during both reactivity and recovery phases was significantly altered by CO_2 inhalation, with HR at 4 min prior to CO_2 inhalation 5.79 units lower than the intercept of 81, and 11.36 units lower than the intercept during the recovery phase.

When each of the covariates exam, PSS and gender were analysed in individual interaction models, the data revealed HR following the CO_2 inhalation test was altered by both exam status and stress scores. Although exam status did not affect HR during recovery, individuals in the exam group exhibited higher HR during the reactivity phase (6.4 units higher than the non-exam group). In effect, exam group HR dropped less in response to the CO_2 inhalation test. Similarly, as stress scores increased, HR increased in the reactivity phase; that is, the higher the stress score the less HR dropped in response to the CO_2 inhalation test. HR during the recovery phase was not significantly altered by stress scores, and no effect was found for gender on HR reactivity and recovery parameters.

When all covariates were included together in a single model, results were consistent with the individual models. Results from this model are displayed in Table 1. HR in the reactivity phase was significantly higher in the exam group although exam status did not alter HR during the recovery phase. This result was also found for stress scores, with higher HR in the reactivity phase as stress scores increased, although this effect almost approached significance. Both perceived stress and exam status resulted in less HR drop following the CO_2 inhalation test.

The analysis also revealed the CO_2 inhalation test significantly altered SBP. Although during the reactivity phase our findings approached significance, SBP was significantly altered by the CO_2 inhalation test during the recovery phase, measuring 4.18 units higher than the intercept of 113.

When each of the covariates exam, PSS and gender were analysed individually, results showed both exam status and gender significantly altered aspects of the SBP response to the CO_2 inhalation test. SBP during the reactivity phase was not significantly affected by exam status, however, exam status significantly affected SBP during the recovery phase with SBP 7.7 units higher in the exam group. SBP reactivity to the CO_2 inhalation test differed significantly between genders, with females showing less reactivity to the task (8.74 units lower in females), although SBP recovery from the task was not affected by gender. Self-reported

Table 1 HR results with all covariates (exam, PSS and gender).

HR $n = 129$	*Coef.*	$p > \|t\|$	(95% confidence interval)	
Reactivity	−14.96	0.001	−23.99	−5.92
Recovery	−16.53	0.00	−25.51	−7.55
PSS	0.12	0.52	−0.26	0.51
Exam status	−1.28	0.67	−7.40	4.83
Gender (female)	2.20	0.47	−3.83	8.25
Reactivity × female	−1.63	0.54	−6.95	3.70
Recovery × female	−0.03	0.99	−5.22	5.16
Reactivity × PSS	0.30	0.07	−0.03	0.64
Recovery × PSS	0.20	0.21	−0.11	0.53
Reactivity × exam	5.66	0.02	0.72	10.60
Recovery × exam	0.54	0.82	−4.32	5.41
Intercept	77.51	0.00	67.08	87.94

Table 2 SBP results with all covariates (exam, PSS and gender).

SBP $n = 129$	*Coef.*	$p > \|t\|$	(95% confidence interval)	
Reactivity	2.67	0.65	−9.14	14.49
Recovery	7.51	0.28	−6.30	21.33
PSS	0.01	0.96	−0.46	0.42
Exam status	0.21	0.95	−5.99	6.41
Gender (female)	−11.63	0.001	−18.52	−4.73
Reactivity × female	−8.87	0.01	−15.82	−1.92
Recovery × female	−5.92	0.22	−15.38	3.54
Reactivity × PSS	0.15	0.53	−0.33	0.63
Recovery × PSS	−0.16	0.54	−0.68	0.36
Reactivity × exam	3.55	0.25	−2.55	9.65
Recovery × exam	8.11	0.03	0.67	15.54
Intercept	120.55	0.00	108.89	132.22

perceived stress did not significantly influence either SBP reactivity or recovery from the CO_2 inhalation test.

When all covariates were included together in a single model, results showed this model agreed with the individual models. Females had significantly lower SBP reactivity compared with males, temporal proximity to examinations significantly affected SBP recovery, and neither SBP reactivity nor recovery were affected by scores on the PSS. Results from this model are displayed in Table 2.

Once again, findings indicated the CO_2 inhalation test significantly altered DBP, with evidence of a significant reduction in the reactivity phase (3.36 units lower than the intercept of 62), although the task did not significantly alter DBP during the recovery phase. Under the individual models neither exam status, scores on the PSS, nor gender, significantly altered DBP reactivity or recovery from the CO_2 task, and these findings were again reflected in a single model that included all covariates. Results from this single model are displayed in Table 3.

When salivary cortisol secretion was analysed in response to the CO_2 inhalation test, findings revealed the task significantly altered cortisol secretion during the reactivity phase (0.07 units higher at 1 min prior to CO_2 inhalation than the intercept of −0.02), although CO_2 inhalation did not significantly alter cortisol secretion in the recovery phase.

When each of the covariates, exam, PSS and gender, were analysed in individual interaction models, cortisol secretion following the CO_2 inhalation test was not altered by any covariate. However, a significant difference in cortisol secretion was found between the exam and non-exam groups, with secretion in the exam group 0.57 units lower than the non-exam group 10 min following the task. Mean cortisol secretion in the exam group was 7.50 (SD = 3.78) at 1 min before inhalation, 7.37 (SD = 4.02) 10 min post-inhalation, and 7.19 (SD = 3.82) 30 min post-inhalation, compared to the non-exam group of 9.82 (SD = 3.11) 1 min before inhalation, 9.41 (SD = 3.46) 10 min post-inhalation and 9.50 (SD = 3.24) 30 min post-inhalation.

Similarly, when all covariates were included together in a single model, the overall cortisol responses in the exam group were 0.55 units lower compared with the non-exam group. Again, there were no significant findings for stress scores and gender under this single model. Results from this model are displayed in Table 4.

Analysis of PSS items revealed high internal consistency, with a cronbach's alpha of 0.85. When PSS scores were

Table 3 DBP results with all covariates (exam, PSS and gender).

DBP $n = 129$	*Coef.*	$p > \|t\|$	(95% confidence interval)	
Reactivity	−2.51	0.65	−13.49	8.48
Recovery	−4.96	0.31	−14.67	4.75
PSS	0.03	0.86	−0.31	0.36
Exam status	1.82	0.49	−3.34	6.98
Gender (female)	−3.40	0.21	−8.78	1.98
Reactivity × female	−1.52	0.66	−8.41	5.37
Recovery × female	−1.68	0.62	−8.44	5.08
Reactivity × PSS	0.01	0.97	−0.45	0.47
Recovery × PSS	0.09	0.62	−0.29	0.49
Reactivity × exam	−0.28	0.93	−7.29	6.72
Recovery × exam	3.13	0.30	−2.85	9.10
Intercept	62.69	0.00	54.37	71.01

Table 4 Cortisol results with all covariates (exam, PSS and gender).

Cortisol (residualised) $n = 129$	*Coef.*	$p > \|t\|$	(95% confidence interval)	
Reactivity	0.13	0.32	−0.13	0.40
Recovery	−0.05	0.73	−0.35	0.24
PSS	−0.007	0.57	−0.03	0.01
Exam status	−0.55	0.003	−0.92	−0.19
Gender (female)	−0.158	0.42	−0.55	0.23
Reactivity × female	0.103	0.15	−0.04	0.24
Recovery × female	0.12	0.24	−0.08	0.32
Reactivity × PSS	−0.003	0.44	−0.01	0.006
Recovery × PSS	0.00	0.99	−0.01	0.01
Reactivity × exam	−0.06	0.41	−0.19	0.08
Recovery × exam	−0.07	0.46	−0.25	0.12
Intercept	0.53	0.12	−0.14	1.21

compared between stress groups, findings revealed the exam group reported significantly higher perceived stress, $t\ (127) = -2.25$, $p = 0.02$, with a mean stress score of 25.22 (SD = 7.13) compared with 22.52 (SD = 6.35) for the non-exam group.

4. Discussion

This study found that individuals undergoing the natural stress of academic examinations exhibited distinct differences in HR and SBP following a brief laboratory stress task compared with individuals during a regular academic period. In line with responses previously reported in studies using the CO_2 inhalation test (Wetherell et al., 2006), both groups produced a drop in HR following CO_2 inhalation, although medical students due to undertake examinations exhibited less of a drop in HR during the reactivity phase. Additionally, the exam group exhibited higher SBP during the recovery phase compared with students undergoing the challenge during a less demanding period. The HR and SBP responses exhibited following the CO_2 inhalation test clearly distinguish students in an examination period, even though academic examinations are transient and medical students are experienced and skilful examinees.

These findings highlight cardiovascular sensitivity to additional stressors in groups undergoing transient periods of stress and may imply some health risk. Research has found that students exhibiting elevated cardiovascular responses following stressful laboratory tasks continued to exhibit elevated ambulatory BP during periods of perceived stress in everyday life (Matthews et al., 1992). Furthermore, increased risk of myocardial infarction can result from surges in blood pressure (BP) in vulnerable individuals (Muller et al., 1989), and primates exposed to repeated BP elevation over weeks and months have shown accelerated atherosclerosis and increased cardiac risk (Kaplan et al., 1991). While our study was conducted on a young healthy population, these results may have important implications for clinical populations or those with compromised health.

As expected students exposed to an examination period reported higher perceived stress. The higher HR exhibited by the exam group during the reactivity phase following the CO_2 inhalation test was similarly found in those who

reported more psychological stress, although no effect was found for SBP or DBP in individuals with higher stress scores. Nonetheless, this finding suggests some physiological alignment in subjective and objective experiences of stress.

In addition, this study found evidence of gender differences in response to the CO_2 inhalation test with women exhibiting lower SBP during the reactivity phase compared with men, although this effect was not found for HR and DBP outcomes. Research on gender-specific cardiovascular responses to stressful encounters has revealed heightened BP in male subjects during exposure to both laboratory and real-life stressors (Stoney et al., 1988; Anishchenko et al., 2001; Matthews et al., 2001). As there are higher numbers of male deaths from cardiac disease (Stoney et al., 1987), our findings encourage future investigation of gender differences in the stress response.

Interestingly, minimal changes in salivary cortisol secretion was exhibited in response to the CO_2 inhalation test, and this was not altered by exam status, gender or perceived stress scores. This finding is contrary to previous studies reporting increased plasma and salivary cortisol secretion in healthy volunteers following 35% CO_2 inhalation (Argyropoulos et al., 2002; Kaye et al., 2004; Wetherell et al., 2006), although cortisol secretion did not increase or was negligible in healthy participants following inhalation of considerably lower concentrations of CO_2 (Sinha et al., 1999; Woods et al., 1988). One study reported no differences in salivary cortisol secretion between groups inhaling either 35% CO_2 or a placebo (van Duinen et al., 2004), however, the same researchers later reported increased serum and salivary cortisol secretion following CO_2 inhalation in a subsequent study, the inconsistent finding explained by methodological changes to the study design (van Duinen et al., 2005). In the present study, the discrepancy in cortisol secretion could be accounted for by the larger participant sample, differing statistical analyses and the inclusion of impending academic assessment as a background stressor. More importantly, the findings revealed consistently lower salivary cortisol secretion in the examination group across the three collection times. Although this is contrary to many investigations reporting increased cortisol secretion in response to academic or laboratory stressors (Kirschbaum et al., 1995; Amario et al., 1996; Lacey et al., 2000; Fortuyn et al., 2004), others report increased plasma cortisol during an examination period only if students reported increased perceived stress (Malarkey et al., 1995), or report no differences in plasma cortisol before, during and after examinations or following a laboratory stressor (Dobbin et al., 1991; Glaser et al., 1994; Larson et al., 2001). Reduced salivary cortisol was found in fire fighters reporting higher stress exposure (Roy, 2004; Roy et al., 2003), and consistent with the present study, reduced salivary cortisol was found in both undergraduate and postgraduate students 2 weeks prior to examinations compared to a regular academic period (Vedhara et al., 2000). Findings from the present study parallel these divergent observations and supplement the growing literature on neuroendocrine responses to stress.

This study has the advantage of comparing groups exposed to a natural real-life stressor and a controlled laboratory stressor, which contributes to a limited literature investigating how responses to an acute stressor are affected by background stress. By investigating responses to the CO_2 inhalation test, activation of the HPA axis and the SNS and PNS was simultaneously examined, evidence not afforded by other laboratory stress tasks. In addition, unlike many other investigations the primary source of background stress was controlled by focussing on the presence/absence of a specific event (the temporal proximity of academic examinations), rather than relying on self-reported stress without knowledge of the event provoking the psychological stress.

A limitation of this study, however, is that the stressed group underwent the laboratory challenge and reported on psychological stress over a period 2–4 weeks prior to the beginning of an examination period when the intensity of stress may be somewhat less than the day of an examination. Future research could therefore focus on stress experienced by students on the day of an examination, in order to establish physiological and psychological responses to an acute stressor during more immediate and extreme periods of stress.

Role of the funding sources

Funding for this study was provided by the Auckland Medical Research Foundation; the AMRF had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

None declared.

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