*Original Research*

# Psychological stress during exercise: immunoendocrine and oxidative responses

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## Abstract

**The purpose of this study was to examine the changes in catecholamines (epinephrine [EPI] and norepinephrine [NE]), interleukin-2 (IL-2) and a biomarker of oxidative stress (8-isoprostane) in healthy individuals who were exposed to a dual challenge (physical and psychological stress). Furthermore, this study also examined the possible relationships between catecholamines (NE and EPI) and 8-isoprostane and between IL-2 and 8-isoprostane following a combined physical and psychological challenge. Seven healthy male subjects completed two experimental conditions. The exercise-alone condition (EAC) consisted of cycling at 60% $VO_{2max}$ for 37 min, while the dual-stress condition (DSC) included 20 min of a mental challenge while cycling. DSC showed greater EPI and 8-isoprostane levels (significant condition by time interaction). NE and IL-2 revealed significant change across time in both conditions. In addition, following dual stress, EPI area-under-the-curve (AUC) demonstrated a positive correlation with NE AUC and IL-2 AUC. NE AUC was positively correlated with IL-2 AUC and peak 8-isoprostane, and peak IL-2 was positively correlated with peak 8-isoprostane in response to a dual stress. The potential explanation for elevated oxidative stress during dual stress may be through the effects of the release of catecholamines and IL-2. These findings may further provide the potential explanation that dual stress alters physiological homeostasis in many occupations including firefighting, military operations and law enforcement. A greater understanding of these responses to stress can assist in finding strategies (e.g. exercise training) to overcome the inherent psychobiological challenges associated with physically and mentally demanding professions.**

**Keywords:** psychological stress, exercise, catecholamines, interleukin-2, oxidative stress, 8-isoprostane

*Experimental Biology and Medicine* 2010; **235**: 1498–1504. DOI: 10.1258/ebm.2010.010176

## Introduction

Psychological stress has been proposed as a major contributor to the progression of cardiovascular diseases.[1,2] Interestingly, numerous occupations (e.g. firefighting, military operations and law enforcement) are often subject to a combination of physical and psychological stress, which may elevate mortality risks.[3] One aspect of fire suppression activities that may contribute to these high mortality rates is the combined physical and psychological challenge,[4] further altering hormonal and immunological responses due to the inability to adapt and maintain allostasis among the hypothalamopituitary (HPA) and sympathoadrenal (SA) axes.[5] Previous studies have shown that the elevations in catecholamine and inflammatory cytokines are linked to oxidative stress[6,7] and these elevations have been seen in response to physical and psychological stressors, independently.[8,9]

Oxidative stress is an imbalance between antioxidants, such as nitric oxide (NO) and reactive oxygen species (ROS), including superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^-$).[10] Intense exercise[9] and psychological stress[11,12] may impact pathogenesis of cardiovascular disease through pathways that include elevated oxidative stress. More specifically, research has previously shown that psychological stress may contribute to the development of atherosclerosis by eliciting an elevation in ROS, which can further induce oxidative DNA damage.[2] Subsequently, in a study on medical students, Sivonova *et al.*[12] demonstrated greater nuclear DNA damage in lymphocytes on the day of an examination (stress condition) compared with during the time between two examination periods (non-stress condition). Furthermore, in response to physical stress the magnitude of the oxidative response is dependent upon exercise intensity and duration. A recent

study by Bloomer *et al.*[13] has observed that both high-intensity aerobic and anaerobic exercise elevated the biomarkers of oxidative stress in blood.

One explanation for elevated oxidative stress during psychological stress could be the effect of high circulating levels of catecholamines (epinephrine [EPI] and norepinephrine [NE]).[6,12,14] This is further supported by Flint *et al.*[15] who have demonstrated that EPI and NE released during psychological stress can induce DNA damage in mice within 10 min. Additionally, evidence supports that inflammation and its subsequent impact on oxidative stress may also play a crucial role in the pathogenesis of cardiovascular disease.[7] The formation of ROS is associated with the levels of proinflammatory cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFN-y) released by type 1 helper T-cells (Th1). For example, IL-2 and IFN-y are thought to be the most potent triggers for macrophage-induced ROS production within the Th1 immune response.[7] Previous research has shown that acute psychological stress exposure is associated with increased IL-2 levels.[16] Recent studies have examined EPI and NE response to a dual challenge (physical and psychological stress) and found a greater increase in EPI and NE corresponding with a higher IL-2 concentration in the dual-stress condition (DSC) compared with exercise-alone condition (EAC).[17,18] These findings suggest that the SA axis is further activated, beyond the EAC, during a dual challenge, and these exacerbations in catecholamines and IL-2 responses may further alter oxidative response. An examination of catecholamines (NE and EPI) and proinflammatory cytokines (e.g. IL-2) following a combination of physical and psychological challenge may provide a greater understanding of the link between physical activity, psychological stress and the oxidative response. Therefore, the purpose of this study was to examine the changes in catecholamines (EPI, NE), IL-2 and a biomarker of oxidative stress (8-isoprostane) in healthy individuals who were exposed to a dual challenge (physical and psychological stress). Furthermore, this study also examined the possible relationships between catecholamines (NE and EPI) and 8-isoprostane and between IL-2 and 8-isoprostane following a combined physical and psychological challenge.

## Materials and methods

### Subjects

Seven healthy male subjects were recruited to participate in the study. Descriptive data are presented in Table 1. To control variability of the dependent measures of interest and because recent studies have shown that women may have different immune reactivity[19] and attenuated cardiovascular responses to mental stress,[20] only male subjects were investigated. Subjects provided informed consent and completed a medical history questionnaire prior to data collection. All experimental procedures were approved by the University Institutional Review Board. Subjects in this study were (a) free of cardiorespiratory and metabolic disorders; (b) free of any known blood disorders (e.g. anemia, hemophilia); (c) without hearing or vision problems (including color-blindness); (d) free of a history of psychological disorders and/or chronic illnesses; (e) native English speakers; (f) had no use of any prescription or non-prescription medication or tobacco products; (g) were non-smokers and with average consumption of less than 10 alcoholic beverages per week; and (h) had no experience of any major life events within 30 d of participation (e.g. death in family, divorce, wedding). Additionally, prior to each testing session, participants were asked to fast overnight for at least eight hours and to abstain from alcohol consumption for at least 48 h. Furthermore, to limit the effect of fitness and physical activity on physiological responses (e.g. reduced activity of the autonomic nervous system) to mental stress, subjects were instructed to maintain their normal physical activity patterns throughout the duration of their involvement in the data collection procedures. In addition, all subjects had not engaged in a combination of physical and psychological stress within the previous 72 h and had not participated in physical activity for the previous 24 h.

**Table 1** Subject descriptive character ($N = 7$)

| Variable | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| Age (years) | 21.43 | 1.99 | 18.00 | 24.00 |
| Height (cm) | 173.14 | 5.43 | 166.00 | 180.00 |
| Weight (kg) | 69.19 | 6.28 | 59.41 | 76.20 |
| $VO_{2max}$ (mL/kg/min) | 45.13 | 7.70 | 35.80 | 52.60 |

### Testing procedures

Three testing sessions comprised the data collection. These sessions consisted of: (1) an initial session to obtain consent to participate, familiarize subjects with all instruments and procedures and assess maximal oxygen uptake ($VO_{2max}$) using a ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT, USA). Subjects reached $VO_{2max}$, when either the primary criterion of a plateau in $VO_2$ with an increase in workload was met or two of the three secondary criteria were achieved. The secondary criteria were (a) reaching predicted maximal heart rate, (b) achieving a respiratory exchange ratio of greater than 1.15 and (c) reporting a rating of perceived exertion (15-point Borg Scale) of 19 or 20; (2) a session that included 37 min of cycle ergometry at 60% $VO_{2max}$ (EAC); and (3) a session that included 37 min of cycle ergometry at 60% $VO_{2max}$ with 20 min of mental challenges (DSC) (see experimental protocol in Figure 1). Subjects were instructed to participate in 5 cycles of the computer-based Stroop Color Word (SCW) and mental arithmetic (MA) tasks (2 min SCW plus 2 min MA), for a total of 20 min of mental challenge. Cardiovascular responses to SCW and MA tasks are highly correlated with responses to real-life stressors.[21] A mental challenge alone condition was not included because of the likelihood that an adaptation to a single exposure of the stress protocol could diminish its physiological effect during the dual-challenge condition and because some occupational duties such as firefighting,

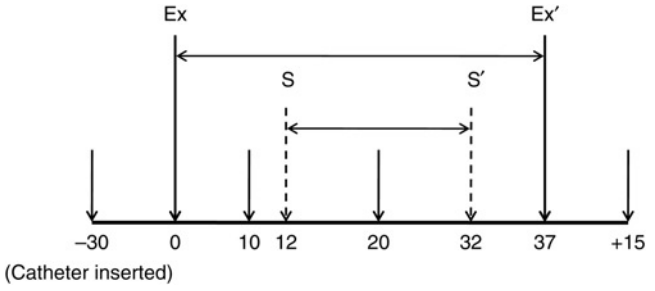


**Figure 1** Time progression of the experimental protocol in minutes. The vertical solid lines extending from the ordinate represent the start of exercise (Ex) and the end of exercise (Ex′). The vertical dotted lines extending from the ordinate represent the start of the mental challenge (S) and the end of the mental challenge (S′)

military operations and law enforcement are most often exposed to mental and physical challenges simultaneously.

Subjects cycled on a stationary cycle ergometer with the workload controlled by CompuTrainer Pro Cycle ergometer software. The workload was calculated using equations developed by the ACSM (2005).[22] One reason for choosing this workload was to limit the possibility of stimulating the HPA axis,[23–25] markers of inflammation,[26,27] and biomarkers of oxidative stress[28] due to high exercise intensity or prolonged exercise. In addition, this exercise intensity (60% $VO_{2max}$) is similar to the intensity experienced during some occupational duties such as firefighting.[29–31]

At least 48 h were allowed to elapse between sessions 1 and 2, and a minimum of one week transpired between sessions 2 and 3. Sessions 2 and 3 were counterbalanced between subjects. For session 1, subjects visited the lab to participate in a graded exercise test on a cycle ergometer designed to elicit maximal exertion within 8–12 min. For sessions 2 and 3, the subjects reported to the lab at 07:00 h, and a venous catheter was inserted by 07:30 h.

### Blood draws and analyses

Blood draws were performed by a physician-approved licensed allied health-care professional using standard technique. An intravenous catheter (Jelco, 20 g, 25 mm) was inserted into an antecubital vein, and a positive pressure adapter (CLC2000, ICU Medical, San Clemente, CA, USA) was attached. The blood was collected prior to exercise (0 min), during exercise (10, 20, 32 and 37 min), and after the conclusion of exercise during recovery (R15 min). IL-2 and 8-isoprostane were collected prior to exercise (0 min), during exercise (32 and 37 min), and after the conclusion of exercise during recovery (R15 min). During each blood draw, the first 1 mL of blood (with saline from the extension set) was collected into a discard tube preceding the sample draw. Each blood draw was divided as follows: (1) 5 ml was collected into tubes containing ethylene glycol-bis-(beta-amino ethyl ether)-N, N, N′, N′-tetraacetic acid and glutathione for subsequent catecholamine analyses and (2) 6 ml of blood was collected into a tube containing ethylenediaminetetraacetic acid (EDTA) for IL-2 and 8-isoprostane. All blood samples were centrifuged for 20 min at 2500 rpm at 4°C, and stored at −80°C for subsequent analyses.

Isolation of catecholamines from human plasma was accomplished by alumina extraction using a Chromosystems reagent kit (Chromsystems, Munchen, Germany). Once extracted, plasma catecholamine concentration was quantified by high-performance liquid chromatography (HPLC). The Waters (Waters Corp., Milford, MA, USA) HPLC system consisted of a pump (model 510), WISP autoinjector (Model 712) with cooling module, column, and an electrochemical detector (Model 460). Data were stored and analyzed using the Waters Millennium software package (V 2.10). The flow rate was 0.8 mL/min, samples in the autoinjector were maintained at 4°C, and the column oven was maintained at 40°C. The column was a 15-cm reversed phase C-18 with 5 m silica particles. The mobile phase consisted of a mixture of water, methanol and buffer salts manufactured by Chromsystems (Part C5001; Hamburg, Germany) and optimized for the separation of the plasma catecholamines. The sensitivity of the assay was 5 pg/mL on a column with a signal to noise ratio of 4 to 1, a between-days coefficient of variation of less than 5% and a within-days variation of less than 3%. The standard curve for the range of 5 to 5000 pg/mL had a correlation coefficient of 0.998.

IL-2 and 8-isoprostane were measured by using enzyme-linked immunosorbent assays (BD Biosciences, San Diego, CA and Cayman Chemical, Ann Arbor, MI, USA, respectively) following manufacturers' recommended protocol. The inter-assay coefficient of variation for IL-2 analysis was 6.71% and the intra-assay coefficient of variation was 4.85%. The inter-assay coefficient of variation for 8-isoprostane analysis was 6.65% and the intra-assay coefficient of variation was 5.53%.

### Statistical analyses

Data analysis was performed using the SPSS version 16.0. To assess differences between the EAC and DSC, 2 ×6 (condition × time) repeated measures analysis of variance (ANOVA) was used to examine EPI and NE, and 2 × 4 (condition × time) repeated measures ANOVAs were used to examine IL-2 and 8-isoprostane. To assess overall release of NE, EPI, IL-2 and 8-isoprostane during the EAC and DSC, integrated trapezoidal area-under-the-curves (AUCs) were calculated with a previously published formula by Pruessner *et al.*[32] Finally, Pearson product-moment correlations were utilized to examine relationships among EPI, NE, IL-2, and 8-isoprostane. The α-level was set at $P \leq 0.05$.

## Results

### EPI and NE responses

EPI revealed a significant condition by time interaction with a greater level in the DSC [$F$ (5, 30) = 2.41, $P < 0.05$] (Figure 2a and Table 2). In addition, NE did not reveal a significant condition by time interaction, but a significant change (main effect) across time was observed [$F$ (5, 30) = 12.10, $P < 0.001$] (Figure 2b and Table 2).

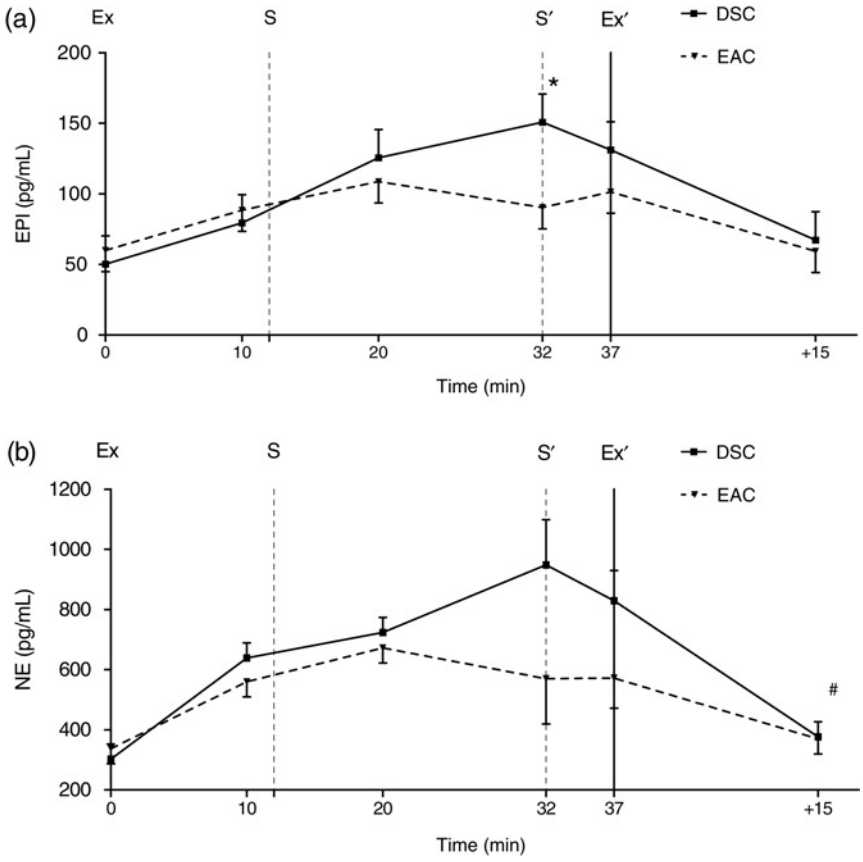


**Figure 2** Norepinephrine (NE) and epinephrine (EPI) responses during exercise in the exercise-alone condition (EAC) and the dual-stress condition (DSC). A significant condition by time interaction was revealed in EPI, with a greater increase in the DSC (a; $^{*}P < 0.05$). A significant increase over time was observed in both conditions (b; $^{\#}P < 0.001$). Points represent the NE and EPI values during the protocol; vertical lines depict standard errors of the means. The vertical solid lines extending from the ordinate represent the start of exercise (Ex) and the end of exercise (Ex′). The vertical dotted lines extending from the ordinate represent the start of the mental challenge (S) and the end of the mental challenge (S′)

### IL-2 and 8-isoprostane responses

IL-2 did not reveal a significant condition by time interaction, but a significant change (main effect) across time was observed [$F$ (3, 18) = 3.33, $P < 0.05$] (Figure 3a and Table 2). In addition, a significant condition by time interaction was revealed for 8-isoprostane with a greater level in the DSC [$F$ (3, 18) = 4.02, $P < 0.05$] (Figure 3b and Table 2).

### Correlation among variables

In response to a dual stress, EPI AUC demonstrated a positive correlation with NE AUC and IL-2 AUC ($r = 0.81$ and $r = 0.67$, $P < 0.01$, respectively). In addition, NE AUC was positively correlated with IL-2 AUC and peak 8-isoprostane ($r = 0.58$ and $r = 0.55$, $P < 0.05$, respectively), and peak IL-2 was positively correlated with peak 8-isoprostane ($r = 0.58$, $P < 0.05$) following a dual stress.

## Discussion

This study examined the changes in catecholamines (EPI, NE), IL-2 and a biomarker of oxidative stress (8-isoprostane) in healthy individuals who were exposed to a dual challenge (physical and psychological stress). DSC activated the SA axis, eliciting greater EPI levels with a significant

**Table 2** Effect of a dual challenge on EPI, NE, IL-2 and 8-isoprostane (mean ± SEM)

| Variable | 0 min | 10 min | 20 min | 32 min | 37 min | R 15 min |
|---|---|---|---|---|---|---|
| EPI (pg/mL) – DSC | 50.19 ± 6.87 | 79.43 ± 17.31 | 125.56 ± 24.64 | 150.74 ± 32.30 | 131.07 ± 24.64 | 67.23 ± 7.65 |
| EPI (pg/mL) – EAC | 59.95 ± 4.89 | 88.39 ± 12.91 | 108.57 ± 19.31 | 90.23 ± 10.79 | 101.27 ± 9.70 | 59.26 ± 7.30 |
| NE (pg/mL) – DSC | 302.48 ± 50.08 | 638.94 ± 96.60 | 723.34 ± 108.16 | 948.64 ± 211.98 | 829.31 ± 133.87 | 376.67 ± 34.92 |
| NE (pg/mL) – EAC | 337.14 ± 15.45 | 559.20 ± 100.45 | 671.63 ± 139.92 | 569.64 ± 84.17 | 571.71 ± 58.01 | 369.70 ± 40.50 |
| IL-2 (pg/mL) – DSC | 3.09 ± 0.73 | | | 5.98 ± 1.31 | 4.45 ± 0.61 | 3.38 ± 0.55 |
| IL-2 (pg/mL) – EAC | 2.82 ± 0.58 | | | 3.76 ± 0.63 | 3.82 ± 0.65 | 3.04 ± 0.58 |
| 8-isoprostane (pg/mL) – DSC | 27.29 ± 2.61 | | | 31.93 ± 3.52 | 32.11 ± 3.54 | 37.33 ± 3.62 |
| 8-isoprostane (pg/mL) – EAC | 26.00 ± 2.55 | | | 22.07 ± 2.49 | 23.84 ± 2.51 | 24.92 ± 2.57 |

EPI, epinephrine; NE, norepinephrine; IL, interleukin; DSC, dual-stress condition; EAC, exercise-alone condition

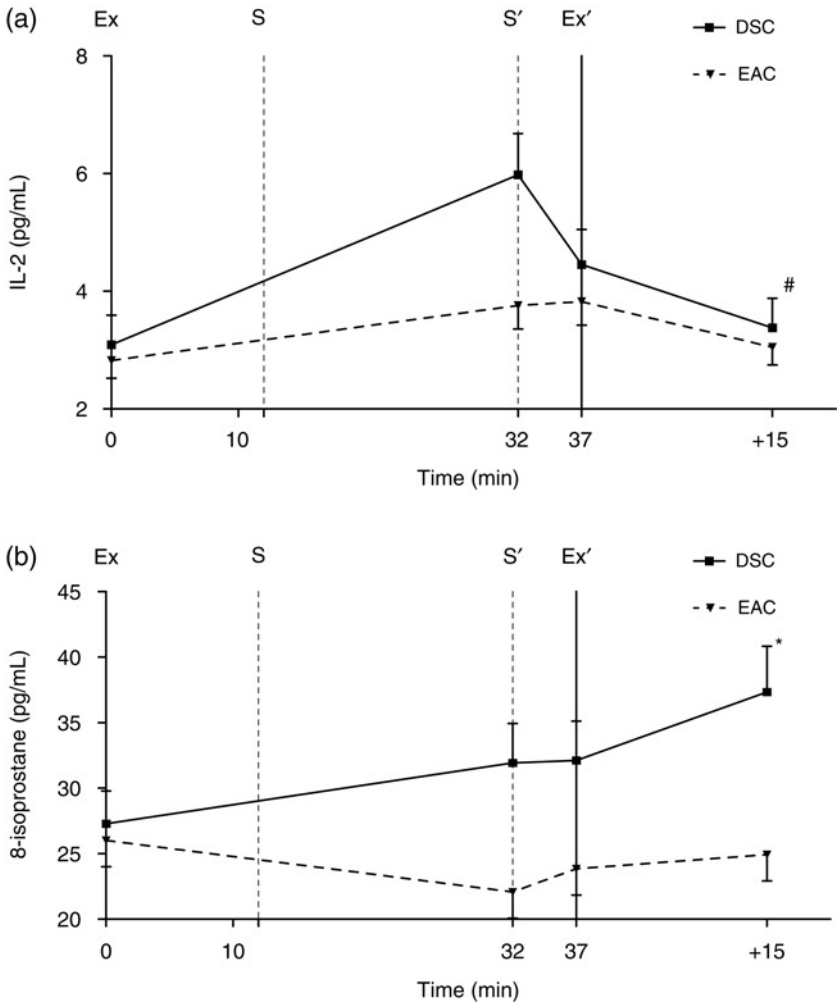


**Figure 3** IL-2 and 8-isoprostane responses during exercise in the exercise-alone condition (EAC) and the dual-stress condition (DSC). A significant change across time was observed in both conditions (a; $^{\#}P < 0.05$). A significant condition by time interaction was revealed in 8-isoprostane, with a greater increase in DSC (b; $^{*}P < 0.05$). Points represent the norepinephrine and epinephrine values during the protocol; vertical lines depict standard errors of the means. The vertical solid lines extending from the ordinate represent the start of exercise (Ex) and the end of exercise (Ex′). The vertical dotted lines extending from the ordinate represent the start of the mental challenge (S) and the end of the mental challenge (S′). IL, interleukin

increase in NE in both conditions. Although both conditions induced elevations in IL-2, higher 8-isoprostane levels were observed following a dual challenge. Furthermore, NE AUC and peak IL-2 were positively associated with peak 8-isoprostane in response to DSC. These findings suggest that the addition of mental challenge to physical stress may elicit greater SA activation and inflammatory response, further providing a possible explanation for elevated oxidative stress.

A DSC revealed a greater EPI elevation with a significant increase in NE in both conditions. This result supports a recent study by Huang *et al.*[33] who have examined firefighters exposed to a decision-making challenge (firefighting strategies and tactics drill) while participating in moderate intensity exercise and found a greater increase for EPI and NE in DSC. This finding is also supported by an earlier study,[34] examining firefighters from India, which observed greater NE and EPI responses in firefighters challenged with a physical and psychological demand. Furthermore, Webb *et al.*[18] have examined healthy individuals in response to a combination of physical and psychological stress and found a greater increase for NE in the DSC. Interestingly, Acevedo *et al.*[35] demonstrated that the addition of a mental challenge elicited an exacerbation in heart rate response during exercise, suggesting that a precursor to the heart rate elevation was an increase in catecholamine levels (EPI and NE). Thus, these findings (elevations in heart rate, EPI and NE) suggest that the SA axes are further activated, beyond the EAC, during a dual challenge. An important consideration for future investigation is to examine the catecholamine response to real-life stressors in some occupations such as military operations and law enforcement.

In the present study a significant increase over time was observed in IL-2 in both the EAC and DSC. Previous research by Heinz *et al.*[16] has shown that acute psychological stress is associated with increased IL-2 concentrations. This elevation may result in the activation of cellular immune responses following acute stress. In response to a dual challenge, Huang *et al.*[17] examined firefighters exposed to a decision-making challenge (firefighting strategies and tactics drill) while participating in moderate

intensity exercise and found a greater increase for IL-2 in the DSC. Although our result did not show a significant condition by time interaction between the two conditions in IL-2, the protocol in this study was different with the protocol by Huang *et al.* and/or our protocol may have been ineffective in eliciting the necessary elevation in IL-2. In addition, IL-2 AUC was correlated with EPI AUC and NE AUC following a dual stress. This result supports a review by Matalka[36] who has summarized that acute stress induces the production of proinflammatory cytokines (e.g. IL-2) via a mild and transient increase in catecholamines (EPI and NE).

Additionally, our result demonstrated a greater 8-isoprostane in response to a dual challenge. The magnitude and direction of the oxidative response to physical stress is dependent upon exercise intensity and duration. This result supports a previous study by Lovlin *et al.*[28] who demonstrated that the release of biomarkers of oxidative stress was limited with moderate intensity exercise, whereas both high-intensity aerobic and anaerobic exercise have been shown to elicit elevated biomarkers of oxidative stress.[13] In response to psychological stress, several studies have demonstrated elevated oxidative stress. More specifically, Sivonova *et al.*[12] investigated a study on medical students and found greater nuclear DNA damage in lymphocytes on the day of an examination (stress condition) compared with during the time between two examination periods (non-stress condition). Since no studies have investigated oxidative response following a dual-stress model, our study presents data demonstrating the exacerbated release of biomarker of oxidative stress to a dual stress and may provide an important contribution to the literature. Further examination of the effects of a dual stress on other forms of oxidative stress will provide a more clear presentation of the oxidative response.

Finally, in support of the role of catecholamines on oxidative stress, Baez and colleagues[37] have demonstrated that catecholamine metabolism generates free radicals and other reactive species. Our result showed that NE AUC was positively correlated with peak 8-isoprostane following a dual challenge. This result supports previous study by Cosentino *et al.*[6] who demonstrated that elevated oxidative stress during psychological stress could be the effect of high circulating levels of catecholamines (EPI and NE). In addition, IL-2 is thought to be the most potent trigger for macrophage-induced ROS production within the Th1 immune response.[7] In the present study peak IL-2 was positively correlated with peak 8-isoprostane following a dual stress. These findings indicate that an elevation in NE and IL-2 may associate with the elevated oxidative stress during a combined physical and psychological stress.

In conclusion, individuals participating in a combination of physical and psychological stress responded with elevations in catecholamines, IL-2 and 8-isoprostane. The elevated oxidative stress may be through the effects of the release of catecholamines and IL-2 during a dual stress. Our results may provide the potential explanation that dual stress alters physiological homeostasis in some occupations including firefighting and law enforcement. This combination of physical and mental stress often experienced by these populations may be a contributing factor in the elevated risk of cardiovascular disease and increased proportionate mortality risks.[4] Further investigation should examine the impact of these changes on cellular function to facilitate a better understanding of the immunoendocrine and oxidative responses to a combined physical and psychological stress. In turn, a greater understanding of these responses to stress can assist in finding strategies (e.g. exercise training) to overcome the inherent psychobiological challenges associated with physically and mentally demanding professions.

**Author contributions:** All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. Conception and design: C-JH, HEW, EOA. Data collection: C-JH, HEW, KAM, SET, GHK, EOA. Data analysis and interpretation: C-JH, HEW, RKE, GHK, EOA. Manuscript writing: C-JH, HEW, RKE, EOA. Final approval of manuscript: C-JH, HEW, RKE, KAM, SET, GHK, EOA.

**Conflict of interest:** Use of trade names does not constitute endorsement of product. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the opinions of the Department of the Army or the Department of Defense.

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*(Received May 31, 2010, Accepted August 9, 2010)*