Hypothalamo-pituitary adrenal axis and sympatho-adrenal medullary system responses to psychological stress were not attenuated in women with elevated physical fitness levels.

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Abstract

Purpose: It is not clear if higher levels of cardiorespiratory fitness are associated with lower hypothalamo-pituitary adrenal (HPA) axis and sympatho-adrenal medullary (SAM) system reactivity to psychological stress in women. The association between cardio-metabolic risk markers and acute physiological responses to psychological stress in women who differ in their cardiorespiratory fitness status has also not been investigated.

Methods: Women with high (n=22) and low (n=22) levels of fitness aged 30-50 years (in the mid-follicular phase of the menstrual cycle) were subjected to a Trier Social Stress Test (TSST) at 1500h. Plasma concentrations of cortisol, adrenaline (Adr) and noradrenaline (NA) and dopamine (DA) were measured in samples collected every 7-15min from 1400h-1700h. Heart rate and blood pressure were measured at the same time points.

Results: Low fit women had elevated serum triglyceride, cholesterol/HDL ratio, fasting glucose and HOMA IR levels compared with high fit women. While cortisol, Adr, NA, HR and blood pressure all demonstrated a significant response to the TSST, the responses of these variables did not differ significantly between high and low fit women in response to the TSST. Dopamine reactivity was significantly higher in the low fit women compared with high fit women. There was also a significant negative correlation between VO_2 max and DA reactivity.

Conclusions: These findings suggest that, for low fit women aged 30-50 years, the response of HPA axis and SAM system to a potent acute psychological stressor is not compromised compared to that in high fit women.

Key words: stress, fitness, women, TSST

Introduction

Optimal stress responses are adaptive [1] whilst frequent unnecessary activation of the stress pathways may lead to cardiovascular disease, type 2 diabetes and anxiety and depression [2-4]. The main mediators of the human physiological stress response are the hypothalamo-pituitary adrenal (HPA) axis which results in the release of cortisol [5] and the sympatho-adrenal medullary (SAM) system which results in the release of the catecholamines adrenaline (Adr), noradrenaline (NA) and dopamine (DA) and increases in the activity of the cardiovascular system [6]. It is important that both the SAM system (plasma catecholamines, heart rate and blood pressure) and the HPA axis (plasma cortisol) are measured simultaneously in stress experiments in order to obtain a complete view of stress responsiveness.

Regular physical activity is thought to be associated with biological adaptations that moderate the physiological response to stress [7,8]. Nevertheless, the role of fitness in the regulation of stress responsiveness is not fully understood. There are studies that have found both beneficial effects of fitness (i.e. reduction in cortisol and heart rate responses to stress) [9-11], as well as no influence on stress responsiveness (norepinephrine, cortisol and heart rate) [12]. Furthermore, in a meta-analysis of 73 studies (mainly in males) that examined whether cardiorespiratory fitness mitigates cardiovascular responses during and after acute psychological stress in humans, Jackson and Dishman reported that higher levels of fitness are related to higher stress reactivity but better recovery of heart rate and blood pressure [13]. Women are underrepresented in investigations regarding fitness and stress responsiveness [13] and the limited research undertaken in women has been inconclusive. Summers and colleagues reported no differences in urine catecholamines in response to a Stroop colour- word task in fit versus unfit women [14]. Nevertheless, only two samples of urine (immediately before and after stress induction) were collected in this study. Thus, these investigators may have missed the physiological changes that may have occurred since urinary catecholamines are less able to reflect acute changes in sympathetic activity compared with blood catecholamines levels [15]. Furthermore, no measures of HPA axis activity were obtained. Traustadottier and colleagues reported lower cortisol responses to a stress test battery that combined mental challenges, a physical challenge, and a psychosocial stressor (Matt Stress Reactivity Protocol), in post-menopausal women with high levels of aerobic fitness compared with their less fit counterparts [16]. As sex steroids can have a marked influence on stress pathway activity [17], the hormonal milieu in pre and post-menopausal women and age related changes in stress physiology [18] make it difficult to extrapolate the findings of this experiment to younger, pre-menopausal women. In summary, the inconclusive

results could be due to differences in the stressor utilised, low frequency of sampling, differences in hormone status of women and suboptimal measures of the SAM system.

Prolonged and sustained activity of the stress pathways can precipitate hyperglycemia [19], promote insulin resistance [20], increase the secretion of pro-inflammatory cytokines [21], increase lipid mobilisation and promote vascular damage [22]. On the other hand, high levels of physical activity are associated with favorable profiles of cardio-metabolic risk [7]. Regular physical activity is associated with improvements in glucose tolerance and insulin activity [23], increased lipid oxidation in muscle [24], attenuation in blood pressure [25] and improvements in endothelial function via increments in bioavailability of nitric oxide [26]. Nevertheless, evidence regarding an association between cardiorespiratory fitness and cardio-metabolic risk markers is non-existent. None of the aforementioned experiments have investigated the association between cardio-metabolic risk markers and acute physiological responses to psychological stress in women who differed in their cardiorespiratory fitness status. Unhealthy levels of cardio-metabolic risk markers combined with excessive stress pathway activity may pose substantial health risk especially in the context of progression to chronic disease. Therefore, it is important to investigate this relationship between responsiveness to psychological stress and cardio-metabolic risk in women who have varying levels of fitness.

This study tested the hypotheses that:

1. Women who have higher levels of cardiorespiratory fitness will have lower cortisol (HPA axis), catecholamine and cardiovascular (SAM system) responses to the Trier Social Stress Test (TSST) compared with their less fit counterparts.
2. Higher physiological stress reactivity is associated with a less favorable profile of cardio metabolic risk markers.

Methods

Women (n=44) aged 30-50 years were recruited using newspaper and online advertisements, emails, fliers in community centers and medical clinics. Exclusion criteria included prior diagnosis of Cushing's syndrome, any stress or anxiety disorder, depression, any diseases of the adrenal gland, type 2 diabetes, heart disease, elevated cholesterol (>5.5 mmol/l), stroke, cancer or any other disorder that may potentially impact the stress pathway activity. Women who were on any form of steroidal contraception (including oral contraceptives, steroidal implants and steroidal IUDs), post-menopausal women, and peri-menopausal women were excluded. Women who were on any medication that could affect stress pathway activity were also excluded. Information about these conditions was obtained by self-report from participants via a telephone interview. Women whose BMI fell outside the range 18-30 (kg/m^2) and women whose resting blood pressure exceeded 160 mmHg for systolic blood pressure or 90 mmHg for diastolic blood pressure were also excluded.

All procedures were approved by the Human Research Ethics Committee of Deakin University (Project code: 2011-242) and conformed to the guidelines of the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research (2007). All women provided informed consent prior to the commencement of the study.

Experimental procedure

Each woman reported to the laboratory on two separate occasions. The first visit (Day 1 testing) was to obtain additional health information (details below), a fasting blood sample (for cardio-metabolic risk markers) and measurement of maximum oxygen consumption (VO_2 max). The stress test (details below) was conducted on the second visit (Day 2 testing) which occurred at least one week after the first visit. No monetary remuneration was provided to the participants.

Day 1 testing

Participants attended this session after an overnight fast (fasting for at least 10 hours). Height (Measurement Concepts, North Bend, Australia) and body mass (TANITA, Wedderburn, Melbourne, Australia) measurements were obtained in order to calculate BMI (body weight/height in $meters^2$). Four resting blood pressure measurements (Criticare systems Inc, Wisconsin, USA) at 2 minute intervals were taken and the average of the second, third and fourth measurements was used to confirm whether resting blood pressure was within the

required range. Participants were instructed to refrain from vigorous intensity physical activity in the 24 hours preceding both Day 1 and Day 2 testing.

A fasting blood sample (22ml) was obtained via a single forearm venipuncture collected into two 9ml serum separator tubes (GreinerBio-One GmbH, Kremsmunster, Austria) and two 2ml FE Sodium Fluoride/K3EDTA tubes (GreinerBio-One GmbH, Kremsmunster, Austria). One serum separator tube was sent to a commercial pathology laboratory (Dorevitch, Melbourne, Australia) for analysis of lipid profile (total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides), fasting serum glucose and C-reactive protein. Plasma was used to measure fasting insulin using a human insulin specific RIA kit (Millipore, Darmstadt, Germany).

Participants were then offered a snack (muesli bars, nuts, dried fruit and/or juice boxes) and asked to complete a Physical Activity Readiness Questionnaire (PAR-Q), an International Physical Activity Questionnaire (IPAQ) [27], a State-Trait Anxiety Inventory (STAI) [28] and a Beck Depression Inventory (BDI-ii) [29]. Information from the IPAQ was used to calculate the total time spent in moderate and vigorous intensity physical activity in a normal week. The STAI and the BDI-ii were used to measure baseline levels of anxiety and depression, respectively. Water was available *ad libitum* throughout the testing session. This was immediately followed by the graded VO_2 max test on an electronically braked cycle ergometer (Lode N.V. Groningen, Netherlands). Maximal aerobic capacity (VO2 max) was measured via an incremental cycle ergometer test to volitional exhaustion. During the test, pulse rate was measured continuously by a heart rate monitor (Polar Electro, Kempele, Finland). Peak oxygen consumption was determined via measurement of expired air connected to a metabolic measurement cart (Vacumed, Ventura, California, USA). The incremental test was performed on an electronically braked cycle ergometer (Lode B.V., Groningen, Netherlands). Participants started the test at 50W. After 2 minutes of riding, the workload was increased to 100W for 2 minutes. Then, increments of 25W were applied each minute until volitional exhaustion. Participants breathed through a Hans-Rudolph two way non-rebreathing valve and the expired air passed through low resistance plastic tubing through to a 4L mixing chamber. Expired air was measured through a flow transducer (KL engineering, Fitchburg, WI, USA), and mixed expired oxygen and carbon dioxide were analysed by a rapidly responding gas analyser (Vacumed, Ventura, California, USA). Ventilatory gas data were averaged every 30s using TurboFit software (Vacumed,

Ventura, California, USA). The gas analyser was calibrated before every testing session using a commercially available gas mixture (Harris Specialty Gas, Brisbane, Australia). The ventilometer was calibrated using a standard 7L syringe (Hans Rudolph INC, Missouri, USA). After ranking women by VO_2 max score, a median split was then used to allocate women evenly into a high fit group (n=22) and a low fit group (n=22) [30].

Day 2 testing

Day 2 testing was booked such that participants were in their mid-follicular phase of their menstrual cycle (Days 5-9, with Day 1 as the first day of menses)[31].

Participants arrived at the laboratory at 1100h. Between 1100h-1145h, measurements of waist and hip circumference and body fat (TANITA, Wedderburn, Melbourne, Australia) were obtained and an intra-venous catheter (Smiths Medical, Ohio, USA) was inserted into an antecubital vein of the forearm for subsequent sampling of blood. Participants also filled in a questionnaire which contained questions about smoking status, alcohol consumption and physical activity in the week leading up to the testing session. The sampling procedures began at 1145h. Between 1200h-1230h participants consumed a standard lunch containing 20% protein, 61% carbohydrates and 19% fat. A period of familiarisation with the sampling procedures took place between 1230h-1330h. A TSST was imposed between 1500h-1530h (details below) and a recovery period extended from 1530h-1700h.

Blood samples (10ml) were collected every 15 minutes from 1145h -1700h except for during and immediately after stress when blood was collected more frequently (1500h, 1507h, 1515h, 1522h, 1530h, 1537h, 1545h, 1552h, and 1600h; Figure 1). Only data from 1400h-1700h is presented here. For the measurement of catecholamines, blood (5ml) was collected in tubes containing reduced glutathione (Sigma-Aldrich, Australia) and Ethylene Glycol Tetra-acetic Acid (EGTA) (Sigma-Aldrich, Australia). Blood samples (5ml) for cortisol assays were collected into separate Lithium Heparin tubes (GreinerBio-One GmbH, Kremsmunster, Austria). All tubes were spun at 3000rpm for 6min. Plasma was separated and stored at -80ºC until assay. Blood pressure was measured at the same times using a clinical blood pressure monitor (Criticare systems Inc, Wisconsin, USA). A continuous electrocardiographic (ECG) measurement was obtained (ADinstruments, NSW, Australia) for the subsequent analysis of heart rate (1400h-1700h). Three ECG electrodes were used (two on wrists and one above the elbow).

TSST

The Trier Social Stress Test (TSST) is a well characterised psychosocial stress protocol [32]. Our laboratory's version of the TSST has been described previously [33]. Briefly, after the 1500h sample collection, participants were introduced to a panel and were given instructions about a speaking task to follow (Figure 1, inset). This was followed by a 10min preparation phase with blood samples collected at the beginning (1507h) and end (1515h) of the preparation phase. Five minutes of public speaking followed after which another blood sample was collected (1522h). Participants were then given instructions regarding a mental arithmetic task and 5 minutes of mental arithmetic followed. Another blood sample was collected at the end of this task (1530h).

Heart rate

A three lead ECG (ADinstruments, NSW, Australia) was used to measure heart rate. A power spectral analysis of the ECG data was conducted using Lab Chart Pro software (ADinstruments, NSW, Australia) to calculate heart rate. For data presentation and analysis, heart rate was calculated as the mean of data for 5min blocks commencing from 1min before to 4min after each blood sample.

Cortisol assays

Plasma concentrations of cortisol were measured using a radio immunoassay (Demeditec Diagnostics, Kiel, Germany). Forty-four assays were conducted (sensitivity 5-2000 nM). The intra-assay coefficient of variation was 9.8% at 92ng/mL and 9.4% at 193ng/ml. The inter-assay coefficient of variation was 10.7% at 146ng/ml and 10.2% at 137ng/ml.

Plasma catecholamine assays

The catecholamines adrenaline (Adr), noradrenaline (NA) and dopamine (DA) and dihydroxyphenylglycol (DHPG), the deaminated metabolite of noradrenaline, were extracted from plasma with alumina adsorption, separated using high performance liquid chromatography (HPLC) and quantified using coulometric detection as previously described [34]. The intra-assay coefficients of variation, determined from 3 repeated measurements of pooled venous plasma, were ±3% for noradrenaline, ±5% for adrenaline, ±3% for DHPG and ±3% for DA. The inter-assay coefficients of variation, determined from 27 consecutive assay runs were ±7% for DHPG, ±5% for noradrenaline, ±13% for adrenaline and ±10% for DA. The assay was linear in the physiological range with a sensitivity (signal-to-noise ratio of 3) of approximately 0.1pmol per 1ml of plasma assayed.

Statistical analysis

Preliminary analysis

Pre-treatment for cortisol, Adr, NA and DA was defined as the average of the four concentrations from 1415h to 1500h (1415h, 1430h, 1445h and 1500h). Pre-treatment for systolic (SBP) diastolic (DBP) and mean arterial pressure (MAP) was defined as the average of the five readings from 1400h-1500h (1400h, 1415h, 1430h, 1445h and 1500h). Pre-treatment for heart rate was defined as the average from the four readings from 1400h to 1445h (1400h, 1415h, 1430h, and 1445h). **Peak height** for cortisol, catecholamines and blood pressures was defined as the highest value obtained for each individual after commencement of stress (1507h-1700h). Peak height for heart rate was defined as the highest reading recorded between 1500h and 1700h inclusive. **Reactivity** was calculated by subtracting the pre-treatment value from the peak height for all parameters. **Area under the curve** (with respect to increase) was calculated for each parameter using values from 1500h – 1700h after the subtraction of the pre-treatment value from each data point. Areas under the curve were calculated using the trapezoid rule utilising Sigmaplot graphing software (Systat Software Inc., California, USA).

Recovery time for all parameters was defined as the time difference from the commencement of the stressor (1500h) to the point at which the relevant parameter returned to within two standard deviations of its pre-treatment value. This was our definition for recovery since 95% of a normally distributed set of data lie within 2 standard deviations of the mean and since 5% error ($p=0.05$) is the generally accepted cut off level for statistical significance. This suggests that once the value has returned to within 2 standard deviations of the pre-treatment level, it has returned to pre-treatment levels. Those who did not exceed two standard deviations of the pre-treatment value between 1500-1700h were excluded from analyses of recovery time. For those who did exceed two standard deviations but did not return to within two standard deviations by 1700h, 120min was used as the recovery time in the analyses.

Analysis

Data were analysed using the Statistical Package for the Social Sciences software version 20.0 (SPSS. Inc, Chicago, USA). Kolmogorov-Smirnov and Shapiro–Wilk tests were conducted to test for normality. Tests for homogeneity of variance were conducted using Levene's test of equality of error variances. Descriptive characteristics were compared between groups using univariate analysis of variance. Plasma cortisol, plasma

catecholamines and cardiovascular parameters were compared within and between groups using repeated measures analysis of variance. The within subjects factor was time and the between subjects factor was cardiorespiratory fitness. Derived plasma cortisol, plasma catecholamine and cardiovascular parameters (pre-treatment, peak height, reactivity and area under the curve) were compared between groups using univariate analysis of variance. Pearson's correlation was used to test for relationships between stress reactivity, cardiorespiratory fitness and cardio metabolic risk markers. $P<0.05$ was considered statistically significant. We estimated that 32 participants in total (16 low fit and 16 high fit) were needed to find a difference between groups in salivary cortisol of the same magnitude as that found by Klaperski et al. [35] with a significance level of 0.05 and a power of 90%.

Results

Participants

A total of 44 women were tested in this study. Women were ranked by VO_2 max and a median split was used to allocate women to either the low fit (n=22) or the high fit group (n=22). One woman from the high fit group was excluded from the cortisol and catecholamine analysis due to cannula failure during sampling which resulted in insufficient volume of plasma to perform the relevant assays. Nine other women (3 from the low fit group and 6 from the high fit group) women were excluded from the analysis of Adr due to complications in measuring levels of adrenaline or not having detectable amounts of adrenaline in plasma samples. One woman in the low fit group was excluded from analyses of HR because of technical difficulties encountered during the ECG recording. Trait anxiety score and insulin data were unavailable for two women (both from the low fit group).

Participant characteristics

Descriptive characteristics of the participants are detailed in Table 1. VO_2 max and number of hours per week of physical activity were significantly higher in women in the high fit group compared with the women in the low fit group (Table 1). As expected, VO_2 max and the number of hours of physical activity per week were significantly positively correlated ($r=0.598$; $p<0.001$). Both groups were in the healthy weight range and had similar BMI levels. However, the low fit women had significantly higher percentage body fat ($p=0.013$), waist circumference ($p=0.021$) and WHR ($p=0.004$) than the high fit women (Table 1). There was a trend towards resting heart rate being higher in the low fit women ($p=0.089$). Resting systolic and diastolic blood pressures and blood pressure variability were comparable between low and high fit women (Table 1). Levels of triglycerides ($p=0.024$), CHOL/HDL ratio ($p=0.001$), fasting levels of glucose ($p=0.024$) and HOMAR-IR ($p=0.049$) were also higher in low fit women compared with high fit women (Table 2). C-reactive protein, total cholesterol levels, insulin levels and depression and anxiety scores were comparable between the groups (Table 2).

Cortisol

Repeated measures analysis of variance revealed that there was a significant effect of time ($p<0.001$; Figure 2). Overall (both groups combined), the peak height of cortisol concentrations (221±86 ng/ml) was significantly higher than pre-treatment concentrations (107±46 ng/ml) ($p<0.001$). Overall, there was a 107% increase in cortisol concentrations from pre-treatment to the peak of the response (both groups combined).

Plasma cortisol in response to the TSST did not differ significantly between low fit women and high fit women (time* cardiorespiratory fitness, $p=0.987$; Figure 2) and accordingly, there were no significant differences between the groups in peak height, reactivity or area under the curve for the cortisol response (data not shown). The mean time to recovery did not differ significantly between the groups (data not shown). There was no significant between subjects effect indicating that there were no significant overall differences between the groups ($p=0.524$).

Catecholamines

Repeated measures analysis of variance revealed that there was a significant effect of time for all of the catecholamines ($p<0.002$ for Adr; $p<0.001$ for NA; $p<0.001$ for DA; Figure 3). Overall (both groups combined), the peak height of, Adr concentrations (133±27pg/ml), NA concentrations (609±37 pg/ml) and DA concentrations (44±4 pg/ml), were significantly higher than their pre-treatment concentrations (54±14, 317±21 and 30±3 pg/ml; respectively) ($p<0.001$ for all). Overall, there was an increase in Adr (146%), NA (92%) and DA (44%) from pre-treatment to the peak of the response (both groups combined).

Plasma concentrations of Adr and NA in response to the TSST did not differ between low fit women and high fit women (time* cardiorespiratory fitness, $p=0.118$ and $p=0.169$; respectively; Figure 3) and accordingly, there were no significant differences between the groups in peak height, reactivity or area under the curve for the Adr and NA response between low and high fit women (data not shown). While repeated measures analysis of variance showed no significant difference between the groups in the DA response to the TSST (time* cardiorespiratory fitness, $p=0.392$; Figure 3), DA reactivity was higher ($p=0.009$) in the low fit women (19±4 pg/ml) compared with the high fit women (8±2 pg/ml). Nevertheless, peak height of DA and area under the curve did not differ between the groups (data not shown). The mean time to recovery did not differ between the groups for any of the parameters (data not shown).

There was no significant between subjects effect for Adr and DA indicating that there were no significant overall differences between the groups (p=0.242 and 0.848; respectively). However, NA for low fit women showed a trend to being higher overall compared with the values for high fit women (p=0.090).

Blood pressure and heart rate

Repeated measures analysis of variance revealed that there was a significant effect of time for all blood pressure parameters and heart rate ($p<0.001$ for all; Figure 4 and 5). Overall (both groups combined), the peak heights of SBP (133±2 mmHg), DBP (80±2 mmHg), MAP (102±2 mmHg) and heart rate (82±2 bpm) were higher than their respective pre-treatment values (105±2, 58±1, 75±1 mmHg and 69±1 bpm) ($p<0.001$). Overall, there was a 27% increase in SBP, a 37% increase in DBP, a 35% increase in MAP and a 20% increase in heart rate from pre-treatment to the peak of the response (both groups combined).

In response to the TSST, none of the blood pressure parameters or heart rate differed between low fit women and high fit women (time* cardiorespiratory fitness, p=0.513 (SBP), p=0.384 (DBP), p=0.263 (MAP) and p=0.832 (HR); Figure 4 and 5) and accordingly, there were no differences between the groups in peak height, reactivity or area under the curve for the blood pressure or heart rate responses (data not shown). The mean time to recovery of all blood pressure parameters and heart rate were also similar between the groups (data not shown). There was no significant between subjects effect in SBP, DBP or MAP indicating that there were no significant overall differences between the groups for any of these variables (p=0.233, 0.251 and 0.164 for SBP, DBP and MAP; respectively). However, overall, the low fit group had higher heart rates (treatment effect p=0.030) compared with the women in the high fit group (Figure 5). This overall difference between the groups was reflected in pre-treatment heart rate being significantly higher (p=0.011) in low fit women (72±2 bpm) compared with high fit women (65±2 bpm).

Correlations/ Associations

VO_2 max and stress reactivity

There were no significant associations between VO_2 max scores and reactivity of cortisol, Adr, NA or any of the cardiovascular parameters (data not shown). Nevertheless, there was a significant ($p=0.007$) negative correlation (Pearson's $r=-0.407$) between VO_2 max and DA reactivity.

VO_2 max and cardio metabolic risk markers

VO_2 max scores were significantly ($p<0.05$ for all) correlated with triglyceride levels, LDL cholesterol, HDL cholesterol, CHOL/HDL ratio and HOMA-IR (Pearson's $r=-0.368$, -0.467, 0.335, -0.533 and -0.380; respectively).

Stress reactivity and cardio metabolic risk markers

Adr reactivity was significantly correlated with HDL cholesterol (Pearson's $r=0.359$, $p=0.037$) and DA reactivity was significantly correlated to plasma levels of CRP (Pearson's $r=0.327$, $p=0.033$). There were no other significant associations between catecholamine reactivity and cardio metabolic risk markers. No significant relationships between cortisol reactivity and any of the cardio metabolic risk markers were found. Systolic and diastolic blood pressure reactivity were not significantly associated with any of the cardio metabolic risk markers. However, MAP reactivity was significantly correlated with fasting glucose levels (Pearson's $r=-0.473$, $p=0.001$). Heart rate reactivity was significantly correlated with levels of insulin (Pearson's $r=-0.367$, $p=0.015$) and HOMA-IR (Pearson's $r=-0.350$, $p=0.023$).

Discussion

We found that women who engaged in higher levels of moderate and vigorous intensity physical activity (high fit women) have comparable HPA axis and SAM system responses to a potent psychological stress compared with aged matched women who engaged in lower levels of physical activity (low fit women). This suggests that physiological responses to psychological stress are not influenced by engaging in moderate and vigorous intensity physical activity ranging from 3 to 8 hours a week. However, we did find that low fit women had higher DA reactivity to stress. Furthermore, greater reactivity of Adr, DA, MAP and HR were significantly associated with higher HDL cholesterol, CRP, fasting glucose and insulin kinetics (fasting insulin and HOMA-IR); respectively. This provides some support for an association between cardio metabolic risk markers and an individual's susceptibility to over activation of the SAM system and excess secretion of DA.

Findings from previous studies partially concur with the results of the current experiment. Our findings are in accordance with Moyna et al 1999 who reported that plasma noradrenaline responses to mental stress were not related to aerobic fitness levels [12]. Similarly Poole et al reported physiological (heart rate, blood pressure and cortisol) responses to psychological stress to be independent of physical activity levels [36]. Heart rate and cortisol results from the current study are in contrast to the findings of Klaperski and colleagues in young females who reported lowered physiological stress responses in higher active individuals [35]. However this study does not report the fitness levels of the participants. Nevertheless, it should be noted that in this study, the women who showed the highest cortisol levels were participating in as little as 22 minutes of physical activity per week. Compared to this, low fit women in the current study took part in an average of 2.5 hours physical activity a week. This is in line with the physical activity levels recommended by the Centres for Disease Control and prevention (accessed 10th March 2015). Therefore, it may be possible that physical activity status will only have a negative effect on stress responsiveness in individuals who take part in extremely low levels of activity. Cortisol results from this investigation also are not consistent with the findings of Traustadottir and colleagues in postmenopausal women [16]. Findings of Traustadottir and colleagues somewhat imply that the changes in the hormone milieu in women after the cessation of menses might be the primary mechanism behind the attenuated neuroendocrine stress responsiveness to stress seen with elevated fitness [16]. Direct comparisons of the aforementioned research and the current study should be approached with caution due to the differences in methodology.

It has been reported previously that high levels of fitness can significantly attenuate HPA axis responses to psychological stress in men (Rimmele, Zellweger et al. 2007, Rimmele, Seiler et al. 2009, Webb, Rosalky et al. 2013). We did not observe such an attenuated HPA axis reactivity pattern in the high fit women in the current experiment. Therefore, it could be speculated that attenuation in HPA axis activity in response to psychological stress mediated by the physical activity status is sex specific and may only occur in males.

We performed an array of SAM system measurements (catecholamines, heart rate and blood pressure) to investigate the role of regular physical activity may have in attenuating physiological responses to psychological stress. Summers and colleagues reported no differences in urinary catecholamine responses to psychological stress in fit and unfit women [14]. This is in line with the comparable changes in Adr and NA observed in the current study in low fit and high fit women. However, a direct comparison of the studies requires a cautious approach due to the difference in the stressors utilised (colour-word stroop vs TSST) and measures of catecholamines (urine vs plasma). The finding that low fit women had a significantly higher DA reactivity compared with high fit women is of interest and warrants further investigation. Both the source of DA circulating in the periphery and the relevance of DA in stress responsiveness are worthy of further investigation.

The majority of past research in the area, albeit mostly in men, revealed that increased fitness/levels of regular physical activity can attenuate heart rate responses to psychological stress [9,10,35,11]. It could be speculated that this is a further illustration of a sex difference in the role that regular exercise has in attenuating physiological responses to mental stress. Current results indicate that despite higher baseline HR in low fit women, the HR responses to TSST were not affected by the fitness status. Current results also indicate that there is no difference in the blood pressure responsiveness to TSST in women with low and high levels of cardiorespiratory fitness. Comparable changes in blood pressure in response to stress further supports findings of the catecholamine measures of the SAM system in this instance. It is also evident from the current results that all parameters (both HPA axis and SAM system) showed substantial increases in responses to the TSST. This highlights the potency of TSST to adequately activate both SAM system and HPA axis. Nevertheless, it cannot be discounted that the lack of differences observed may have been a result of the TSST fully activating

and acutely exhausting stress pathways. Therefore, it might be possible to elucidate subtle differences in stress pathway activity in response to stress if a less potent stressor was used.

Lack of associations between cortisol reactivity and VO_2 max scores suggests that fitness is not associated with HPA axis responses to psychological stress in 30-50 year old women. However, the relationship between SAM system responses to psychological stress seem to be complicated given that there was a significant negative association between VO_2max scores and DA reactivity but not Adr or NA reactivity. Dopamine is not commonly considered a stress hormone. Nevertheless, exposure to psychological stress can activate the mesocorticolimbic DA system in humans [37]. It should, however, be noted that the VO_2 max scores were still associated with a more desirable cardio metabolic risk marker profile as indicated by significant negative correlations between VO_2max and triglycerides, LDL cholesterol, CHOL/HDL ratio and HOMA-IR and a significant positive correlation between VO_2max and HDL cholesterol. This suggests that regular exercise can be beneficial in modifying cardio-metabolic risk independent of the HPA axis and SAM system responses to stress. Although it is not clear as to which precedes the other, previous research shows that increases in sympathetic activity can be associated with metabolic anomalies [3]. The relationship between reactivity of HR and insulin and HOMA-IR and reactivity of MAP and fasting glucose in this research may indicate early signs of such anomalies. This may be suggestive of the need of further research into potential metabolic defects from an excess in sympathetic activation.

In conclusion, while some associations were found between stress reactivity and levels of cardio metabolic risk factors, findings of this research do not support there being a differential effect of the level of physical fitness on SAM system and HPA axis activity in response to psychosocial stress in healthy women between 30-50 years of age.

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Compliance with Ethical Standards

All authors declare no conflicts of interest.

Ethical Approval

All procedures were approved by the Human Research Ethics Committee of Deakin University (Project code: 2011-242), conformed to the guidelines of the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research (2007) and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Tables

Table 1: Mean (±SEM) descriptive characteristics of Low and High fit women

	Low fit (n=22)	High fit (n=22)	P value*
Age (years)	40.4±1.4	38.1±1.3	0.233
Hours of physical activity per week	2.7±0.5	7.1±1.3	0.004
VO_2max (ml/kg*min)	27.4±1.0	41.9±1.6	<0.001
Weight (kg)	62.6±2.3	62.2±1.6	0.833
BMI (kg/m^2)	23.1±0.7	22.2±0.4	0.241
Body fat (%)	30.3±1.4	25.5±1.2	0.013
Waist circumference (cm)	82.5±2.2	76.4±1.3	0.021
Hip circumference (cm)	97.5±1.6	96.1±1.2	0.481
Waist to hip ratio	0.84±0.0	0.79±0.0	0.004
Resting HR (bpm)	71±1.8	66±2.4	0.089
Resting SBP (mmHg)	115±2.7	114±2.4	0.926
Resting SBP variability (SD)	6.5±0.8	6.7±1.3	0.899
Resting DBP (mmHg)	70±2.2	67±1.7	0.232
Resting DBP variability (SD)	4.3±0.5	6.4±1.2	0.137

* Univariate Analysis of Variance, HR = heart rate, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure

Table 2: Mean (±SEM) cardio-metabolic risk markers and depression and anxiety scores of Low and High fit women

	Low fit (n= 22)	High fit (n=22)	P value*
CRP (mg/l)	1.4±0.7	0.6±0.2	0.279
Cholesterol (mmol/l)	5.0±0.1	4.8±0.2	0.256
Triglycerides (mmol/l)	0.9±0.1	0.7±0.1	0.024
CHOL/HDL ratio	3.3±0.2	2.6±0.1	0.001
Fasting glucose (mmol/l)	5.3±0.1	4.7±0.2	0.024
Insulin (µU/ml)	12.7±0.9	11.3±0.5	0.165
HOMA-IR	3.0±0.2	2.5±0.1	0.049
BDI-ii score	4.4±1.1	2.8±0.9	0.271
STAI score (trait)	31.9±1.5	31.7±1.9	0.469
STAI score (state)	32.4±1.2	30.7±2.1	0.475

* Univariate Analysis of Variance, BDI = Beck Depression Inventory, STAI = State-Trait Anxiety Inventory, HOMA-IR = Homeostatic Model Assessment- Insulin Resistance

Figure Legends

Fig 1 Schematic representation of the stress testing day. 'B' and black arrows= blood samples, TSST= Trier Social Stress Test

Fig 2 Mean (± SEM) plasma cortisol concentrations in low and high fit women from 1400h-1700h. TSST: Trier Social Stress Test

Fig 3 Mean (±SEM) a) Adrenaline (Adr), b) Noradrenaline (NA) and c) Dopamine (DA) in low fit and high fit women from 1400h-1700h. TSST: Trier Social Stress Test

Fig 4 Mean (±SEM) systolic, diastolic and mean arterial pressures in low and high fit women from 1400h-1700h. TSST: Trier Social Stress Test, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure

Fig 5 Mean (± SEM) heart rate in low and high fit women from 1400h-1700h. TSST: Trier Social Stress Test