Sleep restriction across a simulated firefighting deployment: The impact on acute stress responses

Alexander Powell Wolkow

Bachelor of Exercise and Sport Science (Hons)

Submitted in fulfilment of the requirements for

the degree of Doctor of Philosophy

Centre for Physical Activity and Nutrition Research

School of Exercise and Nutrition Sciences

Deakin University

December 2015

DEAKIN UNIVERSITY
ACCESS TO THESIS - A

I am the author of the thesis entitled "Sleep restriction across a simulated firefighting deployment: The impact on acute stress responses" submitted for the degree of 'Doctor of Philosophy'.

This thesis may be made available for consultation, loan and limited copying in accordance with the Copyright Act 1968.

'I certify that I am the student named below and that the information provided in the form is correct'

Full Name: Alexander Powell Wolkow

Signed: Signature Redacted by Library

……

Date: 07/12/2015

DEAKIN UNIVERSITY
CANDIDATE DECLARATION

I certify the following about the thesis entitled **"Sleep restriction across a simulated firefighting deployment: The impact on acute stress responses"** submitted for the **degree 'Doctor of Philosophy'** that:

a. I am the creator of all or part of the whole work(s) (including content and layout) and that where reference is made to the work of others, due acknowledgment is given.

b. The work(s) are not in any way a violation or infringement of any copyright, trademark, patent, or other rights whatsoever of any person.

c. If the work(s) have been commissioned, sponsored or supported by any organisation, I have fulfilled all of the obligations required by such contract or agreement.

I also certify that any material in the thesis which has been accepted for a degree or diploma by any university or institution is identified in the text.

'I certify that I am the student named below and that the information provided in the form is correct'

Full Name: Alexander Powell Wolkow

Signature Redacted by Library

Signed: ..... **Date:** 07/12/2015

Acknowledgements

I would like to express my deepest gratitude to the individuals who have supported me on this journey. I am particularly grateful to my supervisors, Dr Luana Main, Dr Brad Aisbett and Prof Sally Ferguson. Each of you have been a huge inspiration to me and have challenged me to grow as a researcher. Together, you have made my supervision experience amazing.

Luana, as my principal supervisor you provided all the support, patience and encouragement I could ever ask for. Your expertise and knowledge in the areas of physiological and psychological stress research has been invaluable and has inspired me to follow a career in stress research. Thank you for always being available for a chat and providing guidance, both professional and personal, through all aspects of my PhD.

Brad, thank you for being with me on this journey from honours. Your faith in me throughout my post-graduate education has been instrumental in the completion of my PhD and inspired me to continue on a path of research. I am most grateful for your wisdom and advice that has steered me through the many challenges I have faced throughout this journey.

Sally, the support, expertise and honest advice you provided me along the way has been integral to my PhD. You have been my gateway to the world of sleep research, which is an area I have developed a passion for and plan to continue researching in.

I would also like to acknowledge the assistance I received from Dr John Reynolds. Your expertise and instruction in the statistical analyses of my data was invaluable.

To the Deakin team Grace Vincent, Brianna Larsen, Cara Lord, Sarah Hall, Jacqueline Tran, Robbie Savage and Regan Tait, I am so lucky to have worked with such an amazing and talented group of people who have helped and encouraged me along the way. You guys are an excellent group of friends that have made my time at Deakin University extremely enjoyable.

To my partner Laura, your understanding and unwavering encouragement and confidence in me, particularly over these final months, has been incredibly important in getting me over the finish line. Thank you for your unconditional love and support and for always being there when I needed you.

Finally, without the love and support from my Mum, Dad and Sister, my accomplishments would have never been possible. Your sacrifices and encouragement over the years have served as the foundation of my academic success. I am truly lucky to have such a supportive family that has always believed in me. I honestly could not have done this without you.

Abstract

Introduction: Exposure to stressors set in motion inflammatory, cortisol, heart rate and mood responses that allow the body to maintain normal healthy function. Severe or chronic stressors however, may adversely alter the action of these stress responses and lead to adverse health outcomes. Periods of sleep restriction and performing physical work are two stressors routinely faced by wildland firefighters, yet the combined impact that these demands have on firefighters' acute stress responses is poorly understood. Therefore, the objective of this thesis was to investigate the effect of physical firefighting work and sleep restriction on firefighters' cytokine, cortisol and heart rate responses, their interactions, and how mood may influence any observed physiological changes.

Methods: Firefighters completed 3-days of simulated wildfire suppression work separated by an 8-h (Control condition; *n*=18; bedtime 22:00-06:00) or 4-h sleep opportunity (Sleep restriction condition; *n*=17; bedtime 02:00-06:00) on each of the 2 nights. During each work day, participants in both conditions completed multiple work circuits that comprised a suite of simulated physical firefighting tasks. Blood samples were collected from participants 4 times a day from which plasma cytokine levels including IL-6, IL-8, IL-1β, TNF-α, IL-4 and IL-10 were determined. Saliva samples for the determination of cortisol were collected 9 times across the day and heart rate was measured continuously each day. Finally, firefighters' mood was assessed 4 times each day using the Mood Scale II and 5 times each day using the Samn-Perelli fatigue scale.

Results: Study 1 investigated how restricted sleep while performing physical firefighting work affected firefighters' acute inflammatory responses by measuring their cytokine levels. Findings revealed that IL-8 was higher among firefighters who

received an 8-h sleep opportunity each night, when compared to those who had a shortened sleep opportunity. Firefighters' IL-6 levels also increased over successive days of work, but no significant differences were found between conditions. Increases across daily time-points were found across both conditions for IL-6 and IL-4, while IL-1β, TNF-α and IL-8 levels decreased across time-points. There were no significant changes observed for IL-10. To examine how multiple days of simulated physical firefighting work and sleep restriction effect neuroendocrine responses, Study 2 examined firefighters' cortisol and heart rate responses as measures of the hypothalamic-pituitary-adrenal-(HPA) axis and sympathetic-adrenal-medullary (SAM) system respectively. No significant differences were found for cortisol between conditions on day 1. However, on day 2 and day 3, the sleep restricted participants exhibited a significantly higher daily area under the cortisol curve (AUC) level and elevated diurnal cortisol profile in the afternoon and evening when compared with the control participants. Firefighters' heart rate decreased across the 3 days, but there were no significant differences found between conditions. Study 3 revealed immune-endocrine relationships between cytokine and cortisol responses during the 3-day deployment with and without restricted sleep. Specifically, a rise in morning IL-6 was related to an elevated evening cortisol among firefighters in the sleep restriction condition, but was associated with a decreased evening cortisol in the control condition. Higher IL-6 levels were related to increased daily cortisol AUC, but this relationship was not different between conditions. Less pronounced relationships were demonstrated between TNF-α, IL-10, IL-4 and cortisol independent of the sleep opportunity and did not persist after adjusting for covariates. In the final study (Study 4), increases in positive mood dimensions were found to influence a rise in IL-6, IL-8 and TNF-α in the sleep restriction condition. A rise in negative mood dimensions in

the sleep restriction condition were also associated with increased IL-6, TNF-α, IL-10 and cortisol levels.

Conclusion: The 4-h sleep opportunity between multiple days of simulated wildland firefighting work did not impact cytokine or heart rate responses in excess of any disturbance caused by the physical work alone. The findings for heart rate demonstrate how exposure to physical work and sleep restriction did not represent an acute risk to elevated SAM system activity. Similar to heart rate, the combined stressors did not represent an acute inflammatory risk for personnel. However, higher IL-8 and IL-6 during the multiday deployment, regardless of the sleep opportunity, highlight the need to further determine if these acute increases in cytokine levels pose a risk to firefighters' long-term health. Findings further highlight the role an 8-h sleep on the fire-ground has in maintaining normal cortisol levels and appropriate immune-endocrine interactions, specifically between morning IL-6 and evening cortisol. An application of the psychophysiological relationships found between mood, cytokine and cortisol demonstrate the potential utility of subjective mood as a fire-ground indicator of physiological changes. Collectively, this PhD indicates for the first time the acute stress response pathways and their interactions through which physical firefighting work, with and without restricted sleep may, over time, impact the health of personnel.

List of Publications

Published

Wolkow, A, Ferguson, SA, Aisbett, B & Main, LC 2015, 'The effects of work-related sleep restriction on acute physiological and psychological stress responses and their interactions: A review among emergency service personnel', *International Journal of Occupational Medicine and Environmental Health*, vol. 28, no. 2, pp. 183-208.

Wolkow, A, Aisbett, B, Reynolds, J, Ferguson, SA & Main, LC 2015, 'The impact of sleep restriction while performing simulated physical firefighting work on cortisol and heart rate responses' *International Archives of Occupational and Environmental Health,* August 2015, doi 10.1007/s00420-015-1085-3

Wolkow, A, Ferguson, SA, Vincent, GE, Larsen, B, Aisbett, BA, & Main, LC 2015, 'The Impact of Sleep Restriction and Simulated Physical Firefighting Work on Acute Inflammatory Stress Responses', *PLoS One,* September 2015, vol. 10 no. 9, e0138128. doi: 10.1371/journal.pone.0138128. eCollection 2015.

Wolkow, A, Aisbett, B, Reynolds, J, Ferguson, SA & Main, LC 2015, 'Relationships between inflammatory cytokine and cortisol responses in firefighters exposed to simulated wildfire suppression work and sleep restriction' Submission under first review at *Physiological Reports,* vol. 3, no. 11, doi:10.14814/phy2.12604.

Under Review

Wolkow, A, Aisbett, B, Reynolds, J, Ferguson, SA, & Main, LC, 'Acute psychophysiological relationships between mood, inflammatory and cortisol changes in response to simulated physical firefighting work and sleep restriction' Submission under second review at *Applied Psychophysiology and Biofeedback*, April 2015.

Conference Presentations

Wolkow A, Ferguson S, Reynolds J, Aisbett B, Main LC. Evidence of psychophysiological relationships between stress responses among sleep restricted firefighters completing simulated wildfire work. Sleep DownUnder 2015 ASM, Cycles, Melbourne, Australia, October 22nd to 24th 2015.

Wolkow A, Aisbett B, Ferguson SA, Main LC. The Impact of Sleep Restriction on Acute Inflammatory Stress Responses to Simulated Physical Firefighting Work (Oral Presentation and Poster). Sleep DownUnder 2014 ASM, Sleep Frontiers, Perth, Australia, October 9th to 11th 2014.

Wolkow A, Aisbett B, Ferguson SA, Main LC. How a lack of sleep on the fire ground may be impacting firefighters' physiological stress response. Australasian Fire Authorities Council/Natural Hazards and Bushfire Co-Operative Research Centre Conference, Wellington, New Zealand, September 2nd to 5th, 2014.

Wolkow A, Aisbett B, Ferguson SA, Main LC. Effects of sleep restriction on cortisol during simulated physical firefighting work (Oral Presentation). Fourth International Conference on Health Wellness, and Society, Vancouver, Canada, March 14th to 15th, 2014.

Wolkow A, Aisbett B, Ferguson SA, Main LC. Sleep Deprivation and Stress Responses: Could emergency work have a negative impact on your health? Australasian Fire Authorities Council/Bushfire Co-Operative Research Centre Conference, Melbourne, Australia, September 2nd to 5th, 2013.

Wolkow A, Aisbett B, Ferguson SA, Main LC. The interactions of physical work, sleep deprivation and stress. Australasian Fire Authorities Council/Bushfire Co-Operative Research Centre Conference, Perth, Australia, August 28th to 31st, 2012.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACTH	Adrenocorticotropic hormone
AIC	Akaike information criterion
ANOVA	Analysis of Variance
AR1	Autoregressive function
AUC	Area under the curve
b	Regression (unstandardized) coefficients
BMI	Body mass index
CFS	Chronic fatigue syndrome
CHD	Coronary heart disease
CHO	Carbohydrates
CON	Control condition
CRH	Corticotropin-releasing hormone
CV	Coefficient of variation
CVD	Cardiovascular disease
df	Degrees of freedom
ELISA	Enzyme-linked immunosorbent assay
HR	Heart rate
HPA	Hypothalamic-pituitary-adrenal axis
IL	Interleukin
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
kg/m^2	Kilogram per square metre
L/min	Litres per minute
LMM	Linear mixed model
nmol/L	Nanomole per litre
pg/mL	Picogram per millilitre
POMS	Profile of mood state questionnaire
PSG	Polysomnography
REM	Rapid-eye-movement sleep
REML	Restricted maximum likelihood
SAM	Sympathetic-adrenal-medullary
SD	Standard deviation
SED	Standard error of difference
SEM	Standard error of the mean
SR	Sleep restriction condition
SWS	Slow-wave sleep
TNF-α	Tumour Necrosis Factor-alpha
µg/dL	Micrograms per decilitre
µL	Micro litres
$VO_{2\ max}$	Maximal oxygen consumption

Chapter 1: Introduction

1.1 Background

Occupational stress is the physiological and psychological responses of an individual to work demands (Huang et al. 2011; World Health Organization 2003). Occupational demands (or stressors) can be physical, including reduced sleep and physical work (Cater et al. 2007; Courtney et al. 2013; Phillips et al. 2012), psychological, such as life threatening situations and critical decisions (Elliott et al. 2009; Liberman et al. 2002), and environmental, for example heat and smoke exposure (Raines et al. 2013; Reisen et al. 2011). Physiological stress responses include stress hormone (e.g., cortisol), cardiovascular (e.g., heart rate), and inflammatory changes (e.g., cytokines; Chandola et al. 2010; Tsigos et al. 2002). These occur in conjunction with observable psychological changes such as variations in mood (Kemeny 2007; Smith et al. 1996). Physiological and psychological changes in response to an occupational stressor allow the body to adapt by achieving renewed stability (or homeostasis) in the function of the physiological and psychological stress systems (Lundberg 1999; McEwen et al. 1999). However, exposure to excessive occupational demands *above* the level a person is capable of coping with, can disturb (e.g., exaggerate or suppress) these physiological and psychological responses. Over time, dysregulation of these responses may have a damaging effect on physical and mental health (Anisman et al. 2003; Black 2006; Chrousos 2000; Mackin et al. 2004; Silverman et al. 2012; Willerson et al. 2004).

Two main views dominate the discussion regarding work-related disturbances to physiological and psychological stress responses (National Institute for Occupational Safety and Health 1999; Salazar et al. 2000). One view centres on worker characteristics such as their personality and their ability to cope mentally (Salazar et al. 2000), while the second focuses on the conditions or stressors of work, such as the physical demands (National Institute for Occupational Safety and Health 1999; Salazar

et al. 2000; Spence 1994). Several strong lines of evidence now point to occupational stressors as a major source of altered physiological and psychological responses among personnel (Karasek et al. 1990; Sauter et al. 1995). Through chronic changes in physiological and psychological stress responses, exposure to occupational stressors may be a contributing factor in the development of adverse health outcomes such as cardiovascular, metabolic and autoimmune diseases (Chandola et al. 2008; Silverman et al. 2012; Willerson et al. 2004) and depression (Anisman et al. 2003; Mackin et al. 2004). The focus of this thesis is therefore to examine how physical stressors inherent to wildland firefighting work (Aisbett et al. 2012) affect physiological and psychological stress responses among personnel. Understanding this will provide an important first step in determining the long-term impact these demands may have on the health of wildland firefighters.

1.2 Wildland firefighting stressors and stress responses

In Australia, there are ~230,000 volunteer and salaried firefighters who protect people and property from a range of natural and manmade threats (Attorney-General's Department and Australasian Fire and Emergency Service Authorities Council 2015; Glass et al. 2014), the most devastating of which are wildfires (Australian Institute of Criminology 2004). With summers becoming longer and hotter (Bureau of Meteorology et al. 2014), the number, severity and length of wildfires are also on the rise (Hennessy et al. 2005). Consequently, firefighters are deployed more frequently to the fire-ground to defend communities in bigger and longer duration incidents. On the fire-ground, wildland firefighters routinely face a unique combination of occupational stressors (e.g., physical work, restricted sleep, heat and smoke exposure; Aisbett et al. 2012; Cater et al. 2007; Cuddy et al. 2007; Reisen et al. 2011). Two common fire-ground stressors for firefighters performing wildfire suppression are

physical work and sleep restriction (Aisbett et al. 2012; Cater et al. 2007; Cuddy et al. 2007). Existing research shows that wildland firefighters often work consecutive long shifts (i.e., 12 to 16-h), which combined with unfamiliar sleeping conditions (i.e., noise, camp beds, light, heat etc.), may contribute to shortened sleep opportunities between shifts on the fire-ground (i.e., 3 to 4 h; Cater et al. 2007). Within shifts, firefighting work can involve short-duration high-intensity physical tasks' performed intermittently for extended periods of time (Cuddy et al. 2007; Phillips et al. 2011; Phillips et al. 2012; Phillips et al. 2015).

The physical work and sleep restriction involved in wildland firefighting may elicit physiological and psychological responses that are adaptive (Chrousos 1995; Maier et al. 1998; McEwen et al. 1999), allowing firefighters to cope with these demands. However, repeated exposure to stressors, such as that reported during multi-day wildfire deployments (Aisbett et al. 2012; Cater et al. 2007; Cuddy et al. 2007) may dysregulate these responses and have deleterious consequences for short- and long-term health. At present however, the effect these common fire-ground demands have on wildland firefighters' acute physiological and psychological stress responses is poorly understood.

Firefighting-based research that assesses a range of inflammatory markers is required to comprehensively understand the impact physical stressors have on innate immune function. Further, determining diurnal cortisol and heart rate responses to occupational stressors is important in interpreting whether these neuroendocrine changes correspond appropriately to physical occupational demands or reveal an abnormal response to stressors. Dysregulation of immune and neuroendocrine function can reflect severe acute or chronic elevations in inflammatory markers such as cytokines

(Brüünsgaard et al. 2003; Mullington et al. 2010), heart rate and cortisol (Chandola et al. 2008; McEwen et al. 1999), as well as an imbalance between inflammatory and cortisol responses (Nijm et al. 2007). Such alterations have been associated with negative physiological (e.g., cardiovascular and metabolic diseases; Mullington et al. 2010; Willerson et al. 2004) and psychological health outcomes (e.g., depression; Anisman et al. 2003; Mackin et al. 2004; Silverman et al. 2012). Indeed, fire suppression activities in the United States of America have been associated with an increased risk of death from cardiovascular disease (CVD)-related events (Kales et al. 2007), while high levels of depression have been reported among firefighters in Australia (for a review see Cook et al. 2013), United States of America (Carey et al. 2011) and South Korea (An et al. 2015). Disruptions to acute immune and cortisol activity have also been related to adverse changes in mood (Kemeny 2007; Vgontzas et al. 2008), supporting the application of a psychophysiological approach to further understand the impact of acute occupational stressors on the body.

1.3 Study aims

The overall objective of this thesis was to investigate the effect that physical wildfire suppression work and sleep restriction have on firefighters' inflammatory, cortisol and heart rate responses, the interactions between the systems, and how mood might influence these physiological changes. Firefighters were studied over a 3-day and 2-night simulated fire-ground deployment in which participants completed physical wildfire suppression work, with or without a restricted sleep opportunity on each of the nights. Exposure to occupational stressors, such as physical work and sleep restriction, is known to elicit an acute inflammatory response causing cells, such as macrophages to release cytokines (Maier and Watkins 1998).

Study 1 *aimed to assess the effect that restricted sleep has on wildland firefighters' inflammatory cytokine levels while performing 3 days of simulated physical firefighting work.*

Stress exposure also activates the hypothalamic-pituitary-adrenal (HPA)-axis, causing the release of cortisol, the major stress hormone. Further, encountering an occupational stressor can activate the sympathetic-adrenal-medullary (SAM) system (Chandola et al. 2010; Faulkner et al. 2014) causing alterations in parasympathetic nervous system activity, reflected by a change in heart rate (Chandola et al. 2010; Faulkner et al. 2014).

Study 2 *aimed to assess the effect that restricted sleep has on wildland firefighters' cortisol and heart rate responses while performing simulated physical firefighting work.*

Interplay between cytokines and cortisol may represent a mechanistic pathway that influences these physiological responses to stressors.

Study 3 sought *to quantify the relationship between firefighters' cytokine and cortisol responses to restricted sleep while performing simulated physical work.*

Psychological stress responses, such as changes in mood, may moderate inflammatory and cortisol responses to stressors.

Study 4 *examined how changes in wildland firefighters' mood influence cytokine and cortisol levels in response to restricted sleep while performing simulated physical work.*

1.4 Significance of the research

Wildland firefighters are routinely exposed to consecutive long shifts of physical work separated by restricted sleep opportunities at night (Aisbett et al. 2012; Cater et al. 2007; Cuddy et al. 2007). At present however, the impact of fire-ground demands on inflammatory, cortisol, heart rate and mood responses is poorly understood. Activation

of physiological stress responses and their interactions with mood can have negative implications to short- (e.g., negative mood; Piazza et al. 2013; Vgontzas et al. 2008) and long-term health (e.g., depression and CVD; Anisman et al. 2003; Mullington et al. 2010; Silverman et al. 2012; Willerson et al. 2004). Certain firefighting populations have been shown to have a high prevalence of CVD and depression (An et al. 2015; Carey et al. 2011; Cook et al. 2013; Kales et al. 2007). Therefore, characterising acute stress responses to consecutive days of simulated physical work and sleep restriction will advance our understanding of how exposure to these demands may be related to adverse health outcomes. Quantifying the relationship between cytokine and cortisol responses to these demands may offer novel insights for occupational-based research into how acute inflammatory responses relate, and potentially signal dysregulated HPA-axis activity. Further examining the relationships between physiological and mood responses may provide useful information to fire agencies about subjective psychological fire-ground indicators of physiological changes.

Together, the potential findings from this thesis will provide insight into the potential consequences of shortened sleep and physical work on acute physiological and psychological stress responses to inform the management of risk associated with sleep restriction during firefighting deployments. In addition to firefighting, the comprehensive assessment of inflammatory, neuroendocrine (i.e., cortisol and heart rate) and psychological stress responses will provide novel findings for applied stress physiology, psychophysiology and sleep research. Furthermore, the results will offer a foundation for future research to investigate these physiological and psychological stress responses among other occupations with similar periods of physical work and sleep restriction to firefighting (e.g., mining, rescue workers).

1.5 Note for readers

Sections of the literature review along with the four experimental studies presented in this thesis have either been accepted for publication (Literature Review, Study 1, Study 2 and Study 3), or are currently under various stages of review (Study 4) in scientific journals. Apart from minor edits, the content presented in each of the study chapters is identical to that submitted to the respective journals. In addition, each study presented has been formatted in accordance with the respective journals submission requirements for referencing. Please also note that each of the completed studies were prepared for submission concurrently. Therefore, in order to reference and discuss how the findings from each of these studies relate to each other, relevant conference abstracts have been appropriately referenced in place of studies yet to be published.

1.6 References

Aisbett, B, Wolkow, A, Sprajcer, M and Ferguson, SA 2012, '"Awake, smoky, and hot": Providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters', *Applied Ergonomics*, vol. 43, no. 5, pp. 916-925.

An, SJ, Chung, YK, Kim, BH, Kwak, KM, Son, JS, Koo, JW, Ju, YS and Kwon, YJ 2015, 'The effect of organisational system on self-rated depression in a panel of male municipal firefighters', *Annals Of Occupational And Environmental Medicine*, vol. 27, pp. 1-7.

Anisman, H and Merali, Z 2003, 'Cytokines, stress and depressive illness: brain-immune interactions', *Annals of Medicine*, vol. 35, pp. 2-11.

Attorney-General's Department and Australasian Fire and Emergency Service Authorities Council 2015, *National Statement Of Capability For Fire And Emergency Services*, Australian Goverment.

Australian Institute of Criminology 2004, *The cost of bushfires*, Australian Institute of Criminology, Canberra, Australian Capital Territory.

Black, P 2006, 'The inflammatory consequences of psychologic stress: Relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II', *Medical Hypotheses*, vol. 67, no. 4, pp. 879-891.

Brüünsgaard, H and Pedersen, B 2003, 'Age-related inflammatory cytokines and disease', *Immunology And Allergy Clinics Of North America*, vol. 23, no. 1, pp. 15-39.

Bureau of Meteorology and Commonwealth Scientific and Industrial Research Organisation (CSIRO) 2014, *State of the Climate 2014*, Australian Goverment.

Carey, MG, Al-Zaiti, SS, Dean, GE, Sessanna, L and Finnell, DS 2011, 'Sleep problems, depression, substance use, social bonding, and quality of life in professional firefighters', *Journal Of Occupational And Environmental Medicine/American College Of Occupational And Environmental Medicine*, vol. 53, no. 8, pp. 928-933.

Cater, H, Clancy, D, Duffy, K, Holgate, A, Wilison, B and Wood, J 2007, 'Fatigue on the fireground: the DPI experience', in R Thornton (ed.), *Bushfire Cooperative Research Centre/Australasian Fire Authorities Council Conference Research Forum*, Hobart, Tasmania.

Chandola, T, Britton, A, Brunner1, E, Hemingway, H, Malik, M, Kumari, M, Badrick, E, Kivimaki, M and Marmot, M 2008, 'Work stress and coronary heart disease: what are the mechanisms?', *European Heart Journal*, vol. 29, no. 5, pp. 640-648.

Chandola, T, Heraclides, A and Kumari, M 2010, 'Psychophysiological biomarkers of workplace stressors', *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 1, pp. 51-57.

Chrousos, GP 1995, 'The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation', *The New England Journal Of Medicine*, vol. 332, no. 20, pp. 1351-1362.

Chrousos, GP. 2000. The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. *International Journal of Obesity and Related Metabolic Disorders,* vol. 24, Supplement 2, pp. 50-55.

Cook, B and Mitchell, W 2013, *Occupational health effects for firefighters: The extent and implications of physical and psychological injuries*, Centre of Full Employment and Equity.

Courtney, J, Francis, A and Paxton, S 2013, 'Caring for the Country: Fatigue, Sleep and Mental Health in Australian Rural Paramedic Shiftworkers', *Journal of Community Health*, vol. 38, no. 1, pp. 178-186.

Cuddy, J, Gaskill, S, Sharkey, B, Harger, S and Ruby, B 2007, 'Supplemental feedings increase self-selected work output during wildfire suppression', *Medicine and Science in Sports and Exercise*, vol. 39, no. 6, pp. 1004-1012.

Elliott, G, Omodei, M and Johnson, C 2009, 'How Human Factors Drive Decision Making at Fire Ground Level', *Bushfire Co-Operative Research Centre FireNote*, p. 4.

Faulkner, SH, Spilsbury, KL, Harvey, J, Jackson, A, Huang, J, Platt, M, Tok, A and Nimmo, MA 2014, 'The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise', *European Journal of Applied Physiology*, vol. 114, no. 6, pp. 1207-1216.

Glass, D, Sim, M, Pircher, S, Del Monaco, A, Dimitriadis, C, Miosge, J, Vander Hoorn, S and Gordon, I 2014, *Australian Firefighters' Health Study*, Monash Centre for Occupational and Environmental Health, Melbourne, Victoria.

Hennessy, K, Lucas, C, Nicholls, N, Bathols, J, Suppiah, R and Ricketts, J 2005, *Climate Change Impacts on Fire-Weather in South-East Australia*, Commonwealth Scientific and Industrial Research Organisation (CSIRO) Marine and Atmospheric Research, Aspendale, Victoria.

Huang, C and Acevedo, E 2011, 'Occupational stress: the influence of obesity and physical activity/fitness on immune function', *American Journal of Lifestyle Medicine*, vol. 5, no. 6, pp. 486-493.

Kales, SN, Soteriades, ES, Christophi, CA and Christiani, DC, . 2007, 'Emergency duties and deaths from heart disease among firefighters in the United States', *The New England Journal Of Medicine*, vol. 356, no. 12, pp. 1207-1215.

Karasek, R and Theorell, T 1990, *Healthy work, stress, productivity and the reconstruction of working life*, Basic Books, New York.

Kemeny, ME 2007, 'Emotions and the Immune System', in R Ader (ed.), *Psychoneuroimmunology Vol 4*, Elsevier, pp. 619-629.

Liberman, AM, Best, SR, Metzler, TJ, Fagan, JA, Weiss, DS and Marmar, CR 2002, 'Routine occupational stress and psychological distress in police', *Policing: An International Journal of Police Strategies and Management*, vol. 25, no. 2, pp. 421-441.

Lundberg, U 1999, 'Coping with stress: neuroendocrine reactions and implications for health', *Noise and Health*, vol. 1, no. 4, pp. 67-74.

Mackin, P and Young, AH 2004, 'The role of cortisol and depression: exploring new opportunities for treatments', *Psychiatric Times*, vol. 21, no. 5, pp. 92-95.

Maier, SF and Watkins, LR 1998, 'Cytokines for Psychologists: Implication of Bidirectional Immune-to-Brain Communication for Understanding Behaviour, Mood, and Cognition', *Psychological Review*, vol. 105, no. 1, pp. 83-107.

McEwen, BS and Seeman, T 1999, 'Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load', *Annals of the New York Academy of Sciences*, vol. 896, pp. 30-47.

Mullington, J, Simpson, N, Meier-Ewert, H and Haack, M 2010, 'Sleep loss and inflammation', *Best Practice and Research Clinical Endocrinology and Metabolism*, vol. 24, pp. 775-784.

National Institute for Occupational Safety and Health 1999, *Stress at work*, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

Nijm, J, Kristenson, M, Olsson, AG and Jonasson, L 2007, 'Impaired cortisol response to acute stressors in patients with coronary disease. Implications for inflammatory activity', *Journal of Internal Medicine*, vol. 262, no. 3, pp. 375-384.

Phillips, M, Netto, K, Payne, W, Nichols, D, Lord, C, Brooksbank, N, Onus, K, Jefferies, S and Aisbett, B 2011, 'Frequency, intensity and duration of physical tasks performed by Australian rural firefighters during bushfire suppression', in *Proceedings of the Bushfire Cooperative Research Center/Australasian Fire Authorities Council Conference Research Forum*, Thornton RP (ed.), Sydney, pp. 205-212.

Phillips, M, Payne, W, Lord, C, Netto, K, Nichols, D and Aisbett, B 2012, 'Identification of physically demanding tasks performed during bushfire suppression by Australian rural firefighters', *Applied Ergonomics*, vol. 43, no. 2, pp. 435-441.

Phillips, M, Payne, WR, Netto, K, Cramer, S, Nichols, D, McConell, GK, Lord, C and Aisbett, B 2015, 'Oxygen uptake and heart rate during simulated wildfire suppression tasks performed by Australian rural firefighters', *Occupational Medicine and Health Affairs*, vol. 3, no. 3.

Piazza, JR, Charles, ST, Stawski, RS and Almeida, DM 2013, 'Age and the association between negative affective states and diurnal cortisol', *Psychology and Aging*, vol. 28, no. 1, pp. 47-56.

Raines, J, Snow, R, Petersen, A, Harvey, J, Nichols, D and Aisbett, B 2013, 'The effect of prescribed fluid consumption on physiology and work behavior of wildfire fighters', *Applied Ergonomics*, vol. 44, no. 3, pp. 404-413.

Reisen, F, Hansen, D and Meyer, CP 2011, 'Exposure to bushfire smoke during prescribed burns and wildfires: Firefighters' exposure risks and options', *Environment International*, vol. 37, no. 2, pp. 314-321.

Salazar, M and Beaton, R 2000, 'Ecological Model of Occupational Stress: Application to Urban Firefighters', *American Association of Occupational Health Nurses Journal*, vol. 48, no. 10, pp. 470-479.

Sauter, S and Murphy, L 1995, *Organizational risk factors for job stress*, American Psychological Association, Washington, DC.

Silverman, MN and Sternberg, EM 2012, 'Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction', *Annals of the New York Academy of Sciences*, vol. 1261, no. 1, pp. 55-63.

Smith, D, Petruzzello, S, Kramer, J and Misner, J 1996, 'Physiological, psychophysical, and psychological responses of firefighters to firefighting training drills', *Aviation, Space, and Environmental Medicine*, vol. 67, no. 11, pp. 1063-1068.

Spence, W 1994, *Stress: A modern epidemic*, Health EDCO, Waco, TX.

Tsigos, C and Chrousos, GP 2002, 'Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress', *Journal of Psychosomatic Research*, vol. 53, pp. 865-871.

Vgontzas, AN, Bixler, EO, Chrousos, GP and Pejovic, S 2008, 'Obesity and sleep disturbances: Meaningful sub-typing of obesity', *Archives of Physiology and Biochemistry*, vol. 114, no. 4, pp. 224-236.

Willerson, JT and Ridker, PM 2004, 'Inflammation as a cardiovascular risk factor', *Circulation*, vol. 109, no. Supplement 1, pp. II2-II10.

World Health Organization 2003, *Work Organization and Stress*, World Health Organization, Geneva.

Chapter 2: Literature Review

Note for readers

Parts of this chapter (i.e., Section 2.5.1) have been published as a review (Appendix D: Wolkow, A, Aisbett, B, Ferguson, S and Main, L. (2015) The effects of work-related sleep restriction on acute physiological and psychological stress responses and their interactions: A review among emergency service personnel*, International Journal of Occupational Medicine and Environmental Health,* vol. 28 no. 2, pp 183-202). However, for comprehensibility of Chapter 2, relevant content from the published paper that relates to sleep restriction and stress responses specific to this PhD have been incorporated into this chapter.

2.1 Overview

The potential for multiple days of physical work and sleep restriction to impact acute stress responses will be discussed in this review of the literature. It begins with an overview detailing what stressors are and how the body responds to stressors through activation of inflammatory, cortisol and heart rate changes. The diurnal rhythm of these physiological changes and how they relate to psychological stress responses (i.e., mood) are then described. The focus of this chapter then narrows to critique and identify gaps in the firefighting-based literature that has investigated acute inflammatory, cortisol and heart rate responses to periods of physical work spanning a single shift through to multiple days. The combined impact physical work together with sleep restriction has on physiological responses and how mood may influence these is then discussed.

Although periods of physical work (i.e., 3 days) and sleep restriction (i.e., 2 nights) reflective of a fire-ground deployment are of primary interest for this review, research investigating comparable periods of up to seven days and six nights are also included. Longer periods however, (e.g., 8-week military training) are not examined, given the impact extended stressor exposure has on chronic changes to physiological and psychological responses, which lie outside the scope of this thesis. While this chapter provides a comprehensive review of firefighting-based literature that has examined physical work and sleep restriction, most studies examining these stressors have been military-based. Consequently, the balance of literature in this review from different groups reflects what is available. Where emergency or firefighting-specific research is not available, findings from the wider sleep and exercise literature that have investigated similar periods of physical activity and sleep restriction are also reviewed and their transferability to firefighters discussed.

2.2 Stress responses

Maintaining stability (or homeostasis) of the physiological and psychological systems is vital to the survival of all living human organisms (Chrousos et al. 1992). Extrinsic demands (or stressors) can threaten the homeostasis of these systems, but in reply, a complex set of physiological and psychological responses are activated to cope with the demands and regain stability. This idea was first developed by Walter Cannon (1939) in his explanation of the 'fight or flight' response to a potentially dangerous situation. Hans Selye (1956) extended the work by Cannon (1939) to describe the body's physiological responses to any type of demand as 'stress' responses. Selye (1956) further explained that while stress responses prepare an organism to adapt, depending on the severity of the stressor, responses can also be excessive and prolonged, or inadequate; and as a result, the response is no longer adaptive and homeostasis is not restored (McEwen et al. 1999). Although first recognised by Selye (1956), this concept has been more recently expanded upon by McEwen et al. (1999) who put forward the allostatic load model. The allostatic load model describes the process by which dysregulation of multiple physiological responses across the body creates a maladaptive state that increases the risk of disease (McEwen et al. 1999). Indeed, this model is supported by a growing body of research indicating that the dysregulation of several inter-related stress responses over time, may lead to pathological processes that contribute to negative health outcomes (Elenkov 2008; Juster et al. 2010; McEwen et al. 1997). Two of the body's principal stress responses include inflammatory and neuroendocrine changes that involve alterations to cytokine, heart rate and cortisol, which are described in further detail below.

Exposure to stressors such as intense physical activity, trauma and infection can cause cells such as macrophages and T-cells to release soluble proteins known as cytokines

in the immune system (Chrousos 1995; Elenkov et al. 2002; Mastorakos et al. 2005). These cytokines facilitate a local inflammatory reaction causing an influx of white blood cells (e.g. lymphocytes, neutrophils, monocytes) to heal tissue and clear the antigen at the site of inflammation (Pedersen et al. 2000). In turn, pro-inflammatory cytokines such as interleukin (IL)-1β, Tumour Necrosis Factor (TNF)-α, IL-8 and IL-6 (Chrousos 1995) induce a systemic response known as the acute-phase response which causes complex and widespread processes throughout the body to return it to a state of homeostasis (Moldoveanu et al. 2001; Pedersen, B et al. 2000). Conversely, anti-inflammatory cytokines are also produced which aid to inhibit the pro-inflammatory cytokine response to stress and attenuate inflammation (Opal et al. 2000). Major anti-inflammatory cytokines include IL-4 and IL-10 (Opal et al. 2000). Furthermore, IL-6 and IL-4 cytokines can display both pro- and anti-inflammatory actions that modulate inflammation (Brown et al. 1997; Paul 1991; Petersen et al. 2005; Tilg et al. 1997). For example, under certain conditions such as 'non-damaging' exercise in the form of moderate intensity cycling, IL-6 has been reported to have anti-inflammatory and immunosuppressive properties that may negatively control the acute-phase response and lower other pro-inflammatory cytokines (Petersen et al. 2005; Starkie et al. 2003). Together, the pro- and anti-inflammatory processes coordinate the body's immune response to stressors (Elenkov et al. 2002; Pedersen et al. 2000; Petersen et al. 2005).

However, severe acute or chronic stress exposure can exacerbate the immune response resulting in elevated cytokine levels (Elenkov et al. 2002; Padgett et al. 2003). Increased cytokine release in response to acute, but severe stressors, has been related to the expression of negative mood states such as fatigue (Robson-Ansley et al. 2009; Thomas et al. 2011; Vgontzas et al. 2008) and depressive symptoms (Fagundes et al.

2013). There is also limited evidence that has linked acute cytokine responses to an increased incidence of upper respiratory tract infections (Smith, LL 2003). Meanwhile, chronic elevations in cytokine levels have been linked with negative physiological (e.g., cadiovascular and metabolic diseases; Grandner et al. 2013) and psychological health outcomes (e.g., depression; Anisman et al. 2003; Pizzi et al. 2008; Zunszain et al. 2011). The mechanisms linking cytokine and physiological health outcomes are not yet fully understood, but growing research indicates that increased cytokine levels play a central role in initiating the release of C-reactive protein which facilitates the formation of atheroslcerotic plaques and increases the risk of cardiovascular disease (CVD; van Leeuwen et al. 2009). Further evidence suggests that increased cytokine levels may dysregulate insulin binding and signalling which can lead to insulin resistance (Black 2006). Elevated cytokine levels have also been implicated in the development of depression via the ability of cytokines to impair neuronal plascity and neuroendocrine and neurotransmitter (e.g., monoamine) activity (Dantzer et al. 2008; Hayley et al. 2005).

The stress-induced release of cytokines stimulates the secretion of corticotropin-releasing hormone (CRH) from the hypothalamus (Chrousos 1995; Elenkov et al. 2002; Mastorakos et al. 2005). The release of CRH activates the hypothalamic-pituitary-adrenal (HPA)-axis, which is associated with the expression of adrenocorticotropic hormone (ACTH) in the anterior pituitary gland (Chrousos 1995; Elenkov et al. 2002; Maier et al. 1998; Mastorakos et al. 2005; Padgett et al. 2003). The ACTH molecules then circulate in the bloodstream to the adrenal cortex where they stimulate the release of cortisol, the major stress hormone (Mastorakos et al. 2005; Padgett et al. 2003). Stress exposure also causes the hypothalamus to activate the sympathetic-adrenal-medullary (SAM) system resulting in the release of

catecholamines (epinephrine and norepinephrine; Chandola et al. 2010; Faulkner et al. 2014). Catecolamines can reduce activity of the parasympathetic nervous system, resulting in an increased heart rate (Chandola et al. 2010; Faulkner et al. 2014). Indeed, heart rate has been found to directly correlate with catecholamine levels in response to simulated and real-world stressors such as physical work (Planz et al. 1975; Salvadori et al. 2003). Furthermore, heart rate as a measure of SAM activity may be less impacted by factors such as diet (e.g., vanilla products, caffeine) nicotine, gender and body weight which strongly influence the measurement of epinephrine and norepinephrine levels in the blood and urine (Lundberg 2008). Therefore, research supports the use of heart rate as a measure of SAM system activity (Sluiter et al. 2000). To cope with a stressor, some activation of these neuroendocrine functions is expected, however exposure to severe acute or chronic stressors can exacerbate the HPA-axis and SAM system resulting in the sustained elevation of cortisol and heart rate (Chandola et al. 2008; Meier-Ewert et al. 2004; van Leeuwen et al. 2009).

Chronically altered cortisol and heart rate responses may have negative consequence for health (Chandola et al. 2008; Mackin et al. 2004). For instance, several lines of enquiry have demonstrated how dysregulated cortisol levels may lead to endothelial dysfunction (for a review see Poitras et al. 2013) and a greater extent of coronary artery calcification (Dekker et al. 2008; Hamer et al. 2010) and intima media thickness (Eller et al. 2005) which are indicators of atherosclerosis. Further evidence indicates that extended exposure to a stressor can dysregulate the cortisol response and contribute to mood disorders such as depression (Hayley et al. 2003; Hayley et al. 2005). Indeed, altered cortisol levels have been linked to structural and functional abnormalities of the prefrontal cortex, amygdala and hippocampus (Dedovic et al. 2010; Holsboer 2000), which along with other potential changes (for a review see McEwen 2005;

Zunszain et al. 2011), are proposed as likely mechanisms responsible for adverse psychological functioning in response to stressors. Furthermore, recent research has reported that an increase in resting heart rate is associated with a heightened risk of future heart failure in asymptomatic individuals (Opdahl et al. 2014). For susceptible people with atherosclerotic disease, hereditary or congenital cardiovascular abnormalities, acute increases in heart rate in response to intense exercise or physical work has been further linked to an increased risk of acute adverse cardiovascular events such as a myocardial infarction (Jouven et al. 2005; Thompson et al. 2007).

An important bi-directional feedback loop exists between cytokines and cortisol that modulates an adequate response to stressors (Elenkov 2008; McEwen et al. 1997; Turnbull et al. 1999). Pro-inflammatory cytokines (e.g., IL-1β, IL-6 and TNF-α) are potent activators of the HPA-axis and trigger the release of cortisol (Maier et al. 1998). In turn, cortisol negatively feeds back on the immune system via glucocoticoid receptors to suppress the production of pro-inflammatory cytokines engaged in activation and proliferation, thereby limiting the extent of the inflammatory response to stressors (Besedovsky et al. 2000; Chandola et al. 2010; Mastorakos et al. 2005; Silverman et al. 2012). This fundamental relationship between cortisol and cytokines is central to maintaining homeostasis of the immune and endocrine systems in response to a stressor (Petrovsky 2001; Turnbull et al. 1999). However, research suggests that exposure to chronic or particularly intense levels of acute stressful stimuli can impair glucocorticoid receptors (e.g., down regulation, reduced expression, nuclear translocation; Kunz-Ebrecht et al. 2004; Mackin et al. 2004; Silverman et al. 2012). As a result, glucocorticoid receptor abnormalities can reduce the capacity of the immune system to lower inflammation in response to a stressor, resulting in persistently increased cytokine and cortisol levels (Elenkov et al. 2002; Silverman et

al. 2012). These two maladaptations, both individually and together, have been linked to adverse health outcomes including depression and CVD (Boscolo et al. 2008; Elenkov et al. 2002; Heinz et al. 2003; Huang et al. 2011; Kunz-Ebrecht et al. 2004; Lutgendorf et al. 2008; Mackin et al. 2004; Maier et al. 1998; Miller et al. 2007; Nijm et al. 2009; Silverman et al. 2012). Interplay between stress systems and their potential combined effects on the body support the concept of allostatic load (McEwen et al. 1999) referred to at the beginning of this section. To understand the effects of stressors on the body, future research should adopt a multivariate approach to study the immune, HPA-axis and SAM system stress responses and how they interact with each other.

2.3 Stress responses and diurnal rhythms

Stress responses, in particular cortisol, follow marked diurnal rhythms under normal conditions (Petrovsky 1998; Petrovsky et al. 1998). Intense or frequent exposure to a stressor may cause diurnal disruption to these responses (Alesci et al. 2005; Miller et al. 2007; Murray 2007; Sapolsky et al. 2000), which over time, has been linked to adverse health outcomes including CVD, diabetes and depression (Cohen et al. 2006; McEwen et al. 1999). Accordingly, the severity and impact of a stressor can be viewed by gauging the disruption it imposes on the normal diurnal rhythm of cortisol (Golden et al. 2011).

The diurnal release of cortisol is characterised by a morning rise, followed by a gradual decline during the afternoon, through to a nadir in the evening (Miller et al. 2007), but exposure to stressors is capable of disrupting this normal, tightly controlled rhythm. For instance, in a meta-analysis, Miller and colleagues (2007) concluded that chronic physical stress lowers the morning peak of cortisol and elevates the evening nadir, resulting in a flattened circadian profile and increased overall daily release of this

hormone. To accurately assess changes in the diurnal profile/curve of cortisol, research firmly supports the collection of multiple cortisol samples (i.e., ≥ 5 samples) across the day (Edwards et al. 2001; Golden et al. 2011; Hruschka et al. 2005; Kraemer et al. 2006; Ranjit et al. 2009). Sampling cortisol frequently across the diurnal curve also allows for determining the cortisol output over a specific time period, such as a day (Pruessner et al. 2003). This is referred as the daily Area Under the cortisol Curve (AUC) and can be calculated from multiple cortisol samples (i.e., ≥ 3) using the trapezoid method (Pruessner et al. 2003). The daily cortisol AUC takes into account unequal time intervals between samples (Smith, TW et al. 2007). Furthermore, the AUC measure provides information on day–to-day changes in overall cortisol release to complement the assessment of time-point changes in the diurnal cortisol profile using multilevel models (Hruschka et al. 2005; Smith, TW et al. 2007).

The diurnal pattern of inflammatory cytokines exhibit an ascending profile during the day and peaks in the late evening or early morning, during the nadir in cortisol (Gudewill et al. 1992; Lemmer et al. 1992; Petrovsky et al. 1998; Sothern et al. 1995). The immuno-suppressive properties of cortisol may explain the inverse relationship between the diurnal rhythm of pro-inflammatory cytokines and cortisol (Petrovsky 1998; Petrovsky et al. 1998). But unlike cortisol, the effect that stressors have on the diurnal rhythm of cytokines is less understood and requires further investigation before clear patterns of diurnal dysregulation can be identified. Research to date has quantified diurnal changes in plasma cytokine levels using protocols that measure two samples per day (i.e., morning and afternoon; Altara et al. 2015) through to collecting plasma at 3-h (Sothern et al. 1995) to 1-h intervals over a 24-h period (Vgontzas et al. 2005).

Similar to physiological responses, psychological responses to stress such as mood have been associated with a diurnal rhythm (Steptoe et al. 2011). For instance, mood dimensions related to positive affect (e.g., happiness, activation) among healthy adults are lowest in the morning followed by a gradual rise through the day to a peak between 12:00 and 13:00, before declining later in the evening (Steptoe et al. 2011; Stone et al. 1996). Meanwhile, mood dimensions related to negative affect (e.g., fear, anxiousness, worried) are most pronounced in the morning and then decrease progressively over the day (Kahneman et al. 2004; Steptoe et al. 2011). Evidence that cortisol, cytokine and mood responses exhibit distinct diurnal profiles supports the use of multiple daily samples (i.e., > 4 samples; Steptoe et al. 2011) to identify potential disruption to diurnal changes in these responses to inherent firefighting stressors such as physical work and sleep restriction.

2.4 Psychophysiological relationships between stress responses

From the time of the ancient Greeks, psychological wellbeing has been believed to interact with physical states (Sternberg et al. 2002). Understanding the relationships between physiological and psychological systems involve research in the field of psychophysiology, which is concerned with the connections between the brain, thoughts, moods and emotions, behaviour and the neuroendocrine and immune systems (Cacioppo et al. 2007). This area of research is in line with the allostatic load model of stress (McEwen et al. 1999) which supports the investigation of interactions between multiple responses to understand the effects that different stressors have on the body.

Mounting evidence indicates that changes in affective states (i.e., moods or emotions as distinguished from cognition or behaviour), are linked to inflammatory and HPA-axis stress responses (for a review see Kemeny 2007 and Marsland et al. 2007; Mittwoch-Jaffe et al. 1995). Evidence indicates that positive (e.g., happiness) and negative emotions (e.g., sadness and provoked anxiety) are associated with distinct patterns of central nervous system activation such as increased activity of the frontal and temporal lobes and specific parts of the limbic system such as the hypothalamus (Canli et al. 2001; Damasio et al. 2000). Neural systems generate regulatory signals to control homeostasis. Therefore, given that activation of these neural systems may impact differently on immune and endocrine responses, it has been proposed that cortisol and cytokines respond in different patterns depending on the affective state (Kemeny 2007).

Moods are affective states that can be categorized as either positive or negative. To date, few studies have explored the relationships between positive mood and physiological changes (Barak 2006; Marsland et al. 2007). Conversely, research linking negative mood to indicators of immune, and to a lesser extent HPA-axis function, has received greater attention (Kemeny 2007; McEwen 2005; Vgontzas et al. 2008; von Känel et al. 2008; Wright et al. 2005). Communication relationships between the brain and changes in cortisol and cytokine levels are known to be bi-directional (Maier 2003; Maier and Watkins 1998). Moreover, research has explored how both positive and negative mood states in simulated laboratory environments may modulate these parameters of the immune system and HPA-axis (Kemeny 2007; Marsland et al. 2007). For example, in simulated laboratory-based settings negative mood such as acute shame has been associated with increases in cortisol (Dickerson and Kemeny 2004; Gruenewald et al. 2004) and TNF-α receptor levels which is an

indicator of pro-inflammatory cytokine activity (Dickerson et al. 2004). Conversely, mild positive mood induction (i.e., watching a humorous video) has been found to elicit a decrease in TNF-α and increases in IL-2 and IL-3 (Mittwoch-Jaffe et al. 1995). However, this research is limited and has not investigated how positive mood moderates other cytokines such as IL-6, IL-1β, IL-10, IL-4, IL-8 or cortisol. Therefore, assessment of the relationships between mood (both positive and negative) and cytokine and cortisol will allow future research to better understand the psychophysiological impact of stressors.

When studying the interactions between mood and physiological systems, it is important to note that stressor-induced changes in psychological processes may coordinate physiological responses that form adaptations necessary for maintaining homeostasis (Dhabhar et al. 2006; Maier et al. 1998). For example, positive mood and immune function may have a curvilinear relationship, with small to medium changes in mood increasing cytokine release to cope with a given stressor (Barak 2006; Koh 1998; Marsland et al. 2007). However, exposure to prolonged or severe stressors may dysregulate responses, such as concomitant acute increases in mood and inflammatory responses, which over time, have been implicated in the pathophysiology of depression in clinical and animal-based studies (Dantzer 2006; Dantzer et al. 2008). Therefore, examining psychophysiological relationships to acute stressors in simulated environments may help predict how longer-term or repetitive stressor exposure (e.g., across a firefighting career) influence these responses and subsequently, impact on long-term health.

2.5 Firefighting stressors and stress responses

Physical wildfire suppression work together with periods of restricted sleep are two stressors routinely faced by firefighters deployed to fight large wildfires (Aisbett et al. 2007; Cater et al. 2007; Cuddy et al. 2007). Observational research from the fire-ground indicates that Australian wildland firefighters are required to work multiple shifts (i.e., 3 to 4 days) separated by compromised sleep opportunities (3 to 6 h sleep between shifts; Cater et al. 2007). Within a shift, firefighting work can involve short-duration high-intensity physical tasks performed intermittently for extended durations (i.e., 12 to 16-h; Aisbett et al. 2007; Cuddy et al. 2007). Occupational stressors such as sleep restriction and physical work are capable of dysregulating cortisol, heart rate (Chandola et al. 2010), inflammatory (Huang et al. 2011) and mood responses (Wellens et al. 2006) linked to adverse health outcomes (McEwen 2005; Opdahl et al. 2014; Rosmond et al. 2003; Violanti et al. 2009; Willerson et al. 2004). Therefore, it is crucial to determine whether physical wildfire suppression work and sleep restriction have an acute adverse effect on the functioning of these stress responses among personnel. At present, however, the effect these common fire-ground stressors have on firefighters' cytokine, cortisol and heart rate responses, their interactions and how mood might influence these physiological changes is poorly understood.

2.5.1 The impact of physical firefighting work on acute inflammatory, cortisol and heart rate stress responses

Firefighting-based research has quantified physiological stress responses following the one-off performance of individual physical work tasks in live-fire and simulated conditions (Glickman-Weiss et al. 1995; Horn et al. 2013; Smith, D et al. 1996). More commonly however, wildfire suppression requires personnel to perform multiple work bouts for extended periods (i.e., up to 12 to 16 h) over multiple days (i.e., 3 to 5 days;

Aisbett et al. 2007; Cater et al. 2007; Cuddy et al. 2007). Therefore, this section of the review will focus on what impact, if any, one or more shifts of physical firefighting work has on physiological stress responses. In Australia, firefighters are typically rostered to work 12-h day shifts, but shifts can also be shorter and last half a day (i.e., 4-h) as fires flare in the afternoon heat or at times last longer periods such as over 16-h to manage increases in the threat of wildfire to people and property (Cater et al. 2007; Phillips et al. 2007). Therefore, for the purposes of this review, a shift is defined as an extended work period lasting between 4 and 16-h performed during the day. Focusing on the impact of physical work on stress responses allows this review to forecast change in cytokine, cortisol and heart rate variables over time (within/between shifts) before examining what additional effect sleep restriction may have. In addition to the limited firefighting research, insights will be provided from exercise- and military-based studies that have investigated the effects that similar physical work demands have on participants. For instance, given that wildland firefighting involves repeated exposures to physical work tasks across a shift (Aisbett et al. 2007; Cater et al. 2007; Cuddy et al. 2007), available research examining multiple bouts of exercise or military tasks across a similar duration will be presented.

2.5.1.1 A single shift of physical work and cortisol, heart rate and cytokine responses

Research is yet to investigate how a single shift of physical wildfire suppression work impacts cytokine levels among firefighters. Repeated bouts of moderate intensity physical activity across a single day can however, have a cumulative positive impact on several pro-inflammatory cytokines (Degerstrom et al. 2006; Ronsen et al. 2002). In particular, plasma levels of IL-8 are known to demonstrate a pronounced increase in response to long duration physical activity such as running (Pedersen et al. 2008). For instance, Degerstrom et al. (2006) investigated cytokine responses following two

30-minute exercise bouts on a treadmill at 80% of maximal volume oxygen consumption (VO_2max) separated by a 4-h rest interval. Participants' IL-8 levels increased 70% above (P = 0.022) baseline after the first bout, but had returned to baseline after the 4-h rest (Degerstrom et al. 2006). The second bout of exercise induced a 100% higher rise in IL-8 than the first bout (P = 0.005), however, no significant change in IL-6 was detected after either bout (Degerstrom et al. 2006). It is possible that longer physical activity durations such as repeated 1-h periods of intermittent physical work completed in firefighting (Aisbett et al. 2007; Phillips et al. 2011), impact the rise in IL-6 differently. For instance, Ronsen et al. (2002) investigated repeated exercise bouts of a similar duration to wildfire work and found that a second 65-minute bout of cycle exercise at 75% VO_2max performed after only 3 h of recovery resulted in a further increase in peak IL-6 (+ 3.6 pg/mL; P = 0.025). Comparisons between the limited exercise-based research and the reported demands involved in wildland firefighting work suggest that firefighters may be at risk of acute increases in IL-6 and IL-8.

Moderate to high working heart rates have been reported among firefighters performing shifts of live (Raines et al. 2013; Rodríguez-Marroyo et al. 2012) and simulated wildfire operations (Budd et al. 1997b). For instance, Budd et al. (1997b) reported mean working heart rate of 152 ± 14 beats/minute among firefighters during an afternoon shift (~ 5 h) of wildfire suppression activities in controlled burn conditions. In live-fire conditions, Raines et al. (2013) reported that firefighters spent 51 ± 41% and 4 ± 9% of the 9 h shift in moderate (55 to 69% of heart rate max) and hard heart rate zones (70 to 89% of heart rate max), respectively. Rodríguez-Marroyo and colleagues (2012) also reported elevated mean heart rate among firefighters completing live wildfire suppression activities lasting < 1 h (133 ± 2 beats/minute), 1-

3 h (128 ± 1 beats/minute), 3-5 h (120 ± 3 beats/minute) and > 5 h (116 ± 32 beats/minute) which are considered near moderate. Interestingly, Rodŕiguez-Marroyo (2012) found firefighters' mean heart rate and percentage mean heart rate max were higher in wildfires lasting shorter periods of time, indicating a trend towards a decrease in relative heart rate with longer duration wildfires. Similar decreases in working heart rate across long periods of intermittent exercise- and physical military-based tasks have also been observed (Dabrowski et al. 2012; Lucas et al. 2008). The decreased heart rate response may be due to the pace (Rodríguez-Marroyo et al. 2012), intensity and nature (e.g., reduced use of hand held firefighting tools; Budd et al. 1997a; Budd et al. 1997b) of wildland fire suppression work decreasing with longer duration fires, resulting in a decreased heart rate response. Furthermore, individuals becoming increasingly more economical in executing the required actions to complete the physical work may also explain the reduced heart rate response. Practice-related effects in response to repeated exercise performance have been found to reduce the internal mechanical work needed to coordinate the limbs, resulting in reduced metabolic energy expenditure, and therefore heart rate (Sparrow et al. 1999). Reduced heart rate could have also resulted from attenuated sympathetic nervous system activation (Dabrowski et al. 2012; Konishi et al. 2013), suggesting a potentially adaptive response of the SAM system when exposed to these demands.

Unlike heart rate, research is yet to investigate firefighters' cortisol levels in response to a single shift of physical wildfire work. Some insights, can be gained from military- and exercise-based studies which have reported an increase in soldiers' mean cortisol levels following multiple bouts of simulated physical work performed over one day (Porta et al. 1993). Porta and colleagues (1993) measured soldiers' cortisol levels following a single 15 minute bout of exhaustive cycle ergometric work (i.e., baseline;

100 watts with 50 watt increment every 3 minutes to a maximum workload of 350 watts). Seven days later, the same soldiers underwent a difficult 2.5-h mountain climb and subsequent 1.5-h rest followed by the same 15 minute cycle ergometric work (Porta et al. 1993). While there was no significant change in cortisol following the one-off performance of the cycle ergometric test, the combined mountain climb and cycle ergometric test, resulted in a 2-fold increase in cortisol from baseline (i.e., before ergometric test; $P < 0.001$). Previous exercise-based research by Ronsen et al. 2001 and Sari-Sarraf et al. 2007 confirmed that following a 2.25-h or 3-h rest period, a second bout of either moderate- or high-intensity physical activity performed for 65 or 90 minute durations respectively, can elicit significant increases in cortisol when compared to a single bout of identical physical activity. Overall, physical activity levels can remain quite stable across a shift of firefighting work (Cuddy et al. 2007; Raines et al. 2013). Therefore, the above findings highlight that two or more repeated bouts of similar intensity physical activity could lead to an exacerbated cortisol response. Moreover, the rest periods between work bouts ranged from 1.5-h to 3-h in the available research (Porta et al. 1993; Ronsen et al. 2001; Sari-Sarraf et al. 2007), which may have been inadequate to restore cortisol levels to normal resulting in the cumulative increases reported.

The available exercise research has examined the inflammatory, cortisol and heart rate responses to similar physical workloads to firefighting (i.e., repeated bouts of physical work over a single day). Further investigations are needed to understand how wildfire-specific work demands affect firefighters' cytokine and cortisol levels. Wildland firefighting work incorporates a large component of intermittent weight bearing manual handling tasks (Phillips et al. 2012) which involves components of muscular endurance not captured by the aerobic activity in exercise studies, such as upper body

eccentric loads. To date, tasks involving eccentric concentrations such as running have been shown to have a more pronounced impact on IL-8 and IL-6 when compared to concentric contractions (Pedersen et al. 2008). Although similarities in the duration of work between firefighting and exercise-based studies exist, additional research needs to confirm if the manual handling-based work involved in wildfire suppression elicits changes in IL-6 and IL-8. Moreover, differences between shorter 1-h repeated bouts of intermittent work involved in firefighting, and the longer (i.e., 1.5 or 2.5-h) periods of activity investigated in exercise and military-based research may impact cortisol levels differently. Therefore, the potential for under- or over-estimating cytokine and cortisol responses for wildfire personnel supports the need for future wildfire-specific research.

2.5.1.2 Consecutive shifts of physical work and cytokine, heart rate and cortisol stress responses

Wildfire suppression work can expose personnel to physical work demands over consecutive days/shifts (Aisbett et al. 2012; Cater et al. 2007; Cuddy et al. 2007). However, little is known regarding the impact repeated long days of intense intermittent physical work has on firefighters' acute inflammatory, cortisol and heart rate responses. Currently, research by Main and colleagues (2013) is the only study to have investigated how consecutive days of live-fire prescribed burn work impacts cytokine levels. Pre- and post-shift blood sampling revealed an increase in firefighters' IL-6, IL-1β, IL-7 levels across the first 12-h work day, while IL-5, IL-10 and TNF-α levels decreased (Main et al. 2013). Firefighters' IL-6 levels, along with IL-1β, IL-8 and IL-4 all exhibited an attenuated response across the second 12-h day of wildfire work relative to the first shift (Main et al. 2013). After an initial rise on day one, the decrease in cytokine levels on the second shift may indicate an appropriate cytokine

response to maintain homeostasis of the immune system. For instance, IL-6 (Pedersen et al. 2007; Petersen et al. 2005; Starkie et al. 2003; Tilg et al. 1997) and IL-4 (Tilg et al. 1997) has anti-inflammatory properties that lower pro-inflammatory cytokines, namely IL-β and TNF-α involved with systemic inflammation (Bruunsgaard 2005; Pedersen et al. 2008). The immune system also interacts with cortisol via the bi-directional feedback loop and in turn, cortisol may suppress cytokine activity (see Section 2.2 for further details; Turnbull et al. 1999) providing further explanation for the attenuated cytokine levels observed by Main and colleagues (2013) among wildland firefighters. The decrease in cytokine levels may be further explained by a possible reduction in the intensity and/or duration of work bouts between days that reflect the later and less intense stages of wildfire suppression activities (Budd et al. 1997a, 1997b; Rodríguez-Marroyo et al. 2012). While the Main study (2013) provides the only insight into how multiple shifts of work on the fire-ground impact firefighters' immune system, it did not control or measure the duration or intensity of work bouts, sleep duration between shifts or environmental demands (e.g., heat, smoke etc.), nor were related stress responses examined (e.g., cortisol). Accordingly, future research should measure physical work through the use of activity monitors and/or observations, and where possible, control the timing of work bouts to understand how these demands, as distinct from other potential stressors (e.g., smoke, ambient temperatures etc.), directly influence cytokine levels.

In addition to cytokines, incorporating related stress system measures such as cortisol (McEwen et al. 1997) may help to further elucidate acute inflammatory changes to occupational demands. For instance, the release of cortisol following acute maximal exercise (i.e., graded treadmill exercise test to 100% VO_2max) was found to suppress IL-1β and TNF-α levels, but had no effect on IL-6, resulting in sustained high levels

of this cytokine (DeRijk et al. 1997). These findings suggest that IL-6 could be more resistant to the effects of cortisol under physical stress (DeRijk et al. 1997). Chronic exposure to physical stressors could therefore restrict the immune system's capacity to react to cortisol and downregulate inflammatory responses resulting in heightened inflammation (Desantis et al. 2012), which could have an adverse impact on health (McEwen et al. 1997; Miller et al. 2002). Alternatively, DeRijk et al. (1997) suggested that if IL-6 is acutely resistant to cortisol, then the sustained release of IL-6 may exert its immunosuppressive effects on IL-1β and TNF-α and protect against an excessive release of these pro-inflammatory cytokines. However, further research is needed to understand the degree to which cytokine and cortisol activity is related. This integrative approach to assessing multiple stress responses affords greater potential insight into the severity of stress system changes on the body after encountering a stressor (McEwen et al. 1999; O'Leary 2014).

To date, there has been limited emergency service-based research investigating the impact consecutive days of physical work has on heart rate or cortisol. Mean heart rate among soldiers exposed to four days of long-distance marching did not differ significantly between days (Väänänen et al. 2002), indicating that the participants were able to successfully maintain a similar intensity of work across the march. In this study, Väänänen et al. (2002) reported an increase in mean cortisol at 13:00 following the first work day, after which there was a decrease in afternoon levels compared to the previous morning sample that continued for the remainder of the study. Furthermore, cortisol levels were reported to remain, for the most part, within the normal reference range for adults (Väänänen et al. 2002). While the authors (Väänänen et al. 2002) suggested that participants' cortisol response may have only taken a single day to adapt to this type of work, resulting in the unchanged response on the subsequent days of

marching, previous exercise-based studies have reported that cortisol stabilises after three or more days of physical work (Fellmann et al. 1992; Marniemi et al. 1984). Therefore, it is possible the findings for cortisol and heart rate indicate that the physical work investigated in this study (Väänänen et al. 2002) may not have been a significant enough stressor to disrupt these responses.

Research by Väänänen and colleagues (2002) provides the only emergency service-based findings relating to the direct impact of consecutive days of physical work on HPA-axis and SAM system responses. Despite this, the long-distance marching investigated (Väänänen et al. 2002) is different to the intermittent, manual handling work involved in wildland firefighting (Phillips et al. 2012). To investigate how these wildfire-specific demands impact heart rate in hot weather, Raines et al. (2015) measured the time firefighters spent at or above 70% of heart rate max across consecutive work days. Firefighters spent more time ≥ 70% heart rate max across the first shift than on day two (Raines et al. 2015). Unlike day two in which firefighters were euhydrated as determined by plasma osmolality, firefighters were not euhydrated on day one (Raines et al. 2015). Raines et al. (2015) therefore suggested that a hypohydration-mediated increase in heart rate may explain the greater amount of time spent in this heart rate zone on day one. While differences did not exist in firefighters' physical activity profile measured objectively across days, Raines and colleagues (2015) acknowledged that it was not possible to determine if work tasks were completed for similar durations on each day, which if different, could impact heart rate. Accordingly, further research, specific to the demands of wildland firefighting is needed to understand the specific impact (if any) these physical work demands have on firefighters' acute cortisol, heart rate and inflammatory stress responses over consecutive days. This research would provide fire agencies with a first insight as to

whether further precautions are needed to mitigate against the possibility of adverse stress responses and the risk they pose to the health of personnel as a result of the physical work.

2.5.2 The impact of sleep restriction and physical work on cytokine, cortisol and heart rate responses, and how psychological changes may influence physiological responses

2.5.2.1 The effects of sleep restriction and physical work on cytokine responses

A high prevalence of sleep disturbances and shortened sleep have been reported among firefighters (both wildland and urban) and personnel in other physically demanding emergency service occupations such as the police or military (Carey et al. 2011; Cater et al. 2007; Neylan et al. 2002). Despite this, no wildland firefighting-based research to date has investigated the specific impact restricted sleep has on inflammatory responses. Pro- and anti-inflammatory cytokine levels have, however, been investigated in response to sleep restriction during physical exercise and military work (Abedelmalek et al. 2013; Bøyum et al. 1996; Gundersen et al. 2006; Lundeland et al. 2012), though the findings remain somewhat equivocal. For example, Bøyum et al. (1996) reported a decrease in IL-6 but no change in IL-1β and IL-4 levels among military personnel during 5 to 7 days of continuous simulated physical military training, shortened sleep (i.e., 2 to 3-h of total sleep) and calorie restriction. The decline in IL-6 reported by Bøyum et al. (1996) is in contrast to findings from studies examining mild to modest (4 to 6-h sleep per night) sleep restriction for similar durations in the absence of physical work, which have demonstrated an increase in daily IL-6 levels among healthy adults (Bhatt et al. 2015; Pejovic et al. 2013) and TNF-α in men (Vgontzas et al. 2004). However, Bøyum and colleagues (1996) did speculate that the findings for inflammatory markers were confounded by occupational factors

not controlled for, such as food and fluid intake. But while Bøyum et al. (1996) proposed an excessive fluid intake may have caused plasma expansion and reduced IL-6, this is questionable as more recent evidence indicates that hemodilution can increase IL-6 concentration (Hightower et al. 2012; Swart et al. 2011), inferring that another uncontrolled factor(s) may explain the findings (e.g., medications or calorie restriction). Alternatively, IL-6 may have been down-regulated by the immunosuppressive actions of anti-inflammatory cytokines (e.g., IL-10 and IL-4) and cortisol. But as these additional biomarkers were not reported by Bøyum et al. (1996), it is difficult to detect, with certainty, the interplay between responses which underlie inflammatory changes to physical work and sleep restriction. Subsequent studies should therefore examine multiple inter-related responses to help interpret the impact of stressors on immune and HPA-axis stress systems.

In contrast to the findings reported by Bøyum and colleagues (1996), Abedelmalek et al. (2013) found that IL-6 along with TNF-α increased (+ 0.5 pg/mL and + 0.7 pg/mL, respectively; estimated from graphs provided) among healthy adults exposed to a restricted 4.5-h sleep opportunity followed by multiple bouts of interval exercise (4 × 250-meter runs). Unlike the available military-based studies (Bøyum et al. 1996; Gundersen et al. 2006; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981), Abedelmalek et al. (2013) included an 8-h sleep opportunity condition for comparison with the restricted sleep condition and closely controlled for variables that can impact immune function, such as the duration and timing of the sleep restriction period and pre-exercise food and fluid intakes. Further, unlike the military-based studies (Bøyum et al. 1996; Gundersen et al. 2006; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981), Abedelmalek et al. (2013) examined an early phase sleep opportunity (22:30-03:00). Wu et al. (2010) found 4 nights of 3-h early phase sleep restriction caused a

larger reduction in rapid-eye-movement (REM) sleep compared with later-night sleep restriction. Research in animal models indicate that a reduced amount of REM sleep may have a positive association with increased IL-6 (Pandey et al. 2011) and TNF-α levels (Venancio et al. 2014; Yehuda et al. 2009) and explain one pathway through which sleep length disrupts sleep architecture, which in turn modulates inflammation. Therefore, the advanced timing of the sleep restriction phase investigated by Abedelmalek et al. (2013), together with the controlled study conditions may explain why, in contrast to Bøyum et al. (1996), there was a rise in IL-6 post-sleep restriction and physical work. While structural firefighting and other occupations (e.g., air crew, train and truck drivers) can require personnel to start work early in the morning (Åkerstedt et al. 2010; Ingre et al. 2004; Kecklund et al. 1997; Paley et al. 1994), shifting sleep to an early-phase sleep restriction is not as common in wildland firefighting. Instead, Australian wildland firefighters deployed to the fire-ground can have their sleep limited to between 02:00 and 06:00, representing a later phase sleep restriction period (Cater et al. 2007; Ferguson et al. 2011).

Over an extended period of simulated physical military work (i.e., 7 days) and minimal sleep (i.e., 1 h sleep per 24 h), Gundersen et al. (2006) reported an increase in soldiers' IL-6 levels from baseline to day 2 (+ 10.6 ± 1.3 pg/ml) and day 4 (+ 6.8 ± 1.6 pg/ml). Lundeland et al. (2012) reported a similar increase in IL-6 on day 3 of a seven day simulated physical military training exercise with limited sleep (i.e., 1 h per 24 h). Multiple days of work and extreme sleep restriction resulted in a greater rise in IL-6 when compared to the more moderate sleep restriction examined over a one-night and one-day protocol by Abedelmalek et al (2013). By the completion of the course however, Gundersen et al. (2006) and Lundeland et al. (2012) both found that IL-6 had returned to baseline levels. Furthermore, plasma concentrations of TNF-α and IL-1β

remained unchanged across the military training scenario investigated by Gundersen et al. (2006). As eluded to earlier, the increase and then decrease in IL-6 and unaltered TNF-a and IL-1β levels demonstrated among soldiers in this study (Gundersen et al. 2006) could provide further evidence of the anti-inflammatory effects that IL-6 can have on other pro-inflammatory cytokines. The findings for IL-6 by Gundersen et al. (2006) and Lundeland et al. (2012) may therefore indicate that over an extended period (i.e., 1 week), soldiers' cytokine levels were able to adjust by responding appropriately to the combination of simulated restricted sleep and physical work and promote homeostasis of the immune system.

2.5.2.2 The effects of sleep restriction and physical work on cortisol and heart rate responses

To date, only one emergency service-based study (not firefighting) has investigated the specific effect that shortened sleep has on the functioning of the HPA-axis (Goh et al. 2001). Although there was no significant difference in overall daily cortisol release between control (i.e., 8 h sleep) and sleep-deprived military personnel (i.e., 40 h), Goh et al. (2001) found increased afternoon cortisol levels following the sleep deprivation period. Elevated cortisol levels in the latter part of the day are consistent with non-emergency service-based studies, that in the absence of physical work, have found that restricted sleep opportunities lasting between 4 and 5 h over 1 to 10 nights result in increased cortisol levels in the afternoon and evening (Buxton et al. 2010; Guyon et al. 2014; Leproult et al. 1997; Reynolds et al. 2012; Spiegel et al. 1999). Chronically increased cortisol levels in the latter part of the day have been positively associated with age-related insulin resistance (Dallman et al. 1993; Kern et al. 1996), highlighting a possible pathogenic process by which restricted sleep may influence the development of diabetes. Restricted sleep opportunities (i.e., 4 and 5 h), without physical work, have

also resulted in elevated resting heart rate (Meier-Ewert et al. 2004; van Leeuwen et al. 2009), which if chronic, has been identified as a risk factor for heart failure (Opdahl et al. 2014). Shortened sleep may therefore pose as a significant stressor for firefighters who have been reported to have an increased risk of CVD-related deaths on duty (Fahy et al. 2013; Kales et al. 2007). But despite wildland firefighting exposing personnel to sleep restriction while performing physical work (Aisbett et al. 2012), considerably less is known regarding the combined impact these stressors have on firefighters' acute cortisol and heart rate responses.

Some insights can be gained from sustained military operation- and exercise-based studies. For instance, unchanged (Plyley et al. 1987) or decreased (Dabrowski et al. 2012; Lucas et al. 2008; Myles 1987) heart rate responses measured throughout extended periods of prolonged physical activity (i.e., 20 to 116 h) and sleep deprivation have been reported. Myles and colleagues (1987) observed a decrease in participants' work rate and an increase in rest time across a 50-h period, which may explain the simultaneous decrease in heart rate. However, neither of the other studies measured or reported a change in work output (Dabrowski et al. 2012; Lucas et al. 2008). As discussed in Section 2.5.1.1, it is therefore possible the decrease in heart rate was the result of other causes, such as participants becoming more economical in completing the actions involved in the physical work. A reduced or unchanged heart rate could have also been caused by attenuated sympathetic nervous system activation (Chandola et al. 2008; Konishi et al. 2013), suggesting a potentially adaptive response of the SAM system when exposed to sleep restriction and sustained physical activity. Even though firefighters are not typically exposed to extended periods of complete sleep deprivation, there are similarities between the physical demands examined in the exercise-based studies (Dabrowski et al. 2012; Lucas et al. 2008; Myles 1987) and the

long periods of physical work involved in firefighting (Aisbett et al. 2012). Similar physical work demands highlight the possibility that the physical work in firefighting may also elicit an attenuation of the sympathetic nervous system, drop in work rate and/or practice-related effects. As a result, wildland firefighters' heart rate while working and at rest may decrease across multiple shifts in a similar way to that demonstrated in military/exercise research.

To date, several military-based studies have investigated soldiers' cortisol changes in response to 1 to 3-h of total sleep across the course of simulated, near continuous physical training lasting 3 to 5 days (Lieberman et al. 2005; Opstad 1994; Opstad et al. 1981). In an early study by Opstad et al. (1981), the normal circadian variation in morning and evening cortisol levels on day 1 and day 4 (i.e., 2 daily samples at 08:00 and 19:00) of a 5-day military training course was reported to have disappeared, indicating an abnormal circadian cortisol release. In a subsequent study, Opstad (1994) employed a higher frequency cortisol sampling method (i.e., 5 daily samples) to further investigate the effect of a simulated, 5-day physical training course with 1 to 3-h of total sleep on military cadets' diurnal cortisol levels. Similar to control conditions involving no physical training and an 8-h sleep opportunity, cortisol levels followed a normal diurnal rhythm on day 1 of the course. However, throughout the rest of the course, mean cortisol levels remained consistently elevated (+130–140% above baseline) and over the final 24-h period, the authors reported that the diurnal rhythm had almost disappeared (Opstad 1994). Moreover, 4 to 5 days after course completion the diurnal release of cortisol remained significantly higher than the control period (Opstad 1994), further suggesting the possibility of chronic disruption to the diurnal cortisol rhythm following four consecutive nights of sleep restriction during physical military training. However, neither this (Opstad 1994) nor the earlier work by Opstad

et al. (1981) statistically analysed multiple time-point changes in cortisol between days to understand detailed circadian alterations such as a shift or flattening in the cortisol profile between days. It is therefore not possible to understand day-to-day changes in cortisol's rhythmicity among military personnel.

Further evidence of disruptions to the cortisol rhythm was found among soldiers completing a 53-h simulated field exercise in the heat in which personnel had 3.0 ± 0.3 h of total sleep measured using activity monitors (Lieberman et al. 2005). Compared to pre-field measurements, Lieberman and colleagues (2005) reported that soldiers had lower morning (06:00; - 0.13 to 0.28 µg/dL) and higher evening cortisol levels (18:00; + 0.16 to 0.22 µg/dL). Lieberman et al. (2005) stated however, that cortisol levels did not exceed the expected range for non-stressed personnel with similar demographic characteristics, nor were they as high as the 2- to 3-fold increase (above baseline) reported by Opstad (1994). But different to the young cadets (22 to 26 years) included in the study by Opstad (1994), Lieberman et al. (2005) examined older personnel (31.6 ± 0.4 years) who had served, on average, 9.2 ± 0.5 years of duty. Research suggests that the HPA-axis can adapt to repetitive prior experience of a stressor (Andersen et al. 2013; McEwen 1998). As suggested by Lieberman et al. (2005), it is therefore likely the experience level among soldiers resulted in a more moderate release of cortisol to the occupational demands. This highlights the importance of recording and, where possible, controlling for occupational experience (i.e., matching experimental groups for experience) when examining the cortisol response to demands such as sleep loss and physical work.

Although Opstad (1994), Opstad et al. (1981) and Lieberman et al. (2005) observed evidence of a dysregulated cortisol response, different discrete parts of the diurnal

cortisol rhythm were investigated in each study. For instance, Opstad (1994) stated that daily cortisol release (i.e., 24-h mean) increased significantly over the 5-day training course. On the other hand, Opstad et al. (1981) and Lieberman et al. (2005) observed a decline in early morning cortisol levels (08:00 and 06:00 respectively), while increases in evening cortisol (18:00) were further reported by Lieberman et al. (2005). Increases and decreases in different cortisol measurements have been demonstrated following stress exposure and could indicate different forms of allostatic load (i.e., wear and tear on the HPA-axis) expressed as either an intensified or suppressed cortisol production in response to a stressor (McEwen et al. 1999). Chronically increased daily cortisol levels have been positively associated with increased CVD risk (Rosmond et al. 2003). Conversely, persons exposed to chronic stress have also demonstrated inadequate morning cortisol levels an hour after awakening (Miller et al. 2002). An inadequate cortisol response occurs when the HPA-axis produces too little cortisol in response to a stressor. As a result, inflammatory responses that are normally contained by cortisol become overactive (McEwen et al. 1999). Despite the known interactions between immune and cortisol responses that regulate an adequate physiological response to stressors (see Section 2.2 for details), research is yet to investigate how sleep restriction combined with physical work specifically, impact the interplay between cortisol and cytokines. Further quantifying this relationship between markers in response to occupational demands may provide insights into the mechanisms underlying altered responses. Identifying possible interactions (i.e., an imbalance between cytokine and cortisol) that have implications for health (Nijm et al. 2009; Silverman et al. 2012) would also help tailor workplace interventions to properly regulate immune-endocrine relationships.

Morning cortisol levels among soldiers were found by Gundersen et al. (2006) to increase from baseline on day 2 (+79%) and day 4 (+74%) of a 7-day simulated training course with 1 h of sleep per 24 h. Lundeland and colleagues (2012) also reported increased morning cortisol levels among soldiers on day 3 (+22%) and day 5 (+73%) of a similar 7-day simulated training program. However, cortisol had moved towards baseline levels by day 7 in both studies (Gundersen et al. 2006; Lundeland et al. 2012). Furthermore, Abedelmalek et al. (2013) reported that cortisol levels were not affected following interval training and a restricted 4.5-h sleep opportunity or an 8-h sleep opportunity. Unaltered cortisol levels (Abedelmalek et al. 2013) could indicate that the single period of 4-h sleep restriction followed by interval exercise were not sufficient to have an acute impact on the functioning of the HPA-axis. Cortisol levels which increased and then decreased (Gundersen et al. 2006; Lundeland et al. 2012) across seven days of combined sleep restriction while performing physical activity may indicate that the HPA-axis requires a longer period to adapt and responded appropriately to ensure homeostasis of this stress system.

Differences in the assessment of cortisol between studies may further explain the conflicting findings for this biomarker (Gundersen et al. 2006; Lieberman et al. 2005; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981). For instance, cortisol levels in the morning can be affected by awakening time, so the use of a single morning sample in the previous research (Gundersen et al. 2006; Lundeland et al. 2012) provide less reliable measures of cortisol when compared to mean total values based on multiple daily samples (Golden et al. 2011), such as that adopted by Opstad (1994). However, in comparison to both mean cortisol levels and single samples, calculating the AUC using multiple (i.e., $\geq$ 3 samples) daily samples provides a more accurate assessment of the overall secretion of cortisol over a specific time period (i.e., each

work day; Pruessner et al. 2003). Therefore, it has been suggested that the assessment of cortisol should be based on the AUC, complemented by an analysis of the diurnal cortisol profile across the day at different time points (Adam et al. 2009). Additionally, collecting multiple (i.e., ≥ 5) cortisol samples across the day on consecutive days allows for the use of mixed statistical models to account for within and between participant differences in cortisol (Hruschka et al. 2005; Smith, TW et al. 2007). However, emergency service-based research is yet to employ either multiday sampling methods or the use of mixed models when assessing cortisol among personnel.

A further aspect of cortisol's circadian rhythm that is important to consider when measuring this hormone is its steep morning rise, which depends on awakening time (Smyth et al. 2013). Variation in diurnal cortisol demonstrated between studies may be further due to differences in the timing of the sleep restriction period and the cortisol sample collection time points. For instance, in previous military-based investigations, morning cortisol has been collected between 06:00 and 10:00 (Gundersen et al. 2006; Lieberman et al. 2005; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981), yet it was not specifically reported when participants slept, making it impossible to account for the impact of the sleep restriction period and awakening time on cortisol. This emphasises the importance in controlling or at least recording/reporting the timing of sleep opportunities and cortisol sampling in future protocols examining HPA-axis function in response to sleep restriction. Furthermore, daily energy intake was restricted during several of the military-based studies (Gundersen et al. 2006; Lundeland et al. 2012; Opstad 1994) which could confound the interpretation of these findings, as energy along with carbohydrate restriction have both been found to increase the daily output of cortisol (Tomiyama et al. 2010). While closely controlling energy and/or carbohydrate intakes may not be ecologically valid in the context of

firefighting, every effort should be made to standardise meals and fluid intakes (e.g., types of food and drink available, meal times, portion sizes etc.) to fire-ground conditions and also between experimental groups.

In addition, previous experiments investigating heart rate were performed in largely uncontrolled field settings (Dabrowski et al. 2012; Lucas et al. 2008). It is therefore possible, that together with physical work and sleep loss, stressors that were not controlled in these studies such as fluid and energy intakes and variations in ambient temperature may influence SAM system activity (Sasaki et al. 1991; Tanaka et al. 2012). There is thus a need for tightly-controlled and firefighting relevant research that examines sleep restriction and physical work to determine the specific impact on concurrent cortisol and heart rate. Established links between adverse health outcomes (e.g., CVD and depression) and elevated cortisol (Hamer et al. 2010; Hayley et al. 2003; Hayley et al. 2005; Mackin et al. 2004; Poitras et al. 2013), and heart rate (Opdahl et al. 2014) highlight the need to examine acute responses to provide an accurate platform from which to assess long-term implications in these firefighter relevant diseases. Simultaneously measuring activity of both HPA-axis and SAM system responses enable greater understanding of potential adaptive or maladaptive functioning in response to fire-ground stressors.

2.5.2.3 The effects of sleep restriction and physical work on psychophysiological stress responses

Research suggests that changes in cytokine and cortisol responses relate to mood states (Kemeny 2007; Mittwoch-Jaffe et al. 1995). Although the relationship is likely to be bi-directional, attention has focused on how both positive and negative mood states moderate immune and endocrine systems (Kemeny 2007; Marsland et al. 2007).

Therefore, to further interpret the physiological impact of a stressor, it is crucial to assess the psychophysiological relationships between mood, cytokine and cortisol responses. Persistent psychophysiological associations between responses (e.g., negative mood, cortisol and cytokines) have been linked to chronic health outcomes such as depression (Lutgendorf et al. 2008; Musselman et al. 2001). Accordingly, several studies have investigated the impact of physical work and sleep restriction separately, on psychophysiological stress responses among healthy participants (Jürimäe et al. 2002; Kajtna et al. 2011; Robson-Ansley et al. 2009; Thomas et al. 2011; Vgontzas et al. 2008; Vgontzas et al. 2002; Vgontzas et al. 2003), but none have investigated sleep restriction and physical work together or have been based in firefighting.

Exercise-based studies of multi-day simulated physical work periods, similar in length to that experienced during wildland firefighting (Aisbett et al. 2012), have demonstrated mixed findings when investigating acute psychophysiological relationships (Jürimäe et al. 2002; Robson-Ansley et al. 2009). For instance, Robson-Ansley et al. (2009) reported that during a 6-day (average exercise duration varied from 160 ± 43 to 391 ± 30 minutes per day) cycling event there was no relationships detected between cyclists' plasma IL-6 or cortisol levels and subjective sensation of post-exercise fatigue measured using an abbreviated Profile of Mood State questionnaire (POMS). Failure to see a psychophysiological relationship was proposed by the authors (Robson-Ansley et al. 2009) to be due to the participants ingesting high carbohydrate drinks during exercise. In addition to influencing cortisol (see Section 2.5.2.2), elevated carbohydrate intakes are known to further dampen IL-6 (Bishop et al. 2001). Specifically, Robson-Ansley et al. (2009) suggested that the increased carbohydrate intake preserved glycaemic homeostasis, reducing IL-6 and cortisol

release (McAnulty et al. 2007; Nieman et al. 1998). Therefore, it is important further research control or at least standardise diet during work conditions when investigating psychophysiological relationships between fatigue and plasma cytokine responses.

Relationships between psychological stress responses and HPA-axis function were further investigated among male athletes completing an intensive 6-day heavy rowing training program (total of 21.5 ± 2.2-h of exercise; Jürimäe et al. 2002). In this study, rowers' resting cortisol levels were positively related to a change in subjective fatigue ($r = 0.64$; Jürimäe et al. 2002) measured on the Recovery Stress Questionnaire for Athletes. However, both the above mentioned exercise-based studies failed to include a control group (Jürimäe et al. 2002; Robson-Ansley et al. 2009). Consequently, how much the psychophysiological relationships (between subjective fatigue and cortisol or IL-6) observed in response to physical work differed from free-living conditions (i.e., no physical training) is unknown. In addition, neither study recorded, in detail, the physical activity performed by participants prior to entering the study (Jürimäe et al. 2002; Robson-Ansley et al. 2009). It is therefore difficult to determine if the psychophysiological effects observed were due to the physical work examined and not previous physical training. In addition to including a control group, prior physical activity as well as sleep are important measures to consider when studying firefighters' stress responses. For example, the on-call nature of firefighting (Dean et al. 2003) may expose personnel to emergencies that affect physical activity and/or sleep prior to beginning an intervention. It may be appropriate, therefore, to either exclude individuals to rule out the possibility of prior stressors influencing the findings or control for these pre-study behaviours in subsequent analyses to isolate the effect additional physical work or sleep restriction has on stress responses.

Sleep research has further investigated interactions between mood and cortisol, but the majority of studies examined participants with either a sleep disorder or mental illness which was not the focus of this thesis. One exception is an analysis of the relationship between subjective mood (assessed using the Brunel mood scale; BRUMS) and cortisol levels pre and post a 40-h period of sleep deprivation among healthy men (Konishi et al. 2013). However, no correlations were found between cortisol and any of the mood states assessed using this scale (Kajtna et al. 2011). A number of factors may have contributed to a lack of observed psychophysiological relationships between responses. Firstly, pervious emergency service-based findings suggest a single night of sleep deprivation is not sufficient duration to alter the overall release of cortisol the next day (Goh et al. 2001). Therefore, the 40-h period of wakefulness examined may explain why a psychophysiological relationship involving this measure of daily cortisol was not reported by Kajtna et al. (2011). On the other hand, elevated afternoon and evening cortisol have been reported following a single night of restricted and complete sleep loss (Goh et al. 2001; Leproult et al. 1997). This highlights the need to examine specific changes in cortisol across time-points in addition to daily cortisol output when investigating psychophysiological responses involving cortisol and mood. Lastly, participants in this laboratory-based study were not reported to have completed any physical activity during the 40-h period of sleep deprivation (Kajtna et al. 2011). Therefore, in the absence of physical activity, sleep loss in isolation may not have been a sufficient stressor to elicit an acute psychophysiological relationship between responses.

In comparison to other psychological responses, fatigue, and its relationship to cortisol and cytokine release has received significant attention (Thomas et al. 2011; Vgontzas et al. 2008; Vgontzas et al. 2002; Vgontzas et al. 2003). In particular, work by

Vgontzas and colleagues (Vgontzas et al. 2008; Vgontzas et al. 2002; Vgontzas et al. 2003) suggests that an elevated daytime release of IL-6 together with increased HPA-axis activation leads to heightened perceptions of fatigue the next day. Although the mechanisms that drive this relationship have not been fully explained, some findings indicate that reduction in slow wave sleep (SWS) might be one pathway through which inflammation leads to perceptions of fatigue (Thomas et al. 2011). For instance, mediation analyses performed by Thomas et al. (2011) showed that reduced SWS mediated the relationship between increased evening levels of IL-6 and elevated daily fatigue levels among healthy adults. Research has also reported a small curtailment in SWS associated with sleep restriction (Kopasz et al. 2010; Mavanji et al. 2013), suggesting a reduction in this sleep stage may be underlying the simultaneous increase in fatigue and inflammation when exposed to shortened sleep. It should be noted however, that sleep research to date (Vgontzas et al. 2008; Vgontzas et al. 2002; Vgontzas et al. 2003) has investigated how physiological responses relate to perceptions of fatigue the next day. As mentioned earlier, the relationship between mood, inflammatory and HPA-axis activation is likely to be bi-directional indicating the need to further explore if, in response to a stressor, subjective fatigue is more acutely related to altered cortisol and cytokine levels.

Although the current literature is limited to separate sleep- (Kajtna et al. 2011; Thomas et al. 2011; Vgontzas et al. 2008; Vgontzas et al. 2002; Vgontzas et al. 2003) or exercise-based protocols of varying durations (Jürimäe et al. 2002; Robson-Ansley et al. 2009), findings demonstrate how changes in mood can be related to cytokine and cortisol responses. However, no study, firefighting-based or otherwise, has investigated the combined impact sleep restriction while performing physical work has on psychophysiological stress responses, indicating the need for further investigation.

Uncovering evidence of psychophysiological relationships in the context of firefighting offers the first step to better equip fire agencies and other workplaces facing similar demands, to accurately assess and monitor physiological stress responses among personnel using subjective psychological questionnaires. Application of this research to the fire-ground may improve fire agencies capacity to monitor personnel and if necessary, intervene before their physical health is impaired.

2.5.2.4 The Mood Scale II and Samn-Perelli Fatigue Scale as subjective measures of mood

While evidence has linked mood and physiological responses during periods of simulated and live physical work and sleep restriction (Jürimäe et al. 2002; Robson-Ansley et al. 2009; Thomas et al. 2011; Vgontzas et al. 2008), there is variability in the type of subjective mood measures used between studies. Consequently, it is difficult to determine which measure is best (i.e., most sensitive and practical) to implement when assessing psychophysiological relationships in response to different occupational stressors. Research conducted in the field however, has tended to use shortened versions of longer mood questionnaires such as the POMS, given their brevity and sensitivity to mood fluctuations in applied settings. For instance, Kajtna et al. (2011) used the BRUMS which is 24-item questionnaire derived from the longer 65-item POMS questionnaire. Likewise, Robson-Ansley et al. (2009) also used an abbreviated version of the POMS questionnaire in their study. Short form POMS measures have been well validated for use in sleep and exercise-based studies (Carpenter et al. 2004; Friedmann et al. 1977). A further measure called the Mood Scale II is comparable to the previously used POMS based questionnaires, but was specifically developed for use in field settings (Thorne et al. 1985). The Mood Scale II takes approximately 5 minutes to complete and has been successfully used to assess

mood responses among military personnel and nurses exposed to a range of stressors including sustained work and sleep deprivation (Paterson et al. 2011; Paterson et al. 2010; Thorne et al. 1985).

The Mood Scale II assesses both positive and negative mood dimensions, which is important to fully understand the structural aspects of these affective experiences (Diener et al. 1984) and their potentially divergent relationship to physiological responses (Kemeny 2007). Specifically, Thorne et al. (1985) explains that this measure presents an individual with 36 mood related adjectives which load onto six sub-dimensions/factors including activation and happiness, which are positive mood dimensions, and depression, anger, fatigue and fear classified as negative mood dimensions. Activation relates to the adjectives 'energetic', 'lively', 'alert', 'cheerful', 'vigorous' and 'active' on the Mood Scale II and is considered a positive factor. Happiness, the other positive mood factor on this measure, is described by the adjectives 'good', 'contented', 'satisfied', 'calm', 'pleased', 'happy' and 'steady'. Depression is associated with negative affect characterised by the adjectives 'miserable', 'blue', 'depressed', 'sad', 'downcast' and 'low' on the Mood Scale II. A further negative factor is angry, related to the adjectives of 'grouchy', 'mean', 'annoyed', 'angry', 'burned up' and 'irritated'. Fatigue is also considered a negative factor typified by feelings of physical and mental tiredness and described by the 'inactive', 'weary', 'lazy', 'drowsy' and 'sluggish' adjectives in the Mood Scale II. Finally, the adjectives 'uneasy', 'alarmed', 'insecure', 'afraid', 'jittery' and 'hopeless' are used to quantify the negative factor fear on the Mood Scale II.

A number of subjective measures have been used to investigate psychophysiological relationships with fatigue (Thomas et al. 2011; Vgontzas et al. 2003). One well-

established measure that was developed for and validated in occupational settings is the Samn-Perelli Fatigue Scale (Samn and Perelli 1982; International Civil Aviation Organization 2011). This brief and easily administered subjective measure asks individuals to rate their level of fatigue on a 7-point scale at a specified time point (Samn et al. 1982). Although evidence indicates that the Samn-Perelli Fatigue Scale (Samn and Perelli 1982; International Civil Aviation Organization 2011) and the Mood Scale II (Carpenter et al. 2004; Friedmann et al. 1977; Thorne et al. 1985) are well-suited (i.e., brief and easily administered) and valid measures for occupational settings such as the fire-ground, neither have been used to investigate psychophysiological relationships among firefighters. Further research adopting these subjective measures suited to occupational settings is needed to understand if, in response to firefighting work and sleep restriction, different factors of negative and positive mood relate to cortisol and cytokine responses.

2.6 Conclusion

It has been established that severe stressors can adversely alter acute cytokine, cortisol and heart rate responses. Over time, dysregulated immune and neuroendocrine function is linked to the development of adverse health outcomes such as cardiovascular and metabolic diseases and mood disorders (Chandola et al. 2008; Dantzer et al. 2008; Grandner et al. 2013; Hamer et al. 2010; Hayley et al. 2005; Mackin et al. 2004; Opdahl et al. 2014; van Leeuwen et al. 2009). Occupational stressors are a major source of altered physiological responses among personnel (Chandola et al. 2010). For wildland firefighters, consecutive long shifts of intense physical work separated by restricted sleep opportunities are two common fire-ground stressors (Aisbett et al. 2012; Cater et al. 2007; Cuddy et al. 2007). To date, military- and exercise-based studies (Abedelmalek et al. 2013; Bøyum et al. 1996; Dabrowski

et al. 2012; Goh et al. 2001; Gundersen et al. 2006; Lieberman et al. 2005; Lucas et al. 2008; Lundeland et al. 2012; Myles 1987; Opstad 1994; Opstad et al. 1981; Plyley et al. 1987) provide the only insights into how sleep restriction while performing simulated and live physical work influence acute physiological responses. For instance, consecutive days of simulated physical military work and severe sleep restriction have been found to result in a disrupted cortisol rhythm (Lieberman et al. 2005; Opstad 1994; Opstad et al. 1981). However, current cytokine and heart rate responses following extended periods of simulated and live physical work and/or shortened sleep need to be clarified. For instance, studies report no change in either cytokine or heart rate responses (Main et al. 2013; Plyley et al. 1987), while others found increases (Abedelmalek et al. 2013; Budd et al. 1997b; Raines et al. 2013; Rodríguez-Marroyo et al. 2012), decreases (Bøyum et al. 1996; Dabrowski et al. 2012; Lucas et al. 2008; Myles 1987) or fluctuations over time (Gundersen et al. 2006; Lundeland et al. 2012; Main et al. 2013). Furthermore, varying durations of sleep loss (Vgontzas et al. 2008; Vgontzas et al. 2002; Vgontzas et al. 2003) and physical activity over multiple days (Jürimäe et al. 2002), indicate how mood may moderate an increase in IL-6 and cortisol.

While military- (Bøyum et al. 1996; Gundersen et al. 2006; Lieberman et al. 2005; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981) and exercise-based studies (Abedelmalek et al. 2013; Bouget et al. 2006; Dabrowski et al. 2012; Jürimäe et al. 2002; Lucas et al. 2008; Robson-Ansley et al. 2009) represent the only experimental research in this area, the periods of shortened sleep and physical work examined are different to those experienced in firefighting (Aisbett et al. 2012; Ferguson et al. 2011; Phillips et al. 2012). Specifically, the extreme sleep restriction investigated among military personnel differs to the moderate, partial sleep restriction (i.e., 3 to 6-h) to

which wildland firefighters are typically exposed (Aisbett et al. 2012; Cater et al. 2007). Moreover, military- and exercise-based research has predominately investigated continuous physical activity or short periods of high-intensity exercise. In contrast, wildland firefighting work involves primarily short-duration high-intensity weight bearing manual handling tasks (e.g., lifting and lowering hoses and firefighting tools; Phillips et al. 2012) performed intermittently across 12 to 16 h shifts, interspersed with sustained periods of low intensity aerobic physical activity. Extrapolating military- or exercise-specific findings to wildland firefighting could therefore under- or over-estimate the potential stress-related implications of this type of firefighting and lead to inappropriate recommendations regarding the management of risk associated with sleep in the field.

Field-based investigations dominate the existing research in this area (Bøyum et al. 1996; Gundersen et al. 2006; Lieberman et al. 2005; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981; Raines et al. 2013; Rodríguez-Marroyo et al. 2012). Although such settings enhance ecological validity, these studies displayed limited control over sleep parameters (e.g., sleep durations and placement/timing of sleep restriction periods) and other potential stressors during testing (e.g., ambient temperature, calorie restriction). In addition, physical activity and sleep prior to beginning interventions have not been recorded or considered in previous studies analyses. Consequently, these issues mean that it is not possible to accurately interpret how the stressors of interest (i.e., sleep restriction and physical work) directly influenced the acute stress responses reported, preventing extrapolation of findings to wildland firefighters. Research in this area could further benefit from utilising a control group and/or comparing results to population norms to accurately quantify the degree

to which acute stress responses to physical work and/or sleep restriction differed from one another and control conditions.

The state of current empirical data points towards the need for further robust research examining controlled periods of sleep restriction across multiple days of wildfire suppression work. In line with the concepts of allostatic load (McEwen et al. 1999), it is imperative future enquiries implement a multivariate (i.e., multiple responses) assessment of acute inflammatory, cortisol and heart rate responses and their interactions to advance this area of applied stress research. Such investigations would provide a comprehensive first insight for fire agencies with industry specific recommendations regarding sleep on the fire-ground. Attaining multiple measures of inflammatory (i.e., cytokine) and neuroendocrine (i.e., heart rate and cortisol) function may help elucidate potential physiological interactions underlying these responses. Research revealing acute relationships between psychological and physiological responses support the application of a psychophysiological approach to assess how mood may relate to changes in cytokines and cortisol among firefighters. Adopting this approach affords new insights for psychophysiology research in an occupational setting, while providing the first steps in identifying subjective mood indicators of cortisol and/or cytokine responses.

2.7 References

Abedelmalek, S, Souissi, N, Chtourou, H, Denguezli, M, Aouichaoui, C, Ajina, M, Aloui, A, Dogui, M, Haddouk, S and Tabka, Z 2013, 'Effects of Partial Sleep Deprivation on Proinflammatory Cytokines, Growth Hormone, and Steroid Hormone Concentrations During Repeated Brief Sprint Interval Exercise', *Chronobiology International: The Journal of Biological and Medical Rhythm Research*, vol. 30, no. 4, pp. 502-509.

Adam, EK and Kumari, M 2009, 'Assessing salivary cortisol in large-scale, epidemiological research', *Psychoneuroendocrinology*, vol. 34, no. 10, pp. 1423-1436.

Aisbett, B, Phillips, M, Raines, J and Nichols, D 2007, 'Work patterns of tanker-based bushfire suppression by Australian volunteer firefighters in south-east Australia', *Human Dimensions of Wildfire Conference*, Fort Collins, Colorado.

Aisbett, B, Wolkow, A, Sprajcer, M and Ferguson, SA 2012, '"Awake, smoky, and hot": Providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters', *Applied Ergonomics*, vol. 43, no. 5, pp. 916-925.

Åkerstedt, T, Kecklund, G and Selén, J 2010, "Early Morning Work—Prevalence And Relation To Sleep/Wake Problems: A National Representative Survey', *Chronobiology International: The Journal of Biological and Medical Rhythm Research*, vol. 27, no. 5, pp. 975-986.

Alesci, S, Martinez, P, Kelkar, S, Ilias, I, Ronsaville, D, Listwak, S, Ayala, A, Licinio, J, Gold, H, Kling, M, Chrousos, G and Gold, P 2005, 'Major depression is associated with significant diurnal elevations in plasma interleukin-6 levels, a shift of its circadian rhythm, and loss of physiological complexity in its secretion: clinical implications', *The Journal Of Clinical Endocrinology And Metabolism*, vol. 90, no. 5, pp. 2522-2530.

Altara, R, Manca, M, Hermans, KCM, Daskalopoulos, EP, Brunner-La Rocca, H-P, Hermans, RJJ, Struijker-Boudier, HAJ and Blankesteijn, MW 2015, 'Diurnal rhythms of serum and plasma cytokine profiles in healthy elderly individuals assessed using membrane based multiplexed immunoassay', *Journal of Translational Medicine*, vol. 13, no. 1, pp. 1-8.

Andersen, JP, Silver, RC, Stewart, B, Koperwas, B and Kirschbaum, C 2013, 'Psychological and physiological responses following repeated peer death', *PLoS ONE*, vol. 8, no. 9.

Anisman, H and Merali, Z 2003, 'Cytokines, stress and depressive illness: brain-immune interactions', *Annals of Medicine*, vol. 35, pp. 2-11.

Barak, Y 2006, 'The immune system and happiness', *Autoimmunity Reviews*, vol. 5, no. 8, pp. 523-527.

Besedovsky, HO and del Rey, A 2000, 'The cytokine-HPA axis feed-back circuit', *Zeitschrift für Rheumatologie*, vol. 59, Supplement 2, pp. 26-30.

Bhatt, V, Diolombi M., Haack M. and Mullington, J 2015, 'Effect of Repeated Exposure to Sleep Restriction on Interleukin 6 Levels in Humans', *The Federation of American Societies for Experimental Biology (FASEB) Journal*, vol. 29, no. 1 Supplement 615.7.

Bishop, NC, Walsh, NP, Haines, DL, Richards, EE and Gleeson, M 2001, 'Pre-Exercise Carbohydrate Status and Immune Responses to Prolonged Cycling: II. Effect on Plasma Cytokine Concentration', *International Journal of Sport Nutrition and Exercise Metabolism*, vol. 11, no. 4, pp. 503-512.

Black, P 2006, 'The inflammatory consequences of psychologic stress: Relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II', *Medical Hypotheses*, vol. 67, no. 4, pp. 879-891.

Boscolo, P, Youinou, P, Theoharides, T, Cerulli, G and Conti, P 2008, 'Environmental and occupational stress and autoimmunity', *Autoimmunity Reviews*, vol. 7, no. 4, pp. 340-343.

Bouget, M, Rouveix, M, Michaux, O, Pequignot, J and Filaire, E, . 2006, 'Realationships among training stress mood and dehydroepiandrosterone sulphate/cortisol ratio in female cyclists', *Journal of Sports Sciences*, vol. 24, no. 12, pp. 1297-1302.

Bøyum, A, Wiik, P, Gustavsson, E, Veiby, OP, Reseland, J, Haugen, AH and Opstad, PK 1996, 'The effect of strenuous exercise, calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines', *Scandinavian Journal Of Immunology*, vol. 43, no. 2, pp. 228-235.

Brown, MA and Hural, J 1997, 'Functions of IL-4 and control of its expression', *Critical Reviews In Immunology*, vol. 17, no. 1, pp. 1-32.

Bruunsgaard, H 2005, 'Physical activity and modulation of systemic low-level inflammation', *Journal of Leukocyte Biology*, vol. 78, no. 4, pp. 819-835.

Budd, GM, Brotherhood, JR, Hendrie, AL, Jeffery, SE, Beasley, FA, Costin, BP, Wu, Z, Baker, MM, Cheney, NP and Dawson, MP 1997a, 'Project Aquarius 4. Experimental bushfires, suppression procedures, and measurements', *International Journal of Wildland Fire*, vol. 7, no. 2, pp. 99-104.

Budd, GM, Brotherhood, JR, Hendrie, AL, Jeffery, SE, Beasley, FA, Costin, BP, Wu, Z, Baker, MM, Cheney, NP and Dawson, MP 1997b, 'Project aquarius 7. Physiological and subjective responses of men supressing wildland fire', *International Journal of Wildland Fire*, vol. 7, no. 133-144.

Buxton, O, Pavlova, M, Reid, E, Wang, W, Simonson, D and Adler, G 2010, 'Sleep restriction for one week reduces insulin sensitivity in healthy men', *Diabetes*, vol. 59, no. 2126–2133.

Cacioppo, J, Tassinary, L and Berntson, G 2007, *Handbook of Psychophysiology*, 3rd edn, Cambridge University Press, New York.

Canli, T, Zhao, Z, Desmond, J, Kang, E, Gross, J and Gabrieli, JDE 2001, 'An fMRI study of personality influences on brain reactivity to emotional stimuli', *Behavioral Neuroscience*, vol. 115, no. 1, pp. 33-42.

Cannon, W 1939, *The Wisdom of the Body*, W.W. Norton and Co, New York.

Carey, MG, Al-Zaiti, SS, Dean, GE, Sessanna, L and Finnell, DS 2011, 'Sleep problems, depression, substance use, social bonding, and quality of life in professional firefighters', *Journal Of Occupational And Environmental Medicine/American College Of Occupational And Environmental Medicine*, vol. 53, no. 8, pp. 928-933.

Carpenter, JS, Elam, JL, Ridner, SH, Carney, PH, Cherry, GJ and Cucullu, HL 2004, 'Sleep, fatigue, and depressive symptoms in breast cancer survivors and matched healthy women experiencing hot flashes', *Oncology Nursing Forum*, vol. 31, no. 3, pp. 591-598.

Cater, H, Clancy, D, Duffy, K, Holgate, A, Wilison, B and Wood, J 2007, 'Fatigue on the fireground: the DPI experience', *Bushfire Cooperative Research Centre/Australasian Fire Authorities Council Conference Research Forum*, Hobart, Tasmania.

Chandola, T, Britton, A, Brunner1, E, Hemingway, H, Malik, M, Kumari, M, Badrick, E, Kivimaki, M and Marmot, M 2008, 'Work stress and coronary heart disease: what are the mechanisms?', *European Heart Journal*, vol. 29, no. 5, pp. 640-648.

Chandola, T, Heraclides, A and Kumari, M 2010, 'Psychophysiological biomarkers of workplace stressors', *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 1, pp. 51-57.

Chrousos, GP 1995, 'The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation', *The New England Journal Of Medicine*, vol. 332, no. 20, pp. 1351-1362.

Chrousos, GP and Gold, PW 1992, 'The concepts of stress and stress system disorders', *Journal of the American Medical Association*, vol. 267, no. 9, pp. 1244-1252.

Cohen, S, Schwartz, J, Epel, E, Kirschbaum, C, Sidney, S and Seeman, T 2006, 'Socioeconomic Status, Race, and Diurnal Cortisol Decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study', *Psychosomatic Medicine*, vol. 68, no. 1, pp. 41-50.

Cuddy, J, Gaskill, S, Sharkey, B, Harger, S and Ruby, B 2007, 'Supplemental feedings increase self-selected work output during wildfire suppression', *Medicine and Science in Sports and Exercise*, vol. 39, no. 6, pp. 1004-1012.

Dabrowski, J, Ziemba, A, Tomczak, A and Mikulski, T 2012, 'Physical performance of healthy men exposed to long exercise and sleep deprivation', vol. 16, no. 1, pp. 6-11.

Dallman, M, Strack, A, Akana, S, Bradbury, M and Hanson, E 1993, 'Feast and Famine: Critical role of glucorticoids with insulin in daily energy flow', *Frontiers in Neuroendocrinology*, vol. 14, pp. 303-347.

Damasio, AR, Grabowski, TJ, Bechara, A, Damasio, H, Ponto, LL, Parvizi, J and Hichwa, RD 2000, 'Subcortical and cortical brain activity during the feeling of self-generated emotions', *Nature Neuroscience*, vol. 3, no. 10, pp. 1049-1056.

Dantzer, R 2006, 'Cytokine, Sickness Behavior, and Depression', *Neurologic Clinics*, vol. 24, no. 3, pp. 441–460.

Dantzer, R, O'Connor, J, Freund, G, Johnson, R and Kelley, K 2008, 'From inflammation to sickness and depression: when the immune system subjugates the brain', *Nature Reviews Neuroscience*, vol. 9, no. 1, pp. 46-56.

Dean, G, Gow, K and Shakespeare-Finch, J 2003, 'Counting the Cost: Psychological Distress in Career and Auxiliary Firefighters ', *The Australasian Journal of Disaster and Trauma Studies*, vol. 1.

Dedovic, K, Engert, V, Duchesne, A, Lue, S, Andrews, J, Efanov, S, Beaudry, T and Pruessner, J 2010, 'Cortisol awakening response and hippocampal volume: Vulnerability for major depressive disorder?', *Biological Psychiatry*, vol. 68, no. 9, pp. 847-853.

Degerstrom, J and Osterud, B 2006, 'Increased Inflammatory Response of Blood Cells to Repeated Bout of Endurance Exercise', *Medicine and Science in Sports and Exercise*, vol. 38, no. 7, pp. 1297-1303.

Dekker, MJHJ, Koper, JW, van Aken, MO, Pols, HAP, Hofman, A, de Jong, FH, Kirschbaum, C, Witteman, JCM, Lamberts, SWJ and Tiemeier, H 2008, 'Salivary cortisol is related to atherosclerosis of carotid arteries', *The Journal Of Clinical Endocrinology And Metabolism*, vol. 93, no. 10, pp. 3741-3747.

DeRijk, R, Michelson, D, Karp, B, Petrides, J, Galliven, E, Deuster, P, Paciotti, G, Gold, PW and Sternberg, EM 1997, 'Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 beta (IL-1 beta), IL-6, and tumor necrosis factor-alpha (TNF alpha) production in humans: high sensitivity of TNF alpha and resistance of IL-6', *The Journal Of Clinical Endocrinology And Metabolism*, vol. 82, no. 7, pp. 2182-2191.

Desantis, AS, Diezroux, AV, Hajat, A, Aiello, AE, Golden, SH, Jenny, NS, Seeman, TE and Shea, S 2012, 'Associations of salivary cortisol levels with inflammatory markers: The Multi-Ethnic Study of Atherosclerosis', *Psychoneuroendocrinology*, vol. 37, pp. 1009-1018.

Dhabhar, FS and McEwen, BS 2006, 'Bidirectional effects of stress on immune function: possible explanations for salubrious as well as harmful effects', in R, Ader (ed.), *Psychoneuroimmunology Vol 2*, Elsevier, pp. 723-760.

Dickerson, SS and Kemeny, ME 2004, 'Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research', *Psychological Bulletin*, vol. 130, no. 3, pp. 355-391.

Dickerson, SS, Kemeny, ME, Aziz, N, Kim, KH and Fahey, JL 2004, 'Immunological effects of induced shame and guilt', *Psychosomatic Medicine*, vol. 66, no. 1, pp. 124-131.

Diener, E and Emmons, R 1984, 'The Independence of Positive and Negative Affect', *Journal of Personality and Social Psychology*, vol. 47, no. 5, pp. 1105-1117.

Edwards, S, Clow, A, Evans, P and Hucklebridge, F 2001, 'Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity', *Life Sciences*, vol. 68, no. 18, pp. 2093-2103.

Elenkov, IJ 2008, 'Neurohormonal-cytokine interactions: Implications for inflammation, common human diseases and well-being', *Neurochemistry International*, vol. 52, pp. 40-51.

Elenkov, IJ and Chrousos, GP 2002, 'Stress Hormones, Proinflammatory and Antiinflammatory Cytokines, and Autoimmunity', *Annals of the New York Academy of Sciences*, vol. 966, no. 1, pp. 290-303.

Eller, NH, Netterstrøm, B and Allerup, P 2005, 'Progression in intima media thickness--The significance of hormonal biomarkers of chronic stress', *Psychoneuroendocrinology*, vol. 30, no. 8, pp. 715-723.

Fagundes, C, Glaser, R, Hwang, B, Malarkey, W and Kiecolt-Glaser, J 2013, 'Depressive symptoms enhance stress-induced inflammatory responses', *Brain, Behavior, and Immunity*, vol. 31, pp. 172-176.

Fahy, RF, Leblanc, PR and Mous, JL 2013, 'Firefighter fatalities in the United States, 2012', *NFPA Journal*, vol. 107, no. 4, pp. 64-73.

Faulkner, SH, Spilsbury, KL, Harvey, J, Jackson, A, Huang, J, Platt, M, Tok, A and Nimmo, MA 2014, 'The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise', *European Journal of Applied Physiology*, vol. 114, no. 6, pp. 1207-1216.

Fellmann, N, Bedu, M, Boudet, G, Mage, M, Sagnol, M, Pequignot, JM, Claustrat, B, Brun, J, Peyrin, L and Coudert, J 1992, 'Inter-relationships between pituitary-adrenal hormones and catecholamines during a 6-day Nordic ski race', *European Journal of Applied Physiology and Occupational Physiology*, vol. 64, no. 3, pp. 258-265.

Ferguson, SA, Aisbett, B, Jay, SM, Onus, K, Lord, C, Sprajcer, M and Thomas, MJW 2011, 'Design of a valid simulation for researching physical, physiological and cognitive performance in volunteer firefighters during bushfire deployment.', in *Proceedings of Bushfire Cooperative Research Centre/Australasian Fire and Emergency Service Authorities Council Conference Research Forum*, ed. RP Thornton, Sydney, pp. 196-204.

Friedmann, J, Globus, G, Huntley, A, Mullaney, D, Naitoh, P and Johnson, L 1977, 'Performance and Mood During and After Gradual Sleep Reduction', *Psychophysiology*, vol. 14, no. 3, pp. 245-250.

Glickman-Weiss, EL, Hegsted, M, Nelson, AG, Hearon, CM, Dunbar, CC and Tulley, R 1995, 'A comparison of a carbohydrate-electrolyte beverage versus a placebo beverage in maintaining thermoregulatory and blood homeostasis during the training of fire fighters', *Wilderness and Environmental Medicine*, vol. 6, no. 4, pp. 377-384.

Goh, VH, Tong, TY, Lim, C, Low, EC and Lee, LK 2001, 'Effects of one night of sleep deprivation on hormone profiles and performance efficiency', *Military Medicine*, vol. 166, no. 5, pp. 427-431.

Golden, SH, Wand, GS, Malhotra, S, Kamel, I and Horton, K, . 2011, 'Reliability of hypothalamic–pituitary–adrenal axis assessment methods for use in population-based studies', *European Journal of Epidemiology*, vol. 26, no. 511-525.

Grandner, M, Sands-Lincoln, M, Pak, V and Garland, S 2013, 'Sleep duration, cardiovascular disease, and proinflammatory biomarkers', *Nature and Science of Sleep*, vol. 5, pp. 93-107.

Gruenewald, TL, Kemeny, ME, Aziz, N and Fahey, JL 2004, 'Acute Threat to the Social Self: Shame, Social Self-esteem, and Cortisol Activity', *Psychosomatic Medicine*, vol. 66, no. 6, pp. 915-924.

Gudewill, S, Pollmächer, T, Vedder, H, Schreiber, W, Fassbender, K and Holsboer, F 1992, 'Nocturnal plasma levels of cytokines in healthy men', *European Archives of Psychiatry and Clinical Neuroscience*, vol. 242, no. 1, pp. 53-56.

Gundersen, Y, Opstad, PK, Reistad, T, Thrane, I and Vaagenes, P 2006, 'Seven days' around the clock exhaustive physical exertion combined with energy depletion and sleep deprivation primes circulating leukocytes', *European Journal of Applied Physiology*, vol. 97, no. 2, pp. 151-157.

Guyon, A, Balbo, M, Morselli, L, Tasali, E, Leproult, R, L'Hermite-Balériaux, M, Van Cauter, E and Spiegel, K 2014, 'Adverse effects of two nights of sleep restriction on the hypothalamic-pituitary-adrenal axis in healthy men', *The Journal Of Clinical Endocrinology And Metabolism*, vol. 99, no. 8, pp. 2861-2868.

Hamer, M, O'Donnell, K, Lahiri, A and Steptoe, A 2010, 'Salivary cortisol responses to mental stress are associated with coronary artery calcification in healthy men and women', *European Heart Journal*, vol. 31, no. 4, pp. 424-429.

Hayley, S, Merali, Z and Anisman, H 2003, 'Stress and Cytokine-elicited Neuroendocrine and Neurotransmitter Sensitization: Implications for Depressive Illness', *Stress: The International Journal on the Biology of Stress*, vol. 6, no. 1, pp. 19-32.

Hayley, S, Poulter, M, Merali, Z and Anisman, H 2005, 'The pathogenesis of clinical depression: stressor- and cytokine-induced alterations of neuroplasticity', *Neuroscience*, vol. 135, no. 3, pp. 659-678.

Heinz, A, Hermann, D, Smolka, MN, Ricks, M, Graf, K, Pohlau, D, Kuhn, W and Bauer, M 2003, 'Effects of acute psychological stress on adhesion molecules, interleukins and sex hormones: implications for coronary heart disease', *Psychopharmacology*, vol. 165, no. 2, pp. 111-117.

Hightower, M, Vázquez, B, Acharya, S, Subramania, S and Intaglietta, M 2012, 'PEG-Albumin Plasma Expansion Increases Expression of MCP-1 Evidencing Increased Circulatory Wall Shear Stress: An Experimental Study', *PLoS ONE*, vol. 7, no. 6, pp. 1-6.

Holsboer, F 2000, 'The Corticosteroid Receptor Hypothesis of Depression', *Neuropsychopharmacology*, vol. 23, no. 5, pp. 477-501.

Horn, G, Blevins, S, Fernhall, B and Smith, D 2013, 'Core temperature and heart rate response to repeated bouts of firefighting activities', *Ergonomics*, vol. 56, no. 9, pp. 1465-1473.

Hruschka, DJ, Kohrt, BA and Worthman, CM 2005, 'Estimating between- and within-individual variation in cortisol levels using multilevel models', *Psychoneuroendocrinology*, vol. 30, no. 7, pp. 698-714.

Huang, C and Acevedo, E 2011, 'Occupational stress: the influence of obesity and physical activity/fitness on immune function', *American Journal of Lifestyle Medicine*, vol. 5, no. 6, pp. 486-493.

Ingre, M, Kecklund, G, Åkerstedt, T and Kecklund, L 2004, 'Variation in Sleepiness during Early Morning Shifts: A Mixed Model Approach to an Experimental Field Study of Train Drivers', *Chronobiology International: The Journal of Biological and Medical Rhythm Research*, vol. 21, no. 6, pp. 973-990.

Jouven, X, Empana, J, Schwartz, P, Desnos, M, Courbon, D and Ducimetière, P 2005, 'Heart-Rate Profile during Exercise as a Predictor of Sudden Death', *New England Journal of Medicine*, vol. 352, no. 19, pp. 1951-1958.

Jürimäe, J, Mäestu, J, Purge, P, Jürimäe, T and Sööt, T 2002, 'Relations among heavy training stress, mood state and performance for male junior rowers', *Perceptual and Motor Skills*, vol. 95, no. 2, pp. 520-526.

Juster, R, McEwen, B and Lupien, S 2010, 'Allostatic load biomarkers of chronic stress and impact on health and cognition', *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 1, pp. 2-16.

Kaciuba-Uscilko, H, Kruk, B, Szczypaczewska, M, Opaszowski, B, Stupnicka, E, Bicz, B and Nazar, K 1992, 'Metabolic, body temperature and hormonal responses to repeated periods of prolonged cycle-ergometer exercise in men', *European Journal of Applied Physiology and Occupational Physiology*, vol. 64, pp. 26-31.

Kahneman, D, Krueger, AB, Schkade, DA, Schwarz, N and Stone, AA 2004, 'A Survey Method for Characterizing Daily Life Experience: The Day Reconstruction Method', *Science*, vol. 306, no. 5702, pp. 1776-1780.

Kajtna, T, Stukovnik, V and Groselj, LD 2011, 'Effect of acute sleep deprivation on concentration and mood states with a controlled effect of experienced stress', *Zdravniski Vestnik*, vol. 80, no. 5, pp. 354-361.

Kales, SN, Soteriades, ES, Christophi, CA and Christiani, DC, . 2007, 'Emergency duties and deaths from heart disease among firefighters in the United States', *The New England Journal Of Medicine*, vol. 356, no. 12, pp. 1207-1215.

Kecklund, G, Akerstedt, T and Lowden, A 1997, 'Morning work: effects of early rising on sleep and alertness', *Sleep*, vol. 20, no. 3, pp. 215-223.

Kemeny, ME 2007, 'Emotions and the Immune System', in R Ader (ed.), *Psychoneuroimmunology Vol 1*, Elsevier, pp. 619-629.

Kern, W, Dodt, C, Born, J and Fehm, HL 1996, 'Changes in cortisol and growth hormone secretion during nocturnal sleep in the course of aging', *The Journals Of Gerontology. Series A, Biological Sciences And Medical Sciences*, vol. 51, no. 1, pp. 3-9.

Koh, KB 1998, 'Emotion and immunity', *Journal of Psychosomatic Research*, vol. 45, no. 2, pp. 107-115.

Konishi, M, Takahashi, M, Endo, N, Numao, S, Takagi, S, Miyashita, M, Midorikawa, T, Suzuki, K and Sakamoto, S 2013, 'Effects of sleep deprivation on autonomic and endocrine functions throughout the day and on exercise tolerance in the evening', *Journal of Sports Sciences*, vol. 31, no. 3, pp. 248-255.

Kopasz, M, Loessl, B, Valerius, G, Koenig, E, Matthaeas, N, Hornyak, M, Kloepfer, C, Nissen, C, Riemann, D and Voderholzer, U 2010, 'No persisting effect of partial sleep curtailment on cognitive performance and declarative memory recall in adolescents', *Journal of Sleep Research*, vol. 19, no. 1, pp. 71-79.

Kraemer, HC, Giese-Davis, J, Yutsis, M, O'Hara, R, Neri, E, Gallagher-Thompson, D, Taylor, CB and Spiegel, D 2006, "Design decisions to optimize reliability of daytime cortisol slopes in an older population': Erratum', *The American Journal of Geriatric Psychiatry*, vol. 14, no. 6.

Kunz-Ebrecht, SR, Kirschbaum, C, Marmot, M and Steptoe, A 2004, 'Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort', *Psychoneuroendocrinology*, vol. 29, no. 4, pp. 516-528.

Lemmer, B, Schwuléra, U, Thrun, A and Lissner, R 1992, 'Circadian rhythm of soluble interleukin-2 receptor in healthy individuals', *European Cytokine Network*, vol. 3, no. 3, pp. 335-336.

Leproult, R, Copinschi, G, Buxton, O and Van Cauter, E 1997, 'Sleep loss results in an elevation of cortisol levels the next evening', *Sleep*, vol. 20, no. 10, pp. 865-870.

Lieberman, HR, Bathalon, GP, Falco, CM, Kramer, MF, Morgan, CA and Niro, P 2005, 'Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat', *Biological Psychiatry*, vol. 57, pp. 422-429.

Lucas, S, Anglem, N, Roberts, W, Anson, J, Palmer, C, Walker, R, Cook, C and Cotter, J 2008, 'Intensity and physiological strain of competitive ultra-endurance exercise in humans', *Journal of Sports Sciences*, vol. 26, no. 5, pp. 477-489.

Lundberg, U 2008, '*Catecholamines and Environmental Stress'*, Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University, retrieved 08 July 2015, <www.macses.ucsf.edu/research/allostatic/catecholamine.php#assessment>.

Lundeland, B, Gundersen, Y, Opstad, PK, Thrane, I, Zhang, Y, Olaussen, RW and Vaagenes, P 2012, 'One week of multifactorial high-stress military ranger training affects Gram-negative signalling', *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 72, no. 7, pp. 547-554.

Lutgendorf, SK, Weinrib, AZ, Penedo, F, Russell, D, DeGeest, K, Costanzo, ES, Henderson, PJ, Sephton, SE, Rohleder, N, Lucci, JA, 3rd, Cole, S, Sood, AK and Lubaroff, DM 2008, 'Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients', *Journal Of Clinical Oncology: Official Journal Of The American Society Of Clinical Oncology*, vol. 26, no. 29, pp. 4820-4827.

Mackin, P and Young, AH 2004, 'The role of cortisol and depression: exploring new opportunities for treatments', *Psychiatric Times*, vol. 21, no. 5, pp. 92-95.

Maier, SF 2003, 'Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition', *Brain, Behavior, and Immunity*, vol. 17, pp. 69-85.

Maier, SF and Watkins, LR 1998, 'Cytokines for Psychologists: Implication of Bidirectional Immune-to-Brain Communication for Understanding Behaviour, Mood, and Cognition', *Psychological Review*, vol. 105, no. 1, pp. 83-107.

Main, L, Raines, J, Della Gatta, P, Wolkow, A, Snow, R and Aisbett, B 2013, 'The Stress of Firefighting - Implications for Long-term Health Outcomes', in *Proceedings of Bushfire Cooperative Research Centre and Australasian Fire and Emergency Service Authorities Council Research Forum*, ed. RP Thornton and LJ Wright, Perth, pp. 160-169.

Marniemi, J, Vuori, I, Kinnunen, V, Rahkila, P, Vainikka, M and Peltonen, P 1984, 'Metabolic changes induced by combined prolonged exercise and low-calorie intake in man', *European Journal of Applied Physiology and Occupational Physiology*, vol. 53, no. 2, pp. 121-127.

Marsland, AL, Pressman, S and Cohen, S 2007, 'Positive Affect and Immune Function', in R Ader (ed.), *Psychoneuroimmunology Vol 2*, Elsevier, pp. 761-779.

Mastorakos, G, Pavlatou, M, Diamanti-Kandarakis, E and Chrousos, G 2005, 'Exercise and the Stress System', *Hormones*, vol. 4, no. 2, pp. 73-89.

Mavanji, V, Teske, JA, Billington, CJ and Kotz, CM 2013, 'Partial sleep deprivation by environmental noise increases food intake and body weight in obesity-resistant rats', *Obesity*, vol. 21, no. 7, pp. 1396-1405.

McAnulty, S, McAnulty, L, Nieman, D, Morrow, J, Dumke, C and Utter, A 2007, 'Carbohydrate Effect: Hormone and Oxidative Changes', *International Journal Of Sports Medicine*, vol. 28, no. 11, pp. 921-927.

McEwen, BS 1998, 'Stress, adaptation, and disease - Allostasis and allostatic load', *Annals of the New York Academy of Sciences*, vol. 840, pp. 33-44.

McEwen, BS 2005, 'Glucocorticoids, depression, and mood disorders: structural remodeling in the brain', *Metabolism*, vol. 54, no. Supplement 1, pp. 20-23.

McEwen, BS, Biron, CA, Brunson, KW, Bulloch, K, Chambers, WH, Dhabhar, FS, Goldfarb, RH, Kitson, RP, Miller, AH, Spencer, RL and Weiss, JM 1997, 'The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions', *Brain Research Reviews*, vol. 23, pp. 79-133.

McEwen, BS and Seeman, T 1999, 'Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load', *Annals of the New York Academy of Sciences*, vol. 896, pp. 30-47.

Meier-Ewert, HK, Ridker, PM, Rifai, N, Regan, MM, Price, NJ, Dinges, DF and Mullington, JM 2004, 'Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk', *Journal of the American College of Cardiology*, vol. 43, no. 4, pp. 678-683.

Miller, GE, Chen, E and Zhou, ES 2007, 'If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans', *Psychological Bulletin*, vol. 133, no. 1, pp. 25-45.

Miller, GE, Cohen, S and Ritchey, AK 2002, 'Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model', *Health Psychology*, vol. 21, no. 6, pp. 531-541.

Mittwoch-Jaffe, T, Shalit, F, Srendi, B and Yehuda, S 1995, 'Modification of cytokine secretion following mild emotional stimuli', *Neuroreport*, vol. 6, no. 5, pp. 789-792.

Moldoveanu, A, Shephard, R and Skek, P 2001, 'The cytokine response to physical activity and training. / Reponses des cytokines a l'activite physique et a l'entrainement', *Sports Medicine*, vol. 31, no. 2, pp. 115-144.

Murray, G, . 2007, 'Diurnal mood variation in depression: A signal of disturbed circadian function?', *Journal Of Affective Disorders*, vol. 102, pp. 47-53.

Musselman, DL, Miller, AH, Porter, MR, Manatunga, A, Gao, F, Penna, S, Pearce, BD, Landry, J, Glover, S, McDaniel, JS and Nemeroff, CB 2001, 'Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: Preliminary findings', *The American Journal of Psychiatry*, vol. 158, no. 8, pp. 1252-1257.

Myles, WS 1987, 'Self-paced work in sleep deprived subjects', *Ergonomics*, vol. 30, no. 8, pp. 1175-1184.

Neylan, TC, Metzler, TJ, Best, SR, Weiss, DS, Fagan, JA, Liberman, A, Rogers, C, Vedantham, K, Brunet, A, Lipsey, TL and Marmar, CR 2002, 'Critical incident exposure and sleep quality in police officers', *Psychosomatic Medicine*, vol. 64, no. 2, pp. 345-352.

Nieman, DC, Nehlsen-Cannarella, SL, Fagoaga, OR, Henson, DA, Utter, A, Davis, JM, Williams, F and Butterworth, DE 1998, 'Influence of mode and carbohydrate on the cytokine response to heavy exertion', *Medicine and Science in Sports and Exercise*, vol. 30, no. 5, pp. 671-678.

Nijm, J and Jonasson, L 2009, 'Inflammation and cortisol response in coronary artery disease', *Annals of Medicine*, vol. 41, no. 3, pp. 224-233.

O'Leary, ÉD 2014, 'Effects of Acute Sleep Restriction on Laboratory and Ambulatory Physiological Reactivity in Young Adults', PhD thesis, Doctor of Philosophy thesis, National University of Ireland.

Opal, SM and DePalo, VA 2000, 'Anti-inflammatory cytokines', *Chest*, vol. 117, no. 4, pp. 1162-1172.

Opdahl, A, Ambale Venkatesh, B, Fernandes, VRS, Wu, CO, Nasir, K, Choi, E-Y, Almeida, ALC, Rosen, B, Carvalho, B, Edvardsen, T, Bluemke, DA and Lima, JAC 2014, 'Resting Heart Rate as Predictor for Left Ventricular Dysfunction and Heart Failure: MESA (Multi-Ethnic Study of Atherosclerosis)', *Journal of the American College of Cardiology*, vol. 63, no. 12, pp. 1182-1189.

Opstad, PK 1994, 'Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men', *European Journal of Endocrinology*, vol. 131, no. 1, pp. 56-66.

Opstad, PK and Aakvaag, A 1981, 'The effect of a high calory diet on hormonal changes in young men during prolonged physical strain and sleep deprivation', *European Journal of Applied Physiology*, vol. 46, no. 1, pp. 31-39.

Padgett, D and Glaser, R 2003, 'How stress influences the immune response', *Trends in Immunology*, vol. 24, no. 8, pp. 444-448.

Paley, M and Tepas, D 1994, 'Fatigue and the shiftworker: firefighters working on a rotating shift schedule', *Human Factors*, vol. 36, no. 2, pp. 269-284.

Pandey, AK and Kar, SK 2011, 'REM sleep deprivation of rats induces acute phase response in liver', *Biochemical and Biophysical Research Communications*, vol. 410, no. 2, pp. 242-246.

Paterson, JL, Dorrian, J, Ferguson, SA, Jay, SM, Lamond, N, Murphy, PJ, Campbell, SS and Dawson, D 2011, 'Changes in structural aspects of mood during 39-66 h of sleep loss using matched controls', *Applied Ergonomics*, vol. 42, no. 2, pp. 196-201.

Paterson, JL, Dorrian, J, PinCombe, J, Grech, C and Dawson, D 2010, 'Mood Change and Perception of Workload in Australian Midwives', *Industrial Health*, vol. 48, pp. 381-389.

Paul, WE 1991, 'Interleukin-4: a prototypic immunoregulatory lymphokine', *Blood*, vol. 77, no. 9, pp. 1859-1870.

Pedersen, B and Hoffman-Goetz, L 2000, 'Exercise and the Immune System: Regulation, Integration, and Adaptation', *Physiological Reviews*, vol. 80, no. 3, pp. 1055-1081.

Pedersen, BK and Febbraio, MA 2008, 'Muscle as an endocrine organ: focus on muscle-derived interleukin-6', *Physiological Reviews*, vol. 88, no. 4, pp. 1379-1406.

Pedersen, BK and Fischer, CP 2007, 'Beneficial health effects of exercise - the role of IL-6 as a myokine', *Trends in Pharmacological Sciences*, vol. 28, no. 4, pp. 152-156.

Pejovic, S, Basta, M, Vgontzas, AN, Kritikou, I, Shaffer, ML, Tsaoussoglou, M, Stiffler, D, Stefanakis, Z, Bixler, EO and Chrousos, GP 2013, 'Effects of recovery sleep after one work week of mild sleep restriction on interleukin-6 and cortisol secretion and daytime sleepiness and performance', *American Journal of Physiology - Endocrinology and Metabolism*, vol. 305, no. 7, pp. 890-896.

Petersen, AMW and Pedersen, BK 2005, 'The anti-inflammatory effect of exercise', *Journal of Applied Physiology*, vol. 98, no. 4, pp. 1154-1162.

Petrovsky, N 1998, 'Diurnal Rhythms of Pro-Inflammatory Cytokines: Regulation by Plasma Cortisol and Therapeutic implications', *Cytokine*, vol. 10, no. 4, pp. 307-312.

Petrovsky, N 2001, 'Towards a unified model of neuroendocrine–immune interaction', *Immunology and Cell Biology*, vol. 79, no. 4, pp. 350-357.

Petrovsky, N and Harrison, L 1998, 'The Chronobiology of Human Cytokine Production', *International Reviews of Immunology*, vol. 16, no. 5, pp. 635-649.

Phillips, M, Netto, K, Payne, W, Nichols, D, Lord, C, Brooksbank, N, Onus, K, Jefferies, S and Aisbett, B 2011, 'Frequency, intensity and duration of physical tasks performed by Australian rural firefighters during bushfire suppression', in *Proceedings of Bushfire Cooperative Research Center/Australasian Fire Authorities Council Conference Research Forum*, ed. RP Thornton, Sydney, pp. 205-213.

Phillips, M, Payne, W, Lord, C, Netto, K, Nichols, D and Aisbett, B 2012, 'Identification of physically demanding tasks performed during bushfire suppression by Australian rural firefighters', *Applied Ergonomics*, vol. 43, no. 2, pp. 435-441.

Phillips, M, Raines, J, Nichols, D and Aisbett, B 2007, 'Work demands of tanker based bushfire suppression', *Bushfire Cooperative Research Center/Australasian Fire Authorities Conference*, Hobart, Tasmania.

Pizzi, C, Manzoli, L, Mancini, S and Costa, GM 2008, 'Analysis of potential predictors of depression among coronary heart disease risk factors including heart rate variability, markers of inflammation, and endothelial function', *European Heart Journal*, vol. 29, no. 9, pp. 1110-1117.

Planz, G, Wiethold, G, Appel, E, Bohmer, D, Palm, D and Grobecker, H 1975, 'Correlation between increased dopamine-beta-hydroxylase activity and catecholamine concentration in plasma: determination of acute changes in sympathetic activity in man', *European Journal of Clinical Pharmacology*, vol. 8, no. 3-4, pp. 181-188.

Plyley, M, Shephard, R, Davis, G and Goode, R 1987, 'Sleep deprivation and cardiorespiratory function', *European Journal of Applied Physiology*, vol. 56, pp. 338-344.

Poitras, V and Pyke, K 2013, 'The impact of acute mental stress on vascular endothelial function: Evidence, mechanisms and importance', *International Journal of Psychophysiology*, vol. 88, no. 2, pp. 124-135.

Porta, S, Emsenhuber, W, Petek, W, Purstner, P, Vogel, W, Schwaberger, G, Salwitsch, P and Korstako, W 1993, 'Detection and evaluation of persisting stress-induced hormonal disturbances by a post stress provocation test in humans', *Life Sciences*, vol. 53, no. 21, pp. 1583-1589.

Pruessner, JC, Kirschbaum, C, Meinlschmid, G and Hellhammer, DH 2003, 'Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change', *Psychoneuroendocrinology*, vol. 28, no. 7, pp. 916-931.

Raines, J, Snow, R, Nichols, D and Aisbett, B 2015, 'Fluid intake, hydration, work physiology of wildfire fighters working in the heat over consecutive days', *The Annals of Occupational Hygiene*, vol. 59, no. 5, pp. 554-554.

Raines, J, Snow, R, Petersen, A, Harvey, J, Nichols, D and Aisbett, B 2013, 'The effect of prescribed fluid consumption on physiology and work behavior of wildfire fighters', *Applied Ergonomics*, vol. 44, no. 3, pp. 404-413.

Ranjit, N, Diez-Roux, AV, Sanchez, B, Seeman, T, Shea, S, Shrager, S and Watson, K 2009, 'Association of salivary cortisol circadian pattern with cynical hostility: multi-ethnic study of atherosclerosis', *Psychosomatic Medicine*, vol. 71, no. 7, pp. 748-755.

Reynolds, AC, Dorrian, J, Liu, P, Van Dongen, H, Wittert, GA, Harmer, L and Banks, S 2012, 'Impact of Five Nights of Sleep Restriction on Glucose Metabolism, Leptin and Testosterone in Young Adult Men', *PLoS ONE*, vol. 7, no. 7, pp. 1-10.

Robson-Ansley, P, Barwood, M, Canavan, J, Hack, S, Eglin, C, Davey, S, Hewitt, J, Hull, J and Ansley, L, . 2009, 'The effect of repeated endurance exercise on IL-6 and sIL-6R and their relationship with sensations of fatigue at rest', *Cytokine*, vol. 45, pp. 111-116.

Rodríguez-Marroyo, JA, López-Satue, J, Pernía, R, Carballo, B, García-López, J, Foster, C and Villa, JG 2012, 'Physiological work demands of Spanish wildland firefighters during wildfire suppression', *International Archives Of Occupational And Environmental Health*, vol. 85, no. 2, pp. 221-228.

Ronsen, O, Haug, E, Pedersen, BK and Bahr, R 2001, 'Increased neuroendocrine response to a repeated bout of endurance exercise', *Medicine and Science in Sports and Exercise*, vol. 33, no. 4, pp. 568-575.

Ronsen, O, Lea, T, Bahr, R and Pedersen Bente, K 2002, 'Enhanced plasma IL-6 and IL-1ra responses to repeated vs. single bouts of prolonged cycling in elite athletes', *Journal of Applied Physiology*, vol. 92, no. 6, pp. 2547-2553.

Rosmond, R, Wallerius, S, Wanger, P, Martin, L, Holm, C and Björntorp , P 2003, 'A 5-year follow-up study of disease incidence in men with an abnormal hormone pattern', *Journal of Internal Medicine*, vol. 254, pp. 386-390.

Salvadori, A, Fanari, P, Giacomotti, E, Palmulli, P, Bolla, G, Tovaglieri, I, Longhini, E 2003. 'Kinetics of catecholamines and potassium, and heart rate during exercise testing in obese subjects. Heart rate regulation in obesity during exercise'. *European Journal of Nutrition,* vol. 42, no. 4, pp. 181-187.

Samn, SW and Perelli, LP 1982, *Estimating aircrew fatigue: a technique with implications to airlift operations*, UASF School of Aerospace Medicine, Texas, United States of America.

Sapolsky, R, Romero, L and Munck, A 2000, 'How do glucorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions ', *Endocrine Reviews*, vol. 21, no. 1, pp. 55-89.

Sari-Sarraf, V, Reilly, T, Doran, DA and Atkinson, G 2007, 'The effects of single and repeated bouts of soccer-specific exercise on salivary IgA', *Archives Of Oral Biology*, vol. 52, no. 6, pp. 526-532.

Sasaki, H, Hotta, N and Ishiko, T 1991, 'Comparison of sympatho-adrenal activity during endurance exercise performed under high- and low-carbohydrate diet conditions', *Journal of Sports Medicine and Physical Fitness*, vol. 31, no. 3, pp. 407-412.

Selye, H 1956, *The stress of life*, McGraw-Hill, New York.

Silverman, MN and Sternberg, EM 2012, 'Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction', *Annals of the New York Academy of Sciences*, vol. 1261, no. 1, pp. 55-63.

Sluiter, JK, Frings-Dresen, MHW, Beek, AJvd and Meijman, TF 2000, 'Reactivity and recovery from different types of work measured by catecholamines and cortisol: a systematic literature overview', *Occupational and Environmental Medicine*, vol. 57, no. 5, pp. 298-315.

Smith, D, Petruzzello, S, Kramer, J and Misner, J 1996, 'Physiological, psychophysical, and psychological responses of firefighters to firefighting training drills', *Aviation, Space, and Environmental Medicine*, vol. 67, no. 11, pp. 1063-1068.

Smith, LL 2003, 'An alternate approach to assessing immune function related to upper respiratory tract infection (URTI)', *International of Sports Medicine Journal*, vol. 4, no. 3, pp. 1-9.

Smith, TW and Uchino, BN 2007, 'Measuring Physiological Processes in Biopsychosocial Research: Basic Principles Amid Growing Complexity', in LJ Luecken and LC Gallo (eds), *Handbook of Physiological Research Methods in Health Psychology*, SAGE, Thousand Oaks, CA, pp. 11-33.

Smyth, N, Clow, A, Thorn, L, Hucklebridge, F and Evans, P 2013, 'Delays of 5-15 min between awakening and the start of saliva sampling matter in assessment of the cortisol awakening response', *Psychoneuroendocrinology*, vol. 38, no. 9, pp. 1476-1483.

Sothern, R, Roitman-Johnson, B, Kanabrocki, E, Yager, J, Fuerstenberg, R, Weatherbee, J, Young, M, Nemchausky, B and Scheving, L 1995, 'Circadian characteristics of interleukin-6 in blood and urine of clinically healthy men', *In Vivo (Athens, Greece)*, vol. 9, no. 4, pp. 331-339.

Sparrow, WA, Hughes, KM, Russell, AP and Le Rossignol, PF 1999, 'Effects of practice and preferred rate on perceived exertion, metabolic variables and movement control', *Human Movement Science*, vol. 18, no. 2-3, pp. 137-153.

Spiegel, K, Leproult, R and Van Cauter, E 1999, 'Impact of sleep debt on metabolic and endocrine function', *The Lancet*, vol. 354, no. 9188, pp. 1435-1439.

Starkie, R, Ostrowski, SR, Jauffred, S, Febbraio, M and Pedersen, BK 2003, 'Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans', *The Federation of American Societies for Experimental Biology (FASEB) Journal*, vol. 17, no. 8, pp. 884-886.

Steptoe, A, Leigh, E and Kumari, M 2011, 'Positive affect and distressed affect over the day in older people', *Psychology and Aging*, vol. 26, no. 4, pp. 956-965.

Sternberg, EM and Gold, PW 2002, 'The Mind-Body Interaction in Disease', *Scientific American Special Edition*, vol. 12, no. 1, pp. 82-89.

Stone, A, Smyth, J, Pickering, T and Schwartz, J 1996, 'Daily Mood Variability: Form of Diurnal Patterns and Determinants of Diurnal Patterns', *Journal of Applied Social Psychology*, vol. 26, no. 14, pp. 1286-1305.

Swart, R, Hoorn, E, Betjes, M and Zietse, R 2011, 'Hyponatremia and Inflammation: The Emerging Role of Interleukin-6 in Osmoregulation', *Nephron Physiology*, vol. 118, no. 1, pp. p45-p51.

Tanaka, H, Shimoda, M and Ishijima, T 2012, 'Influences of raised ambient temperature on cardiorespiratory performance in a 3-minute step test', *Journal Of Human Ergology*, vol. 41, no. 1-2, pp. 67-75.

Thomas, KS, Motivala, S, Olmstead, R and Irwin, MR 2011, 'Sleep depth and fatigue: role of cellular inflammatory activation', *Brain, Behavior, and Immunity*, vol. 25, no. 1, pp. 53-58.

Thompson, PD, Franklin, BA, Balady, GJ, Blair, SN, Corrado, D, Estes, NA, 3rd, Fulton, JE, Gordon, NF, Haskell, WL, Link, MS, Maron, BJ, Mittleman, MA, Pelliccia, A, Wenger, NK, Willich, SN and Costa, F 2007, 'Exercise and acute cardiovascular events placing the risks into perspective: a scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism and the Council on Clinical Cardiology', *Circulation*, vol. 115, no. 17, pp. 2358-2368.

Thorne, DR, Genser, SG, Sing, HC and Hedge, FW 1985, 'The Walter Reed Performance Assessment Battery', *Neurobehavioral Toxicology and Teratology*, vol. 7, pp. 415-418.

Tilg, H, Dinarello, CA and Mier, JW 1997, 'IL-6 and APPs: anti-inflammatory and immunosuppressive mediators', *Immunology Today*, vol. 18, no. 9, pp. 428-432.

Tomiyama, AJ, Mann, T, Vinas, D, Hunger, JM, DeJager, J and Taylor, SE 2010, 'Low calorie dieting increases cortisol', *Psychosomatic Medicine*, vol. 72, no. 4, pp. 357-364.

Turnbull, AV and Rivier, CL 1999, 'Regulation of the Hypothalamic-Pituitary-Adrenal Axis by Cytokines: Actions and Mechanisms of Action', *Physiological Reviews*, vol. 79, no. 1, pp. 1-71.

Väänänen, I, Vasankari, T, Mäntysaari, M and Vihko, V 2002, 'Hormonal responses to daily strenuous walking during 4 successive days. / Reponses hormonales a une longue marche journaliere durant 4 jours successifs', *European Journal of Applied Physiology*, vol. 88, pp. 122-127.

van Leeuwen, WMA, Lehto, M, Karisola, P, Lindholm, H, Luukkonen, R, Sallinen, M, Härmä, M, Porkka-Heiskanen, T and Alenius, H 2009, 'Sleep Restriction Increases the Risk of Developing Cardiovascular Diseases by Augmenting Proinflammatory Responses through IL-17 and CRP', *PLoS ONE*, vol. 4, no. 2, pp. 1-7.

Venancio, D and Suchecki, D 2014, 'Prolonged REM sleep restriction induces metabolic syndrome-related changes: Mediation by pro-inflammatory cytokines', *Brain, Behavior, and Immunity*, vol. 47, pp. 109-117.

Vgontzas, AN, Bixler, EO, Chrousos, GP and Pejovic, S 2008, 'Obesity and sleep disturbances: Meaningful sub-typing of obesity', *Archives of Physiology and Biochemistry*, vol. 114, no. 4, pp. 224-236.

Vgontzas, AN, Bixler, EO, Lin, HM, Prolo, P and Trakada, G 2005, 'IL-6 and Its Circadian Secretion in Humans', *Neuroimmunomodulation*, vol. 12, pp. 131-140.

Vgontzas, AN and Chrousos, GP 2002, 'Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders', *Endocrinology And Metabolism Clinics Of North America*, vol. 31, no. 1, pp. 15-36.

Vgontzas, AN, Zoumakis, E, Bixler, EO, Lin, H, Follett, H, Kales, A and Chrousos, GP 2004, 'Adverse effects of modest sleep restriction on sleepiness, performance and inflammatory cytokines', *The Journal Of Clinical Endocrinology And Metabolism*, vol. 89, no. 5, pp. 2119-2126.

Vgontzas, AN, Zoumakis, M, Bixler, E, Lin, H, Prolo, P, Vela-Bueno, A, Kales, A and Chrousos, GP 2003, 'Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications', *The Journal Of Clinical Endocrinology And Metabolism*, vol. 88, no. 5, pp. 2087-2095.

Violanti, JM, Burchfiel, CM, Fekedulegn, D, Andrew, ME, Dorn, J, Hartley, TA, Charles, LE and Miller, DB 2009, 'Cortisol patterns and brachial artery reactivity in a high stress environment', *Psychiatry Research*, vol. 169, no. 1, pp. 75-81.

von Känel, R, Bellingrath, S and Kudielka, BM 2008, 'Association between burnout and circulating levels of pro- and anti-inflammatory cytokines in schoolteachers', *Journal of Psychosomatic Research*, vol. 65, pp. 51-59.

Wellens, BT and Smith, AP 2006, 'Combined workplace stressors and their relationship with mood, physiology, and performance', *Work and Stress*, vol. 20, no. 3, pp. 245-258.

Willerson, JT and Ridker, PM 2004, 'Inflammation as a cardiovascular risk factor', *Circulation*, vol. 109, Supplement 1, pp. II2-II10.

Wright, CE, Strike, PC, Brydon, L and Steptoe, A 2005, 'Acute inflammation and negative mood: mediation by cytokine activation', *Brain, Behavior, and Immunity*, vol. 19, no. 4, pp. 345-350.

Wu, H, Stone, WS, Hsi, X, Zhuang, J, Huang, L, Yin, Y, Zhang, L and Zhao, Z 2010, 'Effects of Different Sleep Restriction Protocols on Sleep Architecture and Daytime Vigilance in Healthy Men', *Physiological Research*, vol. 59, no. 5, pp. 821-829.

Yehuda, S, Sredni, B, Carasso, RL and Kenigsbuch-Sredni, D 2009, 'REM Sleep Deprivation in Rats Results in Inflammation and Interleukin-17 Elevation', *Journal of Interferon and Cytokine Research*, vol. 29, no. 7, pp. 393-398.

Zunszain, PA, Anacker, C, Cattaneo, A, Carvalho, LA and Pariante, CM 2011, 'Glucocorticoids, cytokines and brain abnormalities in depression', *Progress In Neuro-Psychopharmacology and Biological Psychiatry*, vol. 35, no. 3, pp. 722-729.

Outline of the general research protocol

The four studies completed as part of this degree were based on a single data collection period which tested 35 firefighters (30 males, 5 females). Firefighters were recruited from Australia's state fire agencies and tested in a laboratory located in either South Australia or Victoria. Access to testing space and equipment meant that firefighters were assessed in small groups of 3 to 5, over a 5-day period spent in the testing facility. In total, 9 testing sessions were conducted over a 12-month period to meet the required sample size for the control and sleep restriction condition.

For each session, firefighters arrived at the testing venue at 18:00 on the pre-study day where it was revealed to them what condition they were in. Firefighters then underwent a familiarization session of all testing procedures involved in the study. Throughout the protocol, the daily schedule was strictly adhered to such that wake and sleep periods began at the same time for participants in each condition across testing sessions. Each night, the sleeping environment (e.g., camp beds, bedding) replicated wildfire conditions and was kept consistent across all testing sessions. On the pre-study night, all participants had an 8-h adaptation sleep opportunity. Participants in the control condition then had an 8-h sleep opportunity on nights 2 and 3, whereas those in the sleep restriction condition had a 4-h sleep opportunity. The final night of the study was a recovery night in which all participants had an 8-h sleep opportunity. During each testing day, all participants completed physical, physiological and cognitive test batteries at identical time points during the wake periods. With exception of the cognitive testing that formed part of an unrelated study to be reported elsewhere, the next four chapters describe the physical, physiological and psychological testing procedures as they relate to the specific research questions posed by those chapters.

Chapter 3: Study 1

Study 1

The impact of sleep restriction and simulated physical firefighting work on acute inflammatory stress responses

Alexander Wolkow BExSpSci (Hons), Sally A Ferguson PhD, Grace E Vincent BSc (Hons), Brianna Larsen BExSpSci (Hons), Brad Aisbett PhD and Luana C Main PhD

Published in

Plos One

doi: 10.1371/journal.pone.0138128

Presented here in journal submission format

Abstract

Objectives: This study investigated the effect restricted sleep has on wildland firefighters' acute cytokine levels during 3 days and 2 nights of simulated physical wildfire suppression work.

Methods: Firefighters completed multiple days of physical firefighting work separated by either an 8-h (Control condition; *n*=18) or 4-h (Sleep restriction condition; *n*=17) sleep opportunity each night. Blood samples were collected 4 times a day (i.e., 06:15, 11:30, 18:15, 21:30) from which plasma cytokine levels (IL-6, IL-8, IL-1β, TNF-α, IL-4, IL-10) were measured.

Results: The primary findings for cytokine levels revealed a fixed effect for condition that showed higher IL-8 levels among firefighters who received an 8-h sleep each night. An interaction effect demonstrated differing increases in IL-6 over successive days of work for the SR and CON conditions. Fixed effects for time indicated that IL-6 and IL-4 levels increased, while IL-1β, TNF-α and IL-8 levels decreased. There were no significant effects for IL-10 observed.

Conclusion: Findings demonstrate increased IL-8 levels among firefighters who received an 8- h sleep compared to those who had a restricted 4-h sleep. Firefighters' IL-6 levels increased in both conditions which may indicate that a 4-h sleep restriction duration and/or period (i.e., 2 nights) was not a significant enough stressor to affect this cytokine. Considering the immuno-modulatory properties of IL-6 and IL-4 that inhibit pro-inflammatory cytokines, the rise in IL-6 and IL-4, independent of increases in IL-1β and TNF-α, could indicate a non-damaging response to the stress of simulated physical firefighting work. However, given the link between chronically elevated cytokine levels and several diseases, further research is needed to determine if firefighters' IL-8 and IL-6 levels are elevated following repeated firefighting deployments across a fire season and over multiple fire seasons.

Introduction

Each year, firefighters are deployed to combat the threat of large wildfires to property and lives. These deployments can last multiple days and require firefighters to perform extended hours (i.e., 12 to 16 h) of intense, intermittent, physical work with restricted sleep opportunities between shifts (i.e., 3 to 6 h; 1, 2, 3). Evidence suggests that individually, physical work (4) and sleep restriction (5-7) can elicit an acute inflammatory response causing the release of cytokines.

Pro-inflammatory cytokines such as interleukin (IL)-1β, Tumour Necrosis Factor (TNF)-α and IL-8 facilitate an acute-phase response (8-10). Conversely, anti-inflammatory cytokines such as IL-10 inhibit pro-inflammatory cytokines and attenuate inflammation (9, 11). Furthermore, IL-6 and IL-4 cytokines display both pro- and anti-inflammatory activities that modulate inflammation (12-15). Together, these processes coordinate the body's acute inflammatory response to a stressor to maintain homeostasis of the immune system. However, severe or chronic stress exposure may exacerbate the immune response which could result in chronically elevated cytokine levels and associated adverse health outcomes (9, 16).

Acute increases in IL-6 (7) and TNF-α (5, 6) have been observed after 5-7 nights of sleep restricted to 4 h or 6 h per night in the laboratory, without physical work. Chronically elevated TNF-α and IL-6 levels are markers of systematic inflammation linked to negative health outcomes such cardiovascular disease (CVD) and insulin resistance (17, 18). Increased IL-6 and IL-8 levels were also reported following 3-days of intense physical running training (2.5 h/day) without sleep restriction (19). Chronically elevated IL-8 levels are also associated with atherogenesis and inflammatory changes that may result in CVD (20). In a field setting, Main et al. (4)

reported increased IL-6 across a 12-h shift of physical wildfire work without sleep disruption. However, firefighters' IL-6 levels, along with IL-1β, IL-8 and IL-4 exhibited a decrease over the second shift, possibly indicative of an adaptation to the physical work demands (4).

While firefighting literature is sparse, multi-day military and exercise-based studies have reported an increase (21), decrease (22) or fluctuation in IL-6 (23, 24). Increased or unchanged IL-1β, TNF-α and IL-10 levels were also reported among soldiers completing seven consecutive days of physical work with minimal sleep (e.g., 7 h total; 23, 24). Though it is possible the inflammatory markers in these field-based studies were confounded by other stressors (e.g., fluid and energy intake), an attenuated or unchanged cytokine response to these demands may indicate a non-damaging regulatory response. For instance, the immuno-modulatory properties of IL-6 modulate pro-inflammatory cytokines (12, 13, 25, 26) that underpin systemic inflammation (27, 28). The immune system also interacts with cortisol (29), found to increase during simulated wildland firefighting work (30). An acute increase in cortisol can down-regulate cytokine activity to maintain homeostasis of the immune system (29, 31, 32). While military- and exercise-based research provide some understanding of the effect of physical work and sleep loss on cytokine responses, the demands investigated differ to the sleep restriction and physical work involved in wildfire suppression. Extrapolation of findings to wildland firefighting could, therefore, under- or over-estimate any stress-related implications.

Military-based research mostly investigated long duration marching and running (22-24), whereas wildland firefighting work incorporates a large component of short-duration weight bearing manual handling tasks, in addition to sustained low intensity

aerobic activity (33). Given that eccentric contractions are known to produce a more pronounced increase of IL-6 and IL-8 compared to concentric contractions (27), military-based findings could lead to under-estimates of the cytokine response for wildfire personnel. Furthermore, total sleep deprivation is associated with a greater elevation in IL-6 than partial sleep restriction (34). The almost complete sleep restriction examined in military-based studies (23, 24) could over-estimate the cytokine response for firefighters who report 3 to 6 h of sleep per night (1). Under- or over-estimating inflammation could lead to inappropriate recommendations regarding the management of risk associated with firefighters' sleep during deployments. The aim of the present study was therefore, to assess the effect restricted sleep has on wildland firefighters' inflammatory cytokine levels during 3 days and 2 nights of simulated physical firefighting work.

Materials and Methods

Participants

Male and female volunteer and salaried firefighters from state and territory fire agencies across Australia (Victoria, Tasmania, New South Wales, South Australia and Australian Capital Territory) were recruited for this study (Table 3.1). Firefighters from Western Australia were excluded due to time zone differences between this state and the testing locations in Victoria and South Australia. This allowed for better control over circadian rhythm differences between participants, which is potentially a confounding variable when assessing inflammatory cytokine levels across the day. In addition, because this study was part of a larger project that also examined the impact of firefighting in the heat, firefighters from hotter climates (i.e., Northern Territory and Queensland) were also excluded. However, recruiting firefighters from most

Australian fire agencies maximise generalisability of potential findings to firefighters across Australia.

Once recruited, participants were screened and excluded from the study if they had been diagnosed with any form of heart disease, diabetes, respiratory or sleep disorders which could potentially confound observed cytokine levels. For purposes of analysis, participants in each condition were matched for age, sex and body mass index (BMI) and then randomly assigned to either a control (CON) or sleep restriction (SR) condition based on the participant's availability to attend the testing period, designated at random, to the condition. The randomization method took into account differences in firefighters' availability to volunteer their time for the study (without financial compensation), ensuring an equal chance for each participant of being allocated to either condition. There were no differences with regards to age, firefighting experience and BMI between conditions (Table 3.1). Participants also completed pre- and post-testing health questionnaires to exclude any participants who became ill or sustained an injury directly prior to or during testing that could influence the inflammatory markers measured and confound any subsequent comparisons. As a result, a final sample of 18 firefighters in the CON condition and 17 firefighters in the SR condition completed this study (Table 3.1). While information on injury, illness, and health outcomes were obtained from participants pre- and post-study, this data was only used for study exclusion purposes, and thus will not be described in any further detail. Participation was voluntary and all participants gave written informed consent prior to commencing data collection. This study was approved by the Deakin University Human Research Ethics Committee.

Table 3.1 Characteristics of participants in each condition (mean ± standard deviation)

Characteristic	CON (*n*=18)	SR (*n*=17)
Age (years)	39 ± 16	39 ± 15
Male:Female (*n*)	15:3	15:2
Weight (kg)	85.1 ± 17.7	93.8 ± 20.2
Height (cm)	178.1 ± 7.7	177.8 ± 7.4
BMI (kg/m^2)	26.8 ± 5.0	29.6 ± 5.5
Firefighting experience (years)	8.7 ± 9.3	10.2 ± 6.4

Note: BMI = Body Mass Index

Protocol

Participants in both conditions arrived at the testing venue and completed a familiarisation of all physical work tasks and physiological tests, followed by an adaptation night sleep (8-h sleep opportunity) in the testing environment before the simulation began. All participants were then tested over a 3-day and 2-night simulated fire-ground deployment. On each of the 2 nights, participants in the CON condition had an 8-h sleep opportunity (i.e., 22:00-06:00; Figure 3.1). Conversely, participants in the SR condition had their bed time delayed, resulting in a 4-h sleep opportunity (i.e., 02:00-06:00) on each of the 2 nights (Figure 3.1). Participants in the SR condition were free to perform sedentary leisure activities (e.g., watching television, reading etc.) until the delayed bedtime. The duration of sleep restriction in this study was based on Australian wildland firefighters' self-reported average sleep per rest period on the fire-ground (1). After completing the testing period, both conditions had an 8-h recovery sleep (in which no further measures were collected) to ensure, for the safety of all participants, that they were fully rested before leaving the testing venue and returning

home (Figure 3.1). The testing environment was maintained at moderate temperatures (18-20^{o}C) throughout the testing period in both conditions.

The timing of meals and the types of food and fluid available for consumption during testing were identical in both conditions and based on consultation with subject matter experts from Australian fire agencies. Adhering to fire-ground practices (35), food and drink intake during the study was *ad libitum* and the amount and type of food and drink ingested was recorded. This data was then extracted using the FoodWorks 7 nutrition software (2012 Xyris Software Pty Ltd, Australia). Although total fluid consumption was recorded, (no differences between conditions; $p > 0.05$), the measurement of caffeine intake (i.e., coffee and tea) has only been reported in the results section. Energy and macronutrient intakes have also been described in the results section below.

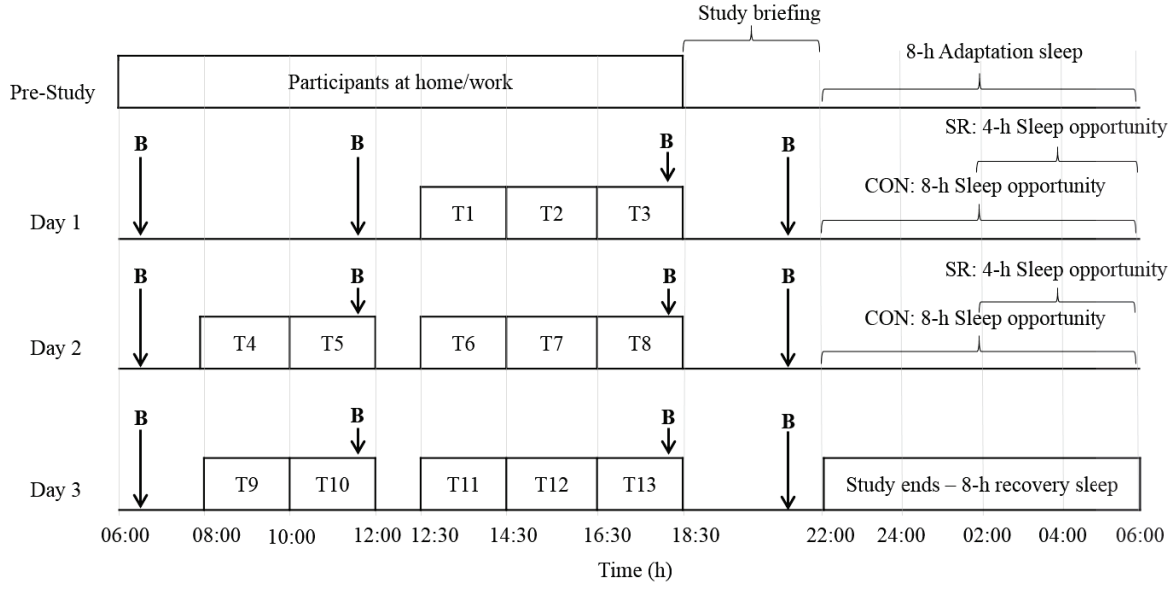

Figure 3.1 Firefighting work protocol for CON and SR conditions

Experimental Procedures

Participants in both conditions were tested in small groups (of 3 to 5) over the 3-day wildland firefighting simulation. During the wake period, participants completed a 2-h testing block, 3 times on day 1 and 5 times on day 2 and day 3 (Figure 3.1). Each 2-h testing block consisted of 55-min of simulated physical wildland firefighting work circuit, immediately followed by 20-25 min of physiological data collection (reported elsewhere; 36), 20-25 min of cognitive testing (outlined elsewhere; 37) and a 15-20 min rest period. The described work-to-rest ratios mimic shift work patterns observed during live wildfire suppression conditions (35, 38, 39). Participants' cytokine levels were measured in both conditions at 4 identical time points across each of the 3 testing days (Figure 3.1).

Simulated Physical Firefighting Work Circuit: The physical work circuit comprised 6 simulated wildland firefighting tasks. The tasks were designed to mimic the different physical demands involved in wildfire suppression work performed by Australia's state and territory fire agencies (33). Each have been recognised by incumbent firefighters and industry experts as being representative of repetitive movements and carry and drag movements that encompass key firefighting tasks frequently performed on the fire-ground (37). The tasks included; lateral repositioning of a hose, rake-hoe work, hose rolling, charged hose advance, black out hose work, and static hold of a hose. These tasks were chosen because they were: 1) deemed to have the highest operational importance 2) the most physically demanding; and 3) the longest, most intense, or most frequently occurring tasks during wildfire suppression work (37, 38). The tasks involved in the physical work circuit were completed in a pre-determined order with task work-to-rest ratios designed to mimic the performance of these tasks on the fire-ground (37, 38).

The performance of each physical task (i.e., repetitions completed for each task within each work period) was self-paced and therefore, a changing variable. Although performance of the physical tasks is not the focus of this study, it is possible the performance during the circuit (i.e., repetitions completed for each task) could interact with the cytokine responses and vice versa. Therefore, physical performance was recorded, but no significant differences in this component were demonstrated between conditions (findings reported elsewhere; 36), therefore it is unlikely physical performance had an impact on the cytokine response. Furthermore, the duration of the physical work circuit was the same for all participants, so it is also unlikely that this component had a confounding impact on the cytokine response.

Blood Sampling and Cytokine Analysis: Participants provided fingertip capillary blood samples for the determination of IL-6, IL-8, IL-1β, TNF-α, IL-4 and IL-10 cytokine levels in blood plasma. Although previous emergency service-based studies have used venous blood samples when investigating cytokine levels (22-24), capillary blood samples were chosen because it is a minimally invasive method to conveniently obtain multiple daily blood samples from participants wearing personal protective clothing and performing repeated bouts of physical work. Some studies suggest that, due to a small local inflammatory response to the action of the pinprick, capillary blood samples can result in higher cytokine levels (40, 41). However, recent evidence indicates a close correlation between venous and capillary plasma IL-6 responses during or post-exercise (40) and at rest (42). Conversely, other reports have found that venous and capillary concentrations of TNF-α (41) and IL-6 (40) differed at rest. However, these studies (40, 41) did not control factors known to impact resting cytokine levels such as the time of day the sample was taken or whether or not the

sample was taken under fasting conditions (43). Control over these factors in the current study limits their potentially confounding influence on cytokine levels at rest. Participants samples were taken at 4 time points each day: a fasting baseline sample in the early morning (i.e., 06:15), late morning (i.e., 11:30), early evening (i.e., 18:15) and at night (i.e., 21:30; Figure 3.1). To avoid acute postprandial changes in cytokine measurements, blood samples at 11:30 and 18:15 were taken pre-lunch and dinner respectively. Prior to sample collection, participants held a heat pack in their hand to aid in blood flow to the fingertips. At every time point, a 500-µL sample of whole blood was taken from each participant in to a microtainer coated with K_2 EDTA (Becton Dickinson ref: 365974). This process took between 1 and 10 minutes to complete and was the same in both conditions. Whole blood samples were centrifuged for 10 min at 5000 revolutions/minute and the plasma was separated and stored frozen at ≤ -80°C.

The Milliplex Human MAP Cytokine immunoassay kit (Millipore, Billerica, MD) was used to profile the expression of inflammatory markers in the plasma samples of participants. The assay kits provide a mixture of microbead populations with distinct fluorescent intensities that are pre-coated with capture antibodies specific for each cytokine. The assay was performed according to the manufacturer's instructions on the Bioplex 200 array reader (V.5.0, Bio-Rad Laboratories, Hercules, CA). The minimal detectable concentrations were 0.06 pg/mL, 0.42 pg/mL, 0.20 pg/mL, 0.05 pg/mL, 0.48 pg/mL and 0.07 pg/mL for IL-1β, IL-4, IL-6, IL-8, IL-10 and TNF-α, respectively. Cytokines intra- and inter-assay coefficients of variation were in acceptable ranges (Intra-assay 4.5 – 10.0%; Inter-assay 9.8 – 20.5%) for all analytes (CV <25%; 44, 45) and comparable to CVs reported for cytokines sampled using venous blood in previous exercise-based literature (21, 40, 42).

Sleep and Activity Monitoring: All participants had their sleep recorded on the adaptation night and the 2 study nights using standard polysomnographic (PSG) equipment (Compumedics E-Series, Australia). The adaptation night was designed to ensure participants' sleep and cytokine responses during the study were not influenced by the lack of familiarity with the PSG equipment. Each night, PSG wire up and recording began at 21:00 for both conditions. From each sleep recording, participants' total sleep time (minutes) was calculated. In addition, participants wore activity monitors (*Actical* MiniMitter/Respironics, Bend, OR, USA) to measure sleep across the 2 nights leading in to the study. Participants' physical activity during the simulation (2-h testing blocks including work and rest periods) was also recorded through the use of activity monitors worn on the wrist. Physical activity data (captured in 1-minute intervals) was downloaded using Actical software (version 3.10 MiniMitter/Respironics, Bend, OR, USA) and expressed as absolute counts. Further details regarding physical activity, including results, can be found in a previous study by Vincent et al. (36).

Statistical Analyses

To decrease the biological variation associated with human plasma samples, outliers that had values greater than 2 standard deviations above the mean were excluded prior to the analysis (46). Values that were below the detectable range of the Milliplex Human MAP Cytokine immunoassay kit were replaced with the minimal detectable concentration as advised in the protocol (Millipore, Billerica, MD). With the exception of TNF-α (for which raw values achieved normality and homogeneity of variance), all cytokines were natural log-transformed to achieve normality, as assessed using Shapiro Wilk tests ($p > 0.05$). Homogeneity of variance and normality of the residuals from the resulting mixed model analyses were further assessed. The resulting

diagnostic plots were visually examined and revealed no departures from the required assumptions. For ease of interpretation, cytokine values were back transformed to pg/mL in the figures presented.

Variables measured just once on an individual, or aggregated over occasions, (e.g., sleep duration, demographic characteristics and food and caffeine intake) were analysed with the Analysis of Variance (ANOVA) method using GenStat software (GenStat *for Windows* 16.1 Edition. VSN International, Hemel Hempstead, UK). For repeated cytokine measurements, linear mixed models (LMM) were fitted by the restricted maximum likelihood (REML) method (47). The LMM approach was used to investigate if participants' individual profiles for each cytokine differed between conditions. This method also allows for the possibility of autocorrelation in the repeated cytokine measurements (i.e., samples and/or days) on each individual by including a model for the covariance structure. In addition, differences between the conditions in their linear trends and deviations from linearity were investigated via the incorporation of smoothing splines (48). Model fit was assessed by Akaike Information Criterion (AIC) and small differences (ΔAIC) in this criterion compared to the minimum observed value in a set of candidate models were used to identify parsimonious models (49). Predicted REML means constructed from the linear models fitted by the REML analysis were calculated and pairwise comparisons of these predicted means (50) were used to examine differences in cytokines at each sample (or time point) and/or day.

The potential fixed effects investigated in the models fitted for each of the individual cytokine profiles were condition (CON or SR), day (day 1, day 2 or day 3) and time of day (06:15, 11:30, 18:15 and 21:30) along with potential interactions of condition

by day, condition by time, day by time and condition by day by time. Random effects of group, profile (or participant), and a group by profile interaction were investigated by applying an independence (banded and unbanned), unstructured and power models for the within-subject autocorrelation. Finally, the REML procedure (47) was used to determine if a common spline was adequate to describe any non-linearity or if separate splines for each group or individual splines were required. Statistical significance was set at $p < 0.05$ and data are presented as means ± standard error of difference (SED) unless otherwise stated.

Results

Cytokines

Several LMM were fit to the cytokine data, but for the majority of cytokines, the model with the lowest AIC and ΔAIC was the unstructured covariance model. Therefore, this model was considered to have the best fit to the data and selected as the final model for the analysis of each cytokine. The LMM demonstrated significant fixed effects for condition ($F = 9.39$, df = 1, $p = 0.02$) and time ($F = 16.57$, df = 3, $p < 0.001$) on log IL-8 levels, but no effect for day ($p = 0.64$). Predicted REML means for each condition showed that the CON condition had higher levels of log IL-8 (Figure 3.2), while predicted REML means at each time-point indicated that log IL-8 levels decreased across the day in both conditions (Figure 3.3A).

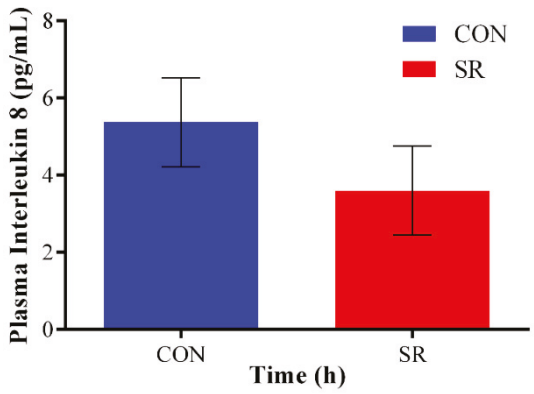

Figure 3.2 Predicted REML means for significant fixed effect of condition for IL-8 level in CON and SR conditions. Data was log-transformed prior to analysis. For ease of interpretation, values were back transformed to pg/mL.

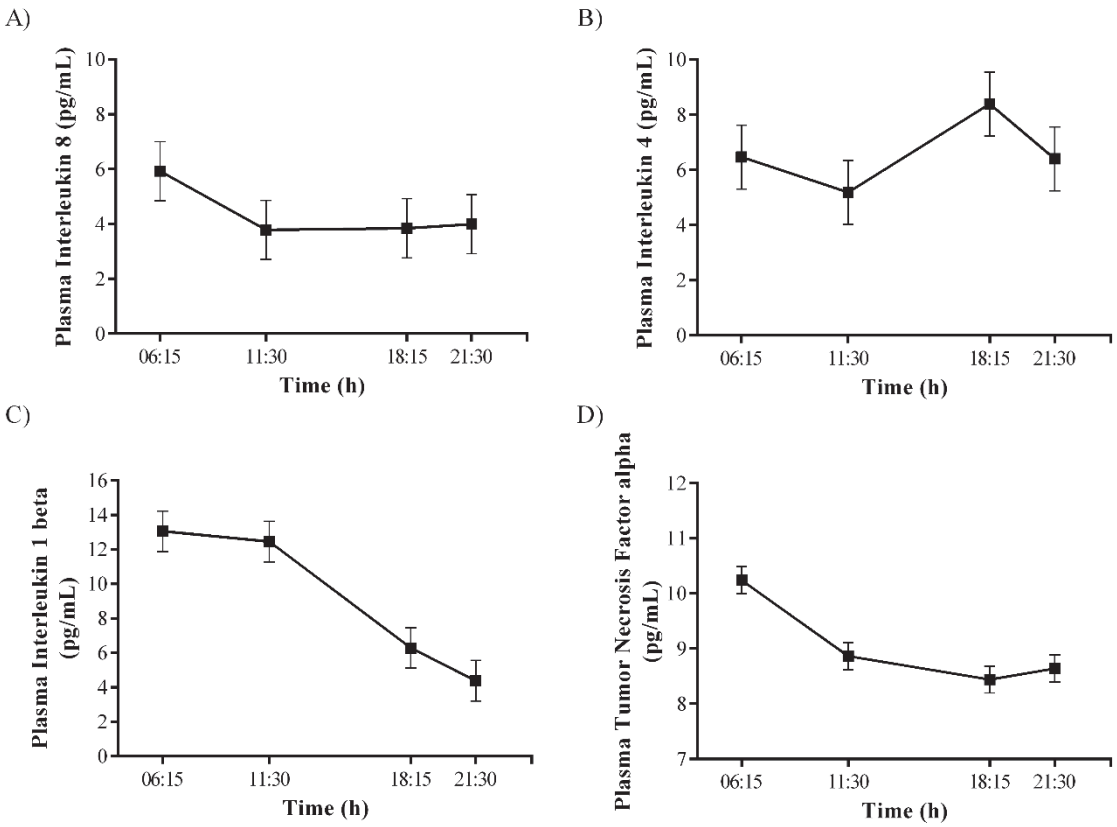

Figure 3.3 Predicted REML means for IL-8 (A), IL-4 (B), IL-1β (C) and TNF-α (D) across each daily time-point. Data was log-transformed prior to analysis. For ease of interpretation, values were back transformed to pg/mL.

The LMM demonstrated that there were significant fixed effects for time ($F = 24.65$, df = 3, $p < 0.001$) and day ($F = 3.10$, df = 2, $p = 0.05$) on log IL-6 levels. Predicted REML means indicated that log IL-6 levels in both conditions increased across time-points within a day and across each day. In addition, there were significant interaction effects for condition by time ($F = 3.72$, df = 3, $p = 0.01$) and day by time ($F = 7.45$, df = 6, $p < 0.001$) on log IL-6 levels. Pairwise comparison of predicted REML means further indicated that log IL-6 levels at 06:15 on day 3 were higher (+ 0.74) than at this time point on day 1 in both the SR (SED = 0.25, $p = 0.03$) and CON conditions (+ 0.67, SED = 0.24, $p = 0.04$; S1; Figure 3.4). In addition, log IL-6 levels in the CON condition on day 2 at 11:30 (+ 0.95, SED = 0.27, $p = 0.01$) were higher than at this time point on day 1 in the CON condition (Figure 3.4). Further pairwise comparisons of predicted REML means indicated that there were no significant differences in log IL-6 levels between conditions at the baseline time point (i.e., day 1 at 06:15) or any of the other sampling time points. Therefore, it is likely the interaction is a reflection of different rates of change in log IL-6 levels between the SR and CON conditions, but not one that yields time point differences between conditions. Furthermore, significant fixed effects for time were found for log IL-1β ($F = 21.79$, df = 3, $p < 0.001$), raw TNF-α ($F = 17.20$, df = 3, $p < 0.001$) and log IL-4 levels ($F = 3.65$, df = 3, $p = 0.02$). For these fixed effects, predicted REML means demonstrated a decrease across time for log IL-1β and raw TNF-α levels, while predicted means for log IL-4 fell slightly from 06:15 to 11:30, followed by higher levels recorded in the evening and at night (i.e., 18:15 and 21:30; Figure 3.3). There were no further fixed or interaction effects demonstrated for the IL-1β, IL-4, TNF-α or IL-8 cytokine profiles, nor were there any significant effects for log IL-10 observed.

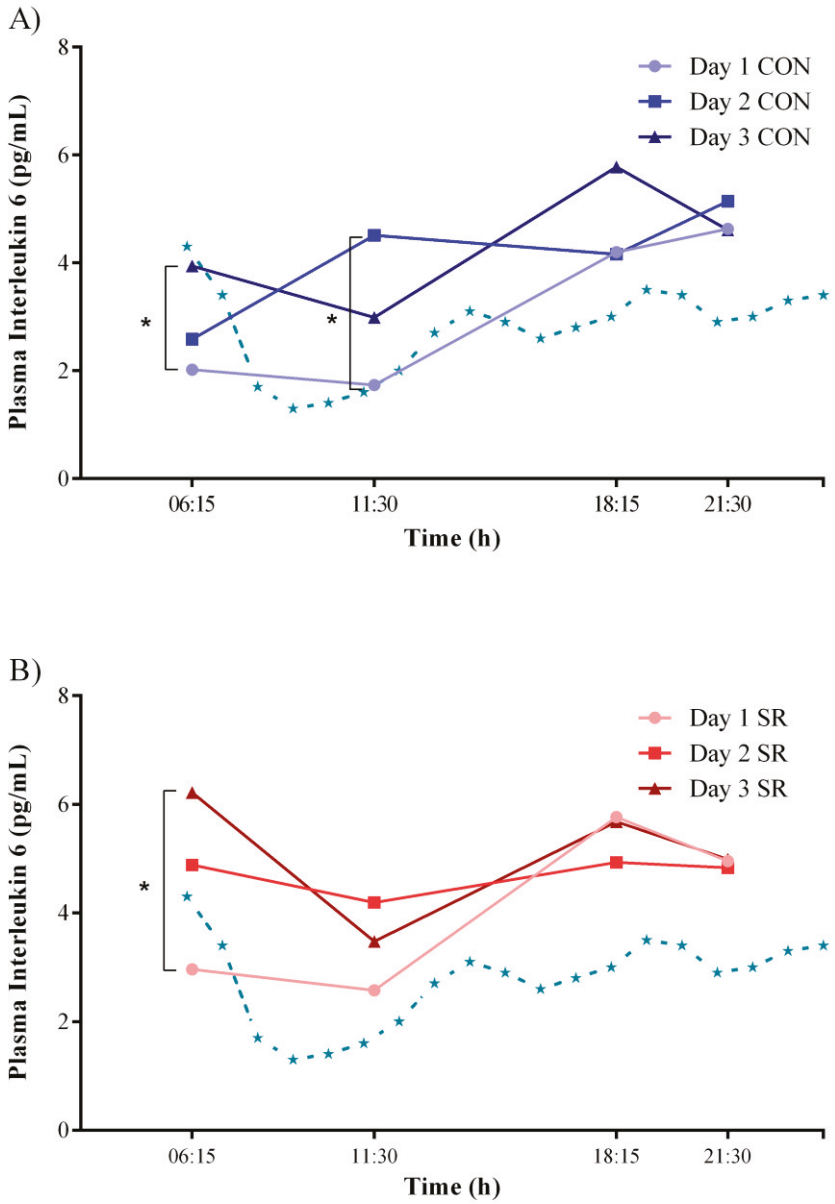

Figure 3.4 Predicted REML means for IL-6 profile across days in CON (A) and SR (B) conditions. Data was log-transformed prior to analysis. For ease of interpretation, values were back transformed to pg/mL. Note: no shortened sleep prior to day 1. Dashed lines represent IL-6 levels for healthy young adults under control conditions (i.e, 8-h sleep opportunity; 51). Significant differences between days within the same condition and time of day are indicated by * ($p < 0.05$).

Food and Caffeine Intake

There was no difference in daily protein and fat intakes between conditions, but differences in carbohydrates ($p = 0.01$), energy ($p = 0.02$) and caffeine ($p = 0.02$) intake were demonstrated. Participants in the SR condition had a lower carbohydrate and energy (both $p = 0.001$) intake on day 3 compared to day 1, while both conditions had lower carbohydrate (SR $p < 0.001$; CON $p = 0.01$) and energy (SR $p < 0.001$; CON $p = 0.001$) intakes on day 3 compared to day 2. Furthermore, participants in the SR condition had lower carbohydrate ($p = 0.01$) and energy ($p = 0.004$) intakes on day 3 compared to this day for the CON condition. Compared to the CON condition, caffeine intake on day 1 and 2 was higher among participants in the SR condition (both $p = 0.01$), but intakes were not different to habitual caffeine consumption for this condition ($p > 0.05$).

Sleep

Sleep duration measured across the 2 nights prior to the study using activity monitors was not significantly different to the adaptation night (i.e., 8-h sleep opportunity) or between conditions (both $p > 0.05$; Table 3.2) and therefore, participants were not sleep restricted before beginning the study. Average total sleep time on the adaptation night was not significantly different between the CON and SR conditions ($p > 0.05$; Table 3.2). The total sleep time on nights 2 and 3 was significantly different, as expected, given the sleep opportunity provided in each condition (Table 3.2).

Table 3.2 Total sleep time (mean ± standard deviation) for each night in both conditions (h)

Night	CON	SR
Pre-study 1	7.3 ± 1.4	6.7 ± 0.9
Pre-study 2	6.7 ± 1.3	6.2 ± 1.4
1 (adaptation)	6.3 ± 0.9	6.4 ± 0.7
2	6.9 ± 0.4	3.6 ± 0.2*
3	6.9 ± 0.5	3.7 ± 0.2*

* = $p < 0.001$ between conditions

Discussion

Higher IL-8 levels were recorded in firefighters who received an 8-h sleep each night when compared to the participants who had their sleep opportunity restricted to 4-h each night. Furthermore, there was an acute increase in IL-6 levels over successive days of firefighting work, irrespective of whether firefighters received an 8-h or 4-h sleep between days.

The higher levels of IL-8 in the 8-h sleep condition (Figure 3.2) may be due to a higher level of physical activity than the sleep restricted condition. While there were no differences in the performance of physical firefighting tasks between conditions, Vincent et al. (36) found that compared to firefighters who had a restricted 4-h sleep, those who had an 8-h sleep displayed: 20.5% greater whole body physical activity (i.e., activity counts) during the rest periods between testing bouts ($p < 0.001$), 5.02% increase in physical activity during the firefighting work circuit ($p < 0.05$) and 11.92% increase across the 2-h testing block ($P < 0.05$) when compared to those who had a 4-h sleep (36). Levels of IL-8 increase in response to long-duration physical activity with

an eccentric contraction component (27). Therefore, a greater amount of physical activity in the CON condition during the rest periods and the work periods (36) may have resulted in elevated IL-8 levels.

The unaltered IL-8 levels in the SR condition are consistent with previous research (52, 53). IL-8 is a bio-marker for cardiovascular health and accumulating evidence links chronically high levels of IL-8 with pathways that may increase the risk of CVD (20, 54). The IL-8 levels observed among the current group of firefighters however, are below those more closely linked with CVD (20, 55) and there is currently no evidence of an increase in cardiovascular mortality (56) or a higher prevalence of CVD risk factors for Australian firefighters when compared to the general population (57). However, in the United States of America (USA), cardiovascular events account for 35% of deaths among all firefighters (i.e., salaried, volunteer and wildland; 58). Furthermore, an analysis of deaths among firefighters in the USA and estimates of time spent in firefighting duties reported that fire suppression activities were associated with an increased risk of death from coronary heart disease (59). Therefore, examining repeated exposures to the acute physical demands involved in wildland firefighting (e.g., over one or more fire seasons) and IL-8 release is warranted to investigate their potential role in the pathogenesis of cardiovascular-related health outcomes.

The increase in firefighters' IL-6 levels across days was irrespective of the duration of sleep between shifts (4-h or 8-h opportunity). Sleep restricted to between 2 and 6-h per night over 5-10 nights, without physical work, has resulted in increased daytime levels of IL-6 (5, 7, 60), TNF-α (5, 6) and IL-1β (61). Collectively, these findings suggest that longer periods of sleep restriction lasting five or more nights, may affect

inflammatory activation, possibly via sleep loss-induced changes to vascular and metabolic function and disrupted sleep architecture (62, 63). Given that firefighters can face extended wildfire deployments (e.g., >5 days; 64), future research should investigate the potential impact of prolonged periods of sleep restriction (i.e., >2 nights) on IL-6, TNF-α and IL-1β (5-7, 60, 61).

The 4-h sleep restriction period did not accentuate the rise in IL-6. Furthermore, the firefighters' IL-6 levels in both conditions were high in comparison to young healthy adults who had an 8-h sleep opportunity and no physical activity (Figure 3.4; 51). But in comparison to healthy adults, firefighters in both conditions had an average BMI classified as overweight (i.e., 25.0 – 29.9 kg/m^2). Given that IL-6 is secreted by adipose tissue (68), testing a potentially overweight group of participants may explain the higher levels of IL-6. However, future research employing the use of more direct body composition measures (e.g., waist circumference and abdominal height) will help further explain the pro-inflammatory state observed among personnel. Alternatively, elevated IL-6 levels in both conditions may indicate that physical work was the major stressor in the current study. In contrast to our findings, Abedelmalek and colleagues (21) reported an increase in IL-6 following a 4.5-h sleep restriction period and four, 250-metre runs on a treadmill at 80% of personal maximal speed. In comparison to the self-paced work in our firefighting simulation, fixed speed work investigated by Abedelmalek et al. (21), although shorter, may have subjected participants to a higher intensity effort. Greater physical demands in combination with sleep restriction may explain why different IL-6 responses were observed between studies. Moreover, while similar durations of sleep restriction were investigated, Abedelmalek et al. (21) examined an early phase sleep opportunity (22:30-03:00). Wu et al. (65) found 4 nights of 3-h early phase sleep restriction caused a larger reduction in rapid-eye-movement

(REM) sleep compared with later-night sleep restriction. Research in animal models indicates that a reduced amount of REM sleep had a positive association with increased IL-6 (66) and TNF-α (63). Firefighting and other occupations (e.g., air crew, train and truck drivers) can require personnel to start work early in the morning, often causing truncated, early sleep phases (67). Accordingly, future research needs to determine how early phase sleep restriction, and the associated changes in sleep architecture, impact the immune system of firefighters' and workers in similar occupations completing physical work.

Morning IL-6 levels in both conditions increased from day 1 to day 3 of testing (Figure 3.4). This finding is in line with Gunderson and colleagues (23) who reported increased morning IL-6 levels among soldiers completing a 7-day training exercise with restricted sleep (1 h sleep per 24 h). However, the reported 11- and 7-fold increase in soldiers' IL-6 levels from day 1 to day 2 and day 4 respectively (23), were higher than the 2-fold increase observed in the current study. The larger increase in soldiers' IL-6 levels (23) could be explained by the long duration semi-continuous physical work (68) and extreme sleep restriction (34) they experienced, which are different to the intermittent physical work and partial sleep restriction typical for wildland firefighting (1) and simulated in the current study. While the observed changes in IL-6 (i.e., 1.9-2.5 pg/mL, Figure 3.4) are toward the low-end of systematic inflammation levels (28, 69), evidence suggests that similar levels of low-grade inflammation in IL-6, if chronic, are sufficient to increase the risk of adverse health outcomes (55, 70). For instance, IL-6 >2.19 pg/mL was associated with an increased risk of all-cause mortality (cardiovascular diseases, cancers and other causes) among older adults across a 9-year period (55). Findings from a 6-year study (70) showed that among healthy men at baseline, IL-6 levels were higher (1.81 pg/mL) in those who experienced a subsequent

myocardial infarction. While firefighters in the USA appear to have a high risk of CHD-related deaths on duty (59), research in Australia is yet to investigate if firefighting demands are associated with cardiovascular events. Further research is needed to understand if wildland firefighters' IL-6 levels are elevated chronically in response to repeated firefighting deployments across a fire season and over multiple fire seasons. Finally, IL-6 and IL-8 can induce white blood cell production (71) and it has been speculated that increased cytokines are responsible for heightened cell counts in CVD and diabetes sufferers (72). Therefore, it is important that future longitudinal studies assess cytokine and blood cell differentials to determine how multiple immune responses interact and potentially impact on health.

Though the change in IL-6 levels was small, there was a significant increase in levels from day 1 to day 3 in both conditions at 06:15 and from day 1 to day 2 in the CON condition at 11:30 (Figure 3.4). While previous firefighting-based research found no change in IL-6 following short bouts of physical work (73), the findings from the current study support exercise and military literature demonstrating significant increases in IL-6 over successive days of both continuous (19) and intermittent (74) physical training. Physical work duration and intensity play a role in regulating IL-6 release (68). Accumulating physical work output/performance across the three days may have contributed to the parallel rise in IL-6 levels in both conditions.

The *ad libitum* consumption of food and drink in the current study adhered to fire-ground practices (35). However, not specifically controlling for nutrient intake is a potential limitation of this study that resulted in differing CHO, energy and caffeine intakes between conditions and testing days. Low or depleted glycogen stores have been associated with an increased immune response following periods of long duration

physical activity (75, 76), which on first inspection, could explain the increased IL-6 response on day 3 in both conditions. While intense periods of physical work can reduce appetite (77), the findings for the SR condition appear in contrast to reports that sleep loss may alter appetite regulation and lead to increased carbohydrate consumption (78). However, the impact of sleep restriction on food intake has been largely investigated in the absence of physical work. In addition, previous energy restriction- and exercise-based studies that have specifically examined macronutrient intake and immune changes (75, 76) indicate that very low levels of carbohydrate (<10% of total energy intake from carbohydrates) during physical activity, with and without sleep loss, are necessary to elicit an acute immune response. In the current study, firefighters' carbohydrate levels were higher in comparison to these studies (56% and 55% of total energy intake from carbohydrates on day 3 in SR and CON respectively; 75, 76) and remained within normal adult levels (79, 80). It is therefore unlikely the lower carbohydrate intake among participants contributed to increased IL-6 levels on day 3. Further, while a high caffeine intake has been shown to increase IL-6 during physical activity (3 to 6 mg/kg body weight; 81), intakes in the current study were much lower (SR 2 mg/kg body weight, CON 1 mg/kg body weight).

Consistent with multi-day exercise training (19), firefighters' IL-6 levels increased across the simulation. Lundeland et al. (24) and Gunderson et al. (23) reported that during a 7-day military training course with minimal sleep, IL-6 levels also increased to day 3 and day 4 respectively, but then decreased towards baseline. Following an increase in IL-6 across the first day of live wildfire suppression work, Main et al. (4) also found IL-6 levels to attenuate over the second day of work. In addition to pro-inflammatory activities, IL-6 has anti-inflammatory properties that lower other pro-inflammatory cytokines to return the immune system to homeostasis (12, 13). For

instance, data suggests that an increased IL-6 response during exercise can exert anti-inflammatory effects that inhibit TNF-α and IL-1β (25-27). The increased levels of IL-6 among firefighters in the current study may have acted to suppress the release of TNF-α and IL-1β, resulting in declining levels of these cytokines across the day (Figure 3.3). Similar to IL-6, the IL-4 cytokine is immuno-modulatory and therefore capable of exerting anti-inflammatory effects that inhibit IL-1β and TNF-α (11). Therefore, increased IL-4 in the evening and at night may further explain the fall in TNF-α and IL-1β (Figure 3.3). Furthermore, compared to the inflammatory response typically related to exercise, severe inflammation (e.g., sepsis) has been associated with a distinctively different cascade of cytokines in the circulation (i.e., TNF-α, IL-1β and IL-6 in that order; 27, 28). Therefore, increased levels of immuno-modulatory cytokines IL-6 and IL-4 among firefighters in the current study, without an increase in TNF-α and IL-1β levels, could indicate a non-damaging adaptive response to the stress of the simulated physical firefighting.

The bidirectional feedback loop between cytokines and cortisol (29) may further explain the acute increase in IL-6 in the current study. Previously we demonstrated that 3 days of physical firefighting work separated by 2 nights of restricted sleep, resulted in an increased cortisol response over successive days of work (30). These findings indicate that the body may be releasing more cortisol to compensate for the added stress of limited sleep while performing physical work. In turn, the increased release of cortisol may be exerting its anti-inflammatory effects (29, 31) and buffering the release of IL-6 in the SR condition. Given that cortisol may down-regulate proteins required for immune cell activation of IL-8 (32), higher cortisol levels previously reported (30) may result in greater inhibition of the IL-8 response among sleep restricted participants. To date, cortisol-induced anti-inflammatory effects for IL-8

have only been demonstrated in lipopolysaccharide (i.e., bacteria)-induced cytokine release (32). Therefore, further research is needed to determine if cortisol has the same mediating impact on plasma IL-8 in response to physical work and sleep restriction. However, the potential for cortisol-induced suppression of IL-6 and possibly IL-8, suggests that the firefighters' physiological stress-related changes were functioning effectively to maintain homeostasis of the immune system in response to simulated firefighting work with sleep restriction.

Conclusion

This is the first wildland firefighting-based study to investigate the effect of sleep restriction and physical work on acute inflammatory stress responses. Findings demonstrate higher levels of IL-8 among participants who received an 8-h sleep each night when compared to those who had a restricted 4-h sleep. Participants' IL-6 levels increased in both conditions suggesting that the sleep restriction duration and/or period may not have been a significant enough stressor to affect this cytokine over and above any disturbance caused by physical work. Considering the immuno-modulatory properties of IL-6 and IL-4 (11, 25-27), the increase of these cytokines and the simultaneous decrease in TNF-α and IL-1β, could indicate a non-damaging response to the stress of simulated physical firefighting work. However, given the link between chronic low-level inflammation of IL-6 and IL-8, and several diseases such as atherosclerosis and Type 2 diabetes (70, 82), further research is needed to determine if wildland firefighters' IL-6 and IL-8 levels are chronically elevated following repeated firefighting deployments across a fire season and over multiple fire seasons.

References

1. Cater H, Clancy D, Duffy K, Holgate A, Wilison B, Wood J. Fatigue on the fireground: the DPI experience. Bushfire Cooperative Research Centre/Australasian Fire Authorities Council Conference Research Forum; 2007; Hobart, Tasmania.
2. Cuddy J, Gaskill S, Sharkey B, Harger S, Ruby B. Supplemental feedings increase self-selected work output during wildfire suppression. Medicine and Science in Sports and Exercise. 2007;39(6):1004-12.
3. Aisbett B, Wolkow A, Sprajcer M, Ferguson SA. "Awake, smoky, and hot": Providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters. Applied Ergonomics. 2012;43(5):916-25.
4. Main L, Raines J, Della Gatta P, Wolkow A, Snow R, Aisbett B. The Stress of Firefighting - Implications for Long-term Health Outcomes. In: Thornton R, Wright L, editors. Bushfire Cooperative Research Centre and Australasian Fire and Emergency Service Authorities Council Research Forum; Perth, Western Australia: Bushfire Cooperative Research Centre; 2013.
5. Vgontzas AN, Zoumakis E, Bixler EO, Lin H, Follett H, Kales A, et al. Adverse effects of modest sleep restriction on sleepiness, performance and inflammatory cytokines. The Journal Of Clinical Endocrinology and Metabolism. 2004;89(5):2119-26.
6. Axelsson J, Rehman J-u, Akerstedt T, Ekman R, Miller GE, Höglund CO, et al. Effects of sustained sleep restriction on mitogen-stimulated cytokines, chemokines and T helper 1/ T helper 2 balance in humans. Plos One. 2013;8(12):e82291-e.
7. Pejovic S, Basta M, Vgontzas AN, Kritikou I, Shaffer ML, Tsaoussoglou M, et al. Effects of recovery sleep after one work week of mild sleep restriction on interleukin-6 and cortisol secretion and daytime sleepiness and performance. American Journal of Physiology - Endocrinology and Metabolism. 2013;305(7):890-6.
8. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. The New England Journal Of Medicine. 1995;332(20):1351-62.
9. Elenkov IJ, Chrousos GP. Stress Hormones, Proinflammatory and Antiinflammatory Cytokines, and Autoimmunity. Annals of the New York Academy of Sciences. 2002;966(1):290-303.
10. Elenkov IJ. Neurohormonal-cytokine interactions: Implications for inflammation, common human diseases and well-being. Neurochemistry International. 2008 1/1/2008;52:40-51.
11. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest. 2000;117(4):1162-72.
12. Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. Journal of Applied Physiology. 2005;98(4):1154-62.
13. Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunology Today. 1997;18(9):428-32.
14. Brown MA, Hural J. Functions of IL-4 and control of its expression. Critical Reviews In Immunology. 1997;17(1):1-32. PubMed PMID: 9034722.
15. Paul WE. Interleukin-4: a prototypic immunoregulatory lymphokine. Blood. 1991;77(9):1859-70. PubMed PMID: 2018830.
16. Padgett D, Glaser R. How stress influences the immune response. Trends in Immunology. 2003;24(8):444-8.

17. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. Circulation. 2004 Jun 1;109(Supplement 1):II2-II10. PubMed PMID: 15173056. Epub 2004/06/03.
18. Chrousos GP. The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. International Journal of Obesity and Related Metabolic Disorders. 2000 Jun;24 (Supplement 2):S50-S5.
19. Nieman DC, Luo BB, Dréau D, Henson DA, Shanely RA, Dew D, et al. Immune and inflammation responses to a 3-day period of intensified running versus cycling. Brain, Behavior, and Immunity. 2014;39:180-5.
20. Inoue T, Komoda H, Nonaka M, Kameda M, Uchida T, Node K. Interleukin-8 as an independent predictor of long-term clinical outcome in patients with coronary artery disease. International journal of cardiology. 2008 Mar 14;124(3):319-25. PubMed PMID: 17442429. Epub 2007/04/20.
21. Abedelmalek S, Souissi N, Chtourou H, Denguezli M, Aouichaoui C, Ajina M, et al. Effects of Partial Sleep Deprivation on Proinflammatory Cytokines, Growth Hormone, and Steroid Hormone Concentrations During Repeated Brief Sprint Interval Exercise. Chronobiology International: The Journal of Biological and Medical Rhythm Research. 2013;30(4):502-9.
22. Bøyum A, Wiik P, Gustavsson E, Veiby OP, Reseland J, Haugen AH, et al. The effect of strenuous exercise, calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines. Scandinavian Journal Of Immunology. 1996;43(2):228-35.
23. Gundersen Y, Opstad PK, Reistad T, Thrane I, Vaagenes P. Seven days' around the clock exhaustive physical exertion combined with energy depletion and sleep deprivation primes circulating leukocytes. European Journal of Applied Physiology. 2006;97(2):151-7. PubMed PMID: 10139971.
24. Lundeland B, Gundersen Y, Opstad PK, Thrane I, Zhang Y, Olaussen RW, et al. One week of multifactorial high-stress military ranger training affects Gram-negative signalling. Scandinavian Journal of Clinical and Laboratory Investigation. 2012;72(7):547-54. PubMed PMID: 82336387.
25. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. The Federation of American Societies for Experimental Biology (FASEB) Journal. 2003;17(8):884-6.
26. Pedersen BK, Fischer CP. Beneficial health effects of exercise--the role of IL-6 as a myokine. Trends in Pharmacological Sciences. 2007;28(4):152-6.
27. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiological Reviews. 2008;88(4):1379-406.
28. Bruunsgaard H. Physical activity and modulation of systemic low-level inflammation. Journal of Leukocyte Biology. 2005;78(4):819-35.
29. Turnbull AV, Rivier CL. Regulation of the Hypothalamic-Pituitary-Adrenal Axis by Cytokines: Actions and Mechanisms of Action. Physiological Reviews. 1999;79(1):1-71. PubMed PMID: 1233803.
30. Wolkow A, Aisbett B, Ferguson SA, Reynolds J, Main LC. Effects of sleep restriction on cortisol during simulated physical firefighting work. Fourth International Conference on Health Wellness, and Society; 2014 14-15 March; Vancouver, Canada.
31. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Annals of the New York Academy of Sciences. 2012;1261(1):55-63.

32. Corsini E, Pinto A, Galbiati V, Viviani B, Galli CL, Marinovich M, et al. Corticosteroids modulate the expression of the PKC-anchoring protein RACK-1 and cytokine release in THP-1 cells. Pharmacological Research: The Official Journal Of The Italian Pharmacological Society. 2014;81:10-6.
33. Phillips M, Payne W, Lord C, Netto K, Nichols D, Aisbett B. Identification of physically demanding tasks performed during bushfire suppression by Australian rural firefighters. Applied Ergonomics. 2012;43(2):435-41.
34. Shearer WT, Reuben JM, Mullington JM, Price NJ, Lee BN, Smith EO, et al. Soluble TNF-alpha receptor 1 and IL-6 plasma levels in humans subjected to the sleep deprivation model of spaceflight. The Journal Of Allergy And Clinical Immunology. 2001;107(1):165-70. PubMed PMID: 11150007.
35. Raines J, Snow R, Petersen A, Harvey J, Nichols D, Aisbett B. The effect of prescribed fluid consumption on physiology and work behavior of wildfire fighters. Applied Ergonomics. 2013;44(3):404-13. PubMed PMID: 23149116.
36. Vincent G, Ferguson SA, Larsen B, Wolkow A, Tran J, Aisbett B. Sleep restriction during simulated wildfire suppression: effect on physical task performance Plos One. 2015;10(1).
37. Ferguson SA, Aisbett B, Jay SM, Onus K, Lord C, Sprajcer M, et al. Design of a valid simulation for researching physical, physiological and cognitive performance in volunteer firefighters during bushfire deployment. In: Thornton R, editor. Bushfire Cooperative Research Centre/ Australasian Fire and Emergency Service Authorities Council Conference Research Forum; Sydney, New South Wales; 2011. p. 196-204.
38. Phillips M, Netto K, Payne W, Nichols D, Lord C, Brooksbank N, et al. Frequency, intensity and duration of physical tasks performed by Australian rural firefighters during bushfire suppression. In: Thornton RP, editor. Bushfire Cooperative Research Center/Australasian Fire Authorities Council Conference Research Forum; Sydney, New South Wales; 2011. p. 205-213.
39. Aisbett B, Phillips M, Raines J, Nichols D, Work patterns of tanker-based bushfire suppression by Australian volunteer firefighters in south-east Australia. Human Dimensions of Wildfire Conference; 2007; Fort Collins, Colorado.
40. Cullen T, Thomas AW, Webb R, Hughes MG. The relationship between interleukin-6 in saliva, venous and capillary plasma, at rest and in response to exercise. Cytokine. 2015 (2):397-400. PubMed PMID: edsgcl.397663920.
41. Eriksson M, Sartono E, Martins CL, Bale C, Garly ML, Whittle H, et al. A comparison of ex vivo cytokine production in venous and capillary blood. Clinical and experimental immunology. 2007 Dec;150(3):469-76.
42. Faulkner SH, Spilsbury KL, Harvey J, Jackson A, Huang J, Platt M, et al. The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise. European Journal of Applied Physiology. 2014 Jun;114(6):1207-16.
43. Zhou X, Fragala MS, McElhaney JE, Kuchel GA. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. Current Opinion in Clinical Nutrition and Metabolic Care. 2010;13(5):541-7.
44. Findlay JW, Smith WC, Lee JW, Nordblom GD, Das I, DeSilva BS, et al. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. Journal Of Pharmaceutical And Biomedical Analysis. 2000;21(6):1249-73. PubMed PMID: 10708409.

45. Chowdhury F, Williams A, Johnson P. Validation and comparison of two multiplex technologies, Luminex and Mesoscale Discovery, for human cytokine profiling. Journal Of Immunological Methods. 2009;340(1):55-64.
46. Nguyen HP, Björkqvist M, Bode FJ, Stephan M, von Hörsten S. Serum levels of a subset of cytokines show high interindividual variability and are not altered in rats transgenic for Huntington´s disease. Plos Currents Huntington Disease. 2010;2:1-5. PubMed PMID: 20981129.
47. Payne R, Welham S, Harding S. A Guide to REML in GenStat (16th Edition). Hempstead, Hertfordshire, UK: VSN International; 2011.
48. Verbyla AP, Cullis BR, Kenward MG, Welham SJ. The Analysis of Designed Experiments and Longitudinal Data by Using Smoothing Splines. Journal of Applied Statistics. 1999;48(3):269-311.
49. Burnham KP, Anderson DR. Model selection and multi-model inference : a practical information-theoretic approach / Kenneth P. Burnham, David R. Anderson. 2nd ed. London, United Kingdom: Springer; 2002.
50. Hsu JC. Multiple Comparisons Theory and Methods. London, United Kingdom: Chapman and Hall; 1996.
51. Vgontzas AN, Zoumakis M, Papanicolaou DA, Bixler EO, Prolo P, Lin HM, et al. Chronic insomnia is associated with a shift of interleukin-6 and tumor necrosis factor secretion from nighttime to daytime. Metabolism - Clinical and Experimental. 2002;51(7):887-92.
52. Faraut B, Boudjeltia KZ, Dyzma M, Rousseau A, David E, Stenuit P, et al. Benefits of napping and an extended duration of recovery sleep on alertness and immune cells after acute sleep restriction. Brain, Behavior, and Immunity. 2011;25(1):16-24.
53. Boudjeltia KZ, Faraut B, Esposito MJ, Stenuit P, Dyzma M, Antwerpen PV, et al. Temporal dissociation between myeloperoxidase (MPO)-modified LDL and MPO elevations during chronic sleep restriction and recovery in healthy young men. Plos One. 2011;6(11):e28230-e. PubMed PMID: 22140557.
54. Apostolakis S, Vogiatzi K, Amanatidou V, Spandidos DA. Interleukin 8 and cardiovascular disease. Cardiovascular Research. 2009;84(3):353-60.
55. Bernhard BT, Rothermundt M, Ladwig K, Meisinger C, Berger K, . Systemic inflammation (Interleukin 6) predicts all-cause mortality in men: results from a 9-year follow-up of the MEMO Study. Age. 2011;33(2):209-17.
56. Glass D, Sim M, Pircher S, Del Monaco A, Dimitriadis C, Miosge J, et al. Australian Firefighters' Health Study. Melbourne, Victoria.: Monash Centre for Occupational and Environmental Health, 2014.
57. Wolkow A, Netto K, Langridge P, Green J, Nichols D, Sergeant M, et al. Coronary Heart Disease Risk in Volunteer Firefighters in Victoria, Australia. Archives of Environmental and Occupational Health. 2014;69(2):112-20.
58. United States Fire Administration, National Fire Programs, National Fallen Firefighters Foundation. Firefighter Fatalities in the United States in 2013. 2014.
59. Kales SN, Soteriades ES, Christophi CA, Christiani DC, . Emergency duties and deaths from heart disease among firefighters in the United States. The New England Journal Of Medicine. 2007;356(12):1207-15.
60. Haack M, Sanchez E, Mullington JM. Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. Sleep. 2007;30(9):1145-52.

61. van Leeuwen WMA, Lehto M, Karisola P, Lindholm H, Luukkonen R, Sallinen M, et al. Sleep Restriction Increases the Risk of Developing Cardiovascular Diseases by Augmenting Proinflammatory Responses through IL-17 and CRP. Plos One. 2009;4(2):1-7. PubMed PMID: 55666635.
62. Mullington J, Simpson N, Meier-Ewert H, Haack M. Sleep loss and inflammation. Best Practice and Research Clinical Endocrinology and Metabolism. 2010;24:775-84.
63. Yehuda S, Sredni B, Carasso RL, Kenigsbuch-Sredni D. REM Sleep Deprivation in Rats Results in Inflammation and Interleukin-17 Elevation. Journal of Interferon and Cytokine Research. 2009;29(7):393-8.
64. Ruby B, Shriver T, Zderic T, Sharkey B, Burks C, Tysk S. Total energy expenditure during arduous wildfire suppression. Medicine and Science in Sports and Exercise. 2002;34(6):1048-54.
65. Wu H, Stone WS, Hsi X, Zhuang J, Huang L, Yin Y, et al. Effects of Different Sleep Restriction Protocols on Sleep Architecture and Daytime Vigilance in Healthy Men. Physiological Research. 2010;59(5):821-9.
66. Pandey AK, Kar SK. REM sleep deprivation of rats induces acute phase response in liver. Biochemical and Biophysical Research Communications. 2011;410(2):242-6. PubMed PMID: edsgcl.260344462.
67. Åkerstedt T, Kecklund G, Selén J. 'Early Morning Work—Prevalence And Relation To Sleep/Wake Problems: A National Representative Survey. Chronobiology International: The Journal of Biological and Medical Rhythm Research. 2010;27(5):975-86. PubMed PMID: 52301468.
68. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB JOURNAL. 2002;16(11):1335-47.
69. Brüünsgaard H, Pedersen B. Age-related inflammatory cytokines and disease. Immunology And Allergy Clinics Of North America. 2003;23(1):15-39.
70. Ridker PM, Rifai N, Stampfer M.J, Hennekens CH. Plasma Concentration of Interleukin-6 and the Risk of Future Myocardial Infarction Among Apparently Healthy Men. Circulation. 2000;101:1767-72.
71. Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, et al. Elevated white blood cell count in subjects with impaired glucose tolerance. Diabetes Care. 2004;27(2):491-6. PubMed PMID: 14747234.
72. Farhangi MA, Keshavarz SA, Eshraghian M, Ostadrahimi A, Saboor-Yaraghi AA. White blood cell count in women: relation to inflammatory biomarkers, haematological profiles, visceral adiposity, and other cardiovascular risk factors. Journal of Health, Population and Nutrition. 2013;31(1):58-64.
73. Huang C-J, Webb HE, Garten RS, Kamimori GH, Evans RK, Acevedo EO. Stress hormones and immunological responses to a dual challenge in professional firefighters. International Journal of Psychophysiology. 2010;75(3):312-8.
74. Laskowski R, Ziemann E, Olek RA, Zembron-Lacny A. The Effect of Three Days of Judo Training Sessions on the Inflammatory Response and Oxidative Stress Markers. Journal of Human Kinetics. 2011;30(65-73):65-73.
75. Bishop NC, Walsh NP, Haines DL, Richards EE, Gleeson M. Pre-Exercise Carbohydrate Status and Immune Responses to Prolonged Cycling: II. Effect on Plasma Cytokine Concentration. International Journal of Sport Nutrition and Exercise Metabolism. 2001;11(4):503-12. PubMed PMID: 6626299.

76. Miles MP, Walker EE, Conant SB, Hogan SP, Kidd JR. Carbohydrate Influences Plasma Interleukin-6 But Not C-Reactive Protein or Creatine Kinase Following a 32-Km Mountain Trail Race. International Journal of Sport Nutrition and Exercise Metabolism. 2006;16(1):36-46.
77. Fry RW, Grove JR, Morton AR, Zeroni PM, Gaudieri S, Keast D. Psychological and immunological correlates of acute overtraining. / Correlation psychologique et immunologique du surentrainement intense. British Journal of Sports Medicine. 1994;28(4):241-6.
78. Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Annals of Internal Medicine. 2004 Dec 7;141(11):846-50.
79. Food and Agricultural Organization. Report on human energy requirements. Rome: FAO, 2004.
80. National Health and Medical Research Council. Eat for health - Australian Dietary Guidelines. Canberra, Australian Capital Territory: 2013.
81. Tauler P, Martinez S, Moreno C, Monjo M, Martinez P, Aguilo A. Effects of caffeine on the inflammatory response induced by a 15-km run competition. Medicine and Science in Sports and Exercise. 2013;45(7):1269-76.
82. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Journal of the American Medical Association. 2001;286(3):327-34.

Chapter 4: Study 2

Study 2

The impact of sleep restriction while performing simulated physical firefighting work on cortisol and heart rate responses

Alexander Wolkow BExSc (Hons), Brad Aisbett PhD, John Reynolds PhD,

Sally Ferguson PhD, and Luana C Main PhD

Published in

International Archives of Occupational and Environmental Health, 2015,

doi: 10.1007/s00420-015-1085-3

Presented here in journal submission format

Abstract

Purpose: Physical work and sleep restriction are two stressors faced by wildland firefighters, yet the combined impact these demands have on firefighters' acute stress responses is poorly understood. The purpose of the present study was to assess the effect firefighting work and sleep restriction has on firefighters' acute cortisol and heart rate (HR) responses during a simulated 3 day and 2 night fire-ground deployment.

Methods: Firefighters completed multiple days of simulated physical work separated by either an 8-h (Control condition; n=18) or 4-h sleep opportunity (Sleep restriction condition; n=17). Salivary cortisol was sampled every 2-h and HR was measured continuously each day.

Results: There were no differences in cortisol between conditions on day 1. However, on day 2 and day 3 the sleep restriction condition exhibited a significantly higher daily area under the curve cortisol level and an elevated cortisol profile in the afternoon and evening when compared with the control condition. Firefighters' HR decreased across the simulation in both conditions.

Conclusion: Findings highlight the protective role an 8-h sleep opportunity between shifts of firefighting work has on preserving normal cortisol levels when compared to a 4-h sleep opportunity which resulted in elevated afternoon and evening cortisol. Given the adverse health outcomes associated with chronically high cortisol, especially later in the day, future research should examine how prolonged exposure to firefighting work (including restricted sleep) affects firefighters' cortisol levels long-term. Furthermore, monitoring cortisol levels post-deployment will determine the minimum recovery time firefighters need to safely return to the fire-ground.

Introduction

Firefighters face a number of occupational stressors in the line of duty. Two common stressors for firefighters performing wildfire suppression are physical work and sleep restriction (Aisbett et al. 2007; Cater et al. 2007; Cuddy et al. 2007). For instance, existing research demonstrates that wildlnd firefighters often work 3 to 4 consecutive long shifts, separated by shortened sleep opportunities (i.e., 3 to 6 h; Cater et al. 2007). Within shifts, firefighting or other emergency response duties (e.g., vehicle accidents and rescues) can involve high-intensity physical work performed intermittently for extended periods (i.e., 12 to 16 h; Aisbett et al. 2007; Cuddy et al. 2007).

Exposure to occupational stressors such as physical work or sleep restriction can trigger acute neuroendocrine stress responses. These include the release of cortisol from the hypothalamic-pituitary-adrenal (HPA)-axis (Chandola et al. 2010; Faulkner et al. 2014; Tsigos and Chrousos 2002) and the secretion of catecholamines (epinephrine and norepinephrine) caused by activation of the sympathetic-adrenal-medullary (SAM) system (Chandola et al. 2010; Faulkner et al. 2014). The release of catecolamines can reduce activity of the parasympathetic nervous system, resulting in an increased heart rate (HR; Chandola et al. 2010; Faulkner et al. 2014). Under normal circumstances activation of these physiological functions is expected to occur, however exposure to particularly intense, or long periods of occupational-related stress over multiple days can exacerbate and dysregulate the HPA-axis and SAM system response resulting in the sustained elevation of cortisol (Chandola et al. 2008; Wolkow et al. 2015) and HR (Meier-Ewert et al. 2004; van Leeuwen et al. 2009). Chronic dysregulation of these systems has been associated with adverse health outcomes such as cardiovascular disease (CVD) and depression (Chandola et al. 2008; Mackin and Young 2004; Silverman and Sternberg 2012; Tanskanen et al. 2011), both prevalent in

firefighting populations (An et al. 2015; Carey et al. 2011; Cook and Mitchell 2013; Kales et al. 2007; Wolkow et al. 2014). As occupational stressors pose a potential risk to firefighters' health, there is a need to understand their effect on neuroendocrine responses in order to inform future evidence-based recommendations aimed at ensuring the safety of firefighting personnel.

Shortened sleep and sleep disturbances are frequently reported among firefighters and personnel in other physically demanding occupations where shift work is common (e.g. military, police; Carey et al. 2011; Neylan et al. 2002). Despite this, no firefighting and only one military-based study (Goh et al. 2001) has investigated how reduced sleep impacts cortisol or HR responses among personnel. For instance, afternoon cortisol levels increased among soldiers following a night of total sleep deprivation (Goh et al. 2001), while further, non-emergency based research indicates that, in the absence of physical work, partial sleep restriction over multiple nights can increase cortisol in the afternoon/evening (Buxton et al. 2010; Leproult et al. 1997; Reynolds et al. 2012; Spiegel et al. 1999). Consecutive nights of restricted sleep have also resulted in elevated daily resting HR levels (Meier-Ewert et al. 2004; van Leeuwen et al. 2009). This indicates that exposure to sleep restriction could be a significant stressor for emergency workers that may contribute to a simultaneous dysregulation of cortisol and HR. The cumulative effects may pose a risk to chronic health outcomes (Chandola et al. 2008; Faulkner et al. 2014; Mackin and Young 2004; Silverman and Sternberg 2012; Tanskanen et al. 2011) and thus the impact of sleep restriction and physical work on HR and cortisol among firefighters requires further investigation.

Research in emergency service cohorts (e.g., firefighting, military) and the general population has investigated the individual impact of physical work (Budd et al. 1997; Fahs et al. 2011; Glickman-Weiss et al. 1995; Perroni et al. 2009; Rodríguez-Marroyo et al. 2012; Smith et al. 2005; Taylor et al. 2008) and sleep restriction (Meier-Ewert et al. 2004; van Leeuwen et al. 2009) on acute cortisol and HR responses. However, wildfire suppression work typically exposes personnel to a combination of these demands over consecutive shifts (Aisbett et al. 2012; Cater et al. 2007; Cuddy et al. 2007). Little is known regarding the impact shortened sleep opportunities between long shifts of intense physical work have on firefighters' acute cortisol and HR responses. Currently, the only insights come from exercise (Myles 1987; Neylan 2008) and sustained military operation studies (Fellmann et al. 1992; Opstad 1994; Opstad and Aakvaag 1981). Findings indicate increased morning and evening cortisol levels among military personnel completing extended periods of near continuous physical work with minimal sleep, in combination with other stressors (e.g., energy restriction; Fellmann et al. 1992; Opstad 1994; Opstad and Aakvaag 1981). Alternatively, unchanged (Cullen et al. 2015) or decreased (Dabrowski et al. 2012; Myles 1987; Neylan 2008) HR responses have been demonstrated during extended periods of exercise and complete or extreme sleep restriction.

While exercise and military-based studies provide the only research in this area, total sleep deprivation and the sustained physical activity investigated are different to the intermittent physical work, manual handling tasks (e.g., lifting and lowering hoses and firefighting tools) and partial sleep restriction experienced in wildland firefighting (Aisbett et al. 2012; Ferguson et al. 2011; Phillips et al. 2012). Different demands may elicit different hormonal and HR responses. For instance, complete sleep deprivation can result in greater evening cortisol levels when compared to partial sleep restriction

(Leproult et al. 1997). Therefore, extrapolating findings from exercise settings or occupations with a different work profile to wildland firefighting may under or overestimate the potential stress related implications of wildland firefighting and lead to inappropriate recommendations regarding the management of risk associated with sleep in the field. Accordingly, more comprehensive research is required to understand the impact sleep restriction while performing physical firefighting work specifically, may have on firefighters' acute stress responses. The aim of the present study was to assess the effect that restricted sleep has on firefighters' cortisol and HR responses during 3 days and 2 nights of simulated physical firefighting work.

Methods

Participants

Rural firefighters from agencies across Australia (Victoria, South Australia, New South Wales, Australian Capital Territory and Tasmania) were recruited for this study (n=35). Including firefighters from most state and territory-based fire agencies maximised the generalisability of potential findings to rural firefighters Australia-wide. For statistical purposes, participants were matched by sex, age and body mass index (BMI) and then randomly allocated to either a control (CON) or sleep restriction (SR) condition based on the participant's availability to attend the testing period, designated at random, to the condition. The randomization procedure took into account differences in firefighters' availability to volunteer their time (without receiving financial compensation), thereby ensuring an equal chance for each participant of being allocated to either condition. One firefighter in the SR condition withdrew and therefore, a final sample of 17 firefighters in the SR group and 18 firefighters in the CON group completed the study and were included in the final analyses. There were no differences in age ($F = 0.829$, $P = 0.913$), sex ($F = 0.663$, $P = 0.689$), firefighting

experience ($F = 0.363$, $P = 0.593$) or BMI ($F = 0.167$; $P = 0.113$) between conditions (Table 4.1). All participants were screened and excluded from testing if they had a diagnosed heart disease or untreated sleep disorder in the 6 months prior to testing. In addition, participants completed pre- and post-testing health questionnaires to exclude participants that may have sustained an injury or illness prior, or during testing that could influence the stress markers measured. Although injury, illness and health outcome data were collected from participants pre- and post-study, this data was used for exclusion purposes only, and therefore will not be described in further detail. This study was approved by the Institutions' Human Research Ethics Committee and written informed consent was provided by all participants prior to data collection.

Protocol

Upon arrival at the testing facility, participants in both conditions completed a familiarisation session of all physical work, cognitive and physiological tests followed by a sleep adaptation night (8-h sleep opportunity in the testing environment). Participants in both conditions were then tested over a 3-day and 2-night simulated fire-ground tour. On each of the 2 nights, participants in the CON condition had an 8-h sleep opportunity (i.e., 22:00-06:00). In comparison, participants in the SR condition had their bed time delayed, resulting in a 4-h sleep opportunity (i.e., 02:00-06:00) on each of the 2 nights (Figure 4.1). Participants in the SR condition were free to perform sedentary leisure activities (e.g., watching television, reading etc.) until the delayed bedtime (i.e., 02:00). The duration of partial sleep deprivation (i.e., 4-h) was based on Australian firefighters' self-reported average sleep per rest period on the fire-ground (Cater et al. 2007). Following the completion of the 3-day testing period, participants had an 8-h recovery sleep before leaving the testing facility. In both conditions, the

testing environment was maintained at mild temperatures (18-20^{o}C) with normal room light (i.e., windows blacked out) during the wake period.

The timing of meals and the types of food and fluid available for consumption during testing were standardised across conditions. Similar to the fire-ground, food and drink intake during the study was *ad libitum.* Throughout testing, the types and quantities of ingested food and fluid were recorded and food and drink data was analysed using the FoodWorks 7 nutrition software (2012 Xyris Software Pty Ltd, Australia). Total fluid intake was recorded and not found to display differences between conditions ($P > 0.05$). However, differences in caffeine intake (i.e., coffee and tea) have been reported in the results section along with energy and macronutrient intakes.

Table 4.1. Demographic characteristics for participants in CON and SR conditions

Characteristic	CON (n = 18)	SR (n = 17)
Age (years)	39 ± 16	39 ± 15
Men:Women (n)	15:3	15:2
Weight (kg)	84.9 ± 17.8	93.8 ± 20.2
Height (cm)	178.1 ± 7.7	177.8 ± 7.4
BMI (kg/m^2)	26.8 ± 5.0	29.6 ± 5.5
Firefighting experience (years)	6 (min-max 1.0-39.0)	10 (min-max 1.0-20.0)

Note: BMI = Body Mass Index; Age, Weight, Height and BMI are presented as mean ± standard deviation; Firefighting experience is presented as median years and minimum-maximum years.

Experimental Procedures

Participants in both conditions were tested in groups of 3 to 5 over the firefighting simulation. During the wake period, participants completed a 2-h testing block 3 times on day 1 and 5 times on day 2 and day 3 (Figure 4.1). Each testing block consisted of 55 minutes of simulated physical firefighting work circuit, 20-25 minutes of physiological data collection, 20-25 minutes of cognitive testing (to be reported elsewhere) and a 15-20 minute rest period. The time allocated to physical work and non-physical data collection described above is reflective of physical activity patterns observed across a shift on the fire-ground (Aisbett et al. 2007; Phillips et al. 2011; Raines et al. 2013). Participants' saliva was collected for the determination of cortisol prior to the first 2-h testing block, at the completion of each 55-minute physical work circuit and at additional pre-determined time points throughout the wake periods (Figure 4.1). In addition, participants' HR was monitored continuously throughout each of the 2-h testing blocks.

Simulated Physical Firefighting Work Circuit: The physical work circuit comprised 6 simulated firefighting tasks designed to mimic the physical demands involved in wildfire suppression work performed by each of Australia's state and territory-based fire agencies. These tasks have been recognized by industry experts and incumbent firefighters as being representative of repetitive lift, and carry and drag movements that comprise key wildland firefighting tasks frequently performed on the fire-ground (Ferguson et al. 2011). The six physical tasks included; rake hoe work, charged hose advance, black out hose work, hose rolling and lateral repositioning and static hold of a hose. These tasks were chosen because they were the most physically demanding; deemed to have the highest operational importance; and were the longest, most intense, or most frequently occurring tasks during wildfire suppression work (Phillips et al.

2015a; Phillips et al. 2012). The average intensity for several of the physical work tasks involved in this study have been previously characterised in terms of mean absolute VO_2 (L/min) and HR (beats/min; Phillips et al. 2015b). For instance, rake hoe work elicited a mean VO_2 of 2.6 ± 0.3 L/min and HR of 162 ± 15 beats/min, charged hose advance elicited a mean VO_2 of 2.1 ± 0.6 L/min and HR of 141 ± 18 beats/min and black out hose work elicited a mean VO_2 of 1.6 ± 0.3 ml/kg/min and HR of 129 ± 14 beats/min (Phillips et al. 2015b). Each of the six tasks involved in the physical work battery were completed in a pre-devised circuit consisting of task work to rest ratios designed to mimic the performance of these tasks on the fire-ground (Phillips et al. 2015a; Phillips et al. 2012). The performance of each physical task (i.e., repetitions completed within each work period) was self-paced and therefore, a changing variable. Although the performance of physical tasks was not the focus of this study, it is possible performance on the circuit (i.e., repetitions) could interact with the stress responses measured and vice versa. Therefore, work was recorded, but no differences in physical work performance were demonstrated between the CON and SR conditions (findings reported in previous study; Vincent et al. 2015).

Saliva Sampling and Cortisol Analysis: Salivary cortisol samples were collected using a cotton swab (Salivette; Sarstedt, Nümbrecht, Germany) at baseline each morning (i.e., 07:30) and at the completion of each physical work circuit (i.e., 09:00, 11:15, 13:30, 15:30, 17:30). Additional daily samples for both conditions were taken following wakening (i.e., 06:30) and in the evening (i.e., 19:30, 21:30; Figure 4.1). The number of samples and sampling time points have been determined to reliably assess the change in diurnal cortisol levels (Golden et al. 2011). The number and timing of samples also allowed for the calculation of the Area Under the cortisol Curve (AUC) with respect to ground for each participant on each day in both conditions using

the trapezoidal rule (Pruessner et al. 2003). Salivary cortisol is a convenient and minimally-invasive collection method that provides a valid and reliable measure of the biologically active unbound form of cortisol in the body (Golden et al. 2011; Kirschbaum and Hellhammer 1994). To prevent sample contamination from acidic or high sugar food and/or fluid intake, participants were not allowed to eat or drink 15 minutes prior to saliva collection. Samples were centrifuged for 10 minutes at 5000 revolution/minutes (83 hertz) and stored at ≤ -80°C. Salivary cortisol concentration was determined using a high sensitivity enzyme immunoassay ELISA kit (IBL International, Hamburg, Germany). The assay was performed according to the manufacturer's directions and read at 450 nm on a luminescence microplate reader (Synergy™ 2 SL, BioTek, Winooski, VT). Analytical sensitivity (lower limit of detection) was 0.14 nmol/L, accuracy was 5.2% and intra- and inter-assay precision coefficient of variations (CV) were 7.2% and 10.7% (both mean 13.8 nmol/L) respectively, which are each within their acceptable ranges (i.e., Accuracy <15%; Intra-assay CV <10%; Inter-assay CV <15%; Biopharmaceutics Coordinating Committee 2001; Nicolson 2008)

Heart Rate: Participants' HR was monitored in beats per minute (beats/min) continuously each testing day using a HR strap and transmitter (Polar, Kempele, Finland). Data collection began at 12:00 on day 1 and 08:00 on day 2 and day 3, and continued though to 17:30 each day. Participants' HR was sampled in 5-second epochs and averaged over 5-minute intervals.

Sleep Monitoring: Participants' sleep was recorded using the Siesta Portable EEG system (Compumedics E-Series; Melbourne, Victoria, Australia) and standard polysomnographic (PSG) montage. PSG recording began each night at 21.00 for both

conditions. From each sleep period, participants' total sleep time (minutes) was calculated. In addition, participants wore activity monitors (*Actical* MiniMitter/Respironics, Bend, OR, USA) to measure sleep across the 2 nights prior to the study.

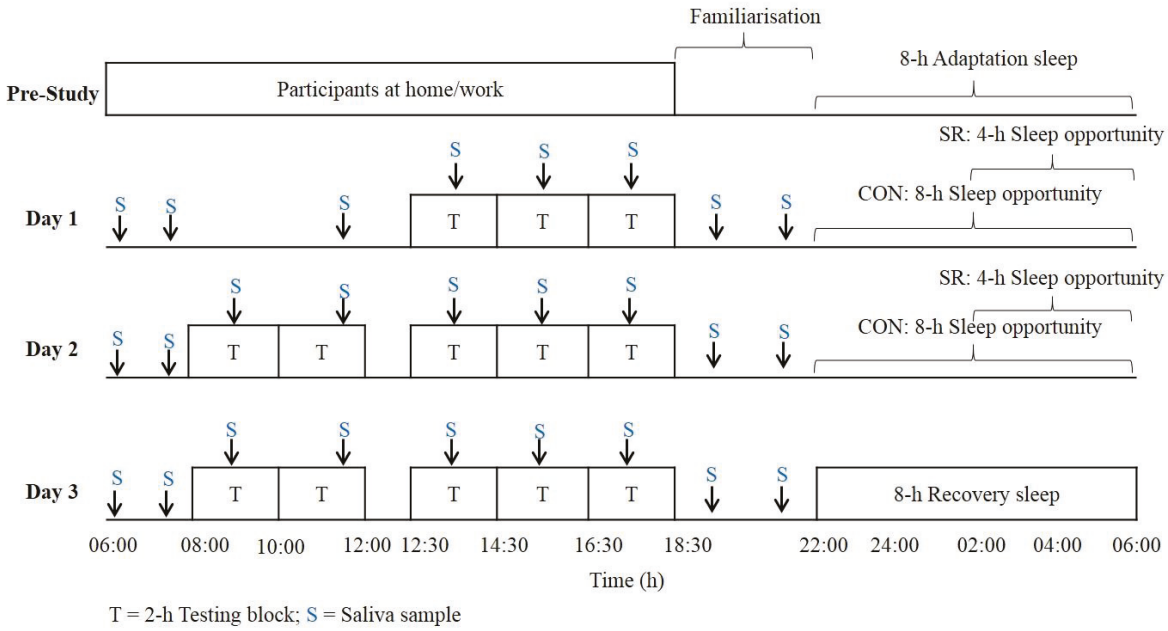

Figure 4.1 Firefighting work protocol for CON and SR condition

Statistical Analysis

Prior to analysis, salivary cortisol measurements were adjusted using a natural log transformation to achieve normality, as assessed using the Shapiro Wilk tests ($P > 0.05$). Homogeneity of variance and normality of the residuals from the resulting mixed model analysis were further assessed and the resultant diagnostic plots revealed no departures from these required assumptions (Field 2009). Due to the sampling design, a single cortisol measurement at 09:00 was missing on day 1 for all participants. Consequently, the missing combinations were appended to the data, but missing value codes were associated with these additional records. This did not affect the hypothesis tests and estimates for the cortisol profile, but it facilitated the fitting of various models for the autocorrelated errors.

Variables measured just once on an individual, or aggregated over occasions (e.g., sleep duration, demographic characteristics) were analysed with Analysis of Variance (ANOVA) using GenStat software (GenStat, 16.1 Edition). For repeated measurements which included cortisol, HR and food and fluid intake, linear mixed models (LMM) were fitted by the restricted maximum likelihood (REML) method using GenStat software (GenStat 16.1 Edition; Payne et al. 2011). The LMM approach was used to investigate if participants' individual cortisol and HR profiles and cortisol AUC differed between conditions. The method also allows for the possibility of autocorrelation in the repeated measurements (i.e., samples and/or days) on each individual by including a model for the covariance structure. In addition, differences between the groups in their linear trends and deviations from linearity were investigated via the incorporation of smoothing splines for the cortisol and HR profile data (Gurrin et al. 2005; Verbyla et al. 1999). Model fit was assessed by the Akaike Information Criterion (AIC). Small differences (ΔAIC) in values of this criterion

compared to the minimum observed value in a set of candidate models, were used to identify parsimonious models (Burnham and Anderson 2002). Pairwise comparisons of the predicted means, constructed from the linear models fitted by the REML analysis, were also conducted (Hsu 1996) to examine differences in the cortisol profile and AUC at each sample (or time point) and/or day. Standard errors of the predicted means were calculated and the corresponding lower limit and an upper limit for the mean (i.e., mean ± standard errors of the mean; SEM) was determined. For ease of interpretation, back-transformations of the means to nmol/L ± standard errors (asymmetric) bars are presented graphically. Significant comparisons of HR between days within the same time were investigated using least significant differences (1.96 × max standard error of difference; SED).

The fixed effects in the models fitted to the individual cortisol and HR profile data were condition (CON or SR), day (day 1, day 2 or day 3) and time of day along with interactions of condition by day, condition by time, day by time and condition by day by time. For cortisol, random effects of group, profile (or subject), and a group by profile interaction were investigated with and without a power model for the within-subject autocorrelation. Random effects of subject and a subject by day interaction were investigated for HR with and without the inclusion of the autoregressive function (AR1). Finally, the REML procedure (Payne et al. 2011) was used to determine if a common spline was adequate to describe any nonlinearity or if separate splines for each group (condition and day) or individual splines were required (i.e., no commonality across individuals in the non-linear departures from the linear trend).

A LMM was fitted to the cortisol AUC data to investigate if participants' individual AUCs differed between conditions allowing for the possibility of autocorrelation in

the repeated measures on each individual by applying identity and unstructured covariance structures. Fixed effects for these models included condition and day and a condition by day interaction effect, along with random effects of group, subject, and a group by subject interaction. Statistical significance was set at $P \leq 0.05$.

Results

Sleep

Sleep duration measured across the 2 nights prior to the study was not significantly different to the adaptation night or between conditions ($P > 0.05$; data not presented). The average total sleep time measured for the CON and SR conditions was similar on the adaptation night (CON 6.3 ± 0.9 h; SR 6.4 ± 0.7 h; $P > 0.05$). On nights 2 and 3 of the simulation, total sleep time was as expected, given the sleep opportunity provided in each condition (CON 6.9 ± 0.4 h; SR 3.6 ± 0.3 h; $P < 0.001$).

Food and Caffeine Intake

While there was no difference in daily protein and fat intakes between conditions, differences in carbohydrate (CHO; $P = 0.01$), energy ($P = 0.02$) and caffeine ($P = 0.02$) were demonstrated (data not presented). The CON condition had a higher CHO ($P = 0.01$) and energy ($P = 0.004$) intake than the SR condition on day 3 of the protocol. Participants in the SR condition had a higher CHO and energy (both $P = 0.001$) intake on day 1 compared to day 3, while both conditions had higher CHO (SR $P < 0.001$; CON $P = 0.01$) and energy (SR $P < 0.001$; CON $P = 0.001$) intakes on day 2 compared to day 3. Compared to the CON condition, caffeine intake on day 1 and day 2 was higher in the SR condition (both $P = 0.01$), but not different to habitual caffeine intakes ($P > 0.05$; data not presented).

Cortisol

Individual Cortisol Profiles over Time

Several LMM were fitted to the log cortisol data, but the model with lowest AIC was the power model and after inspection of the ΔAIC values, it was considered to have the best fit to the data. Furthermore, when time was catered for as a continuous covariate, linear trends with time differed between conditions and also between days. But the nonlinear departures from these different trends were common to all subjects. As a result, a power model with a common spline term fitted for all profiles was selected as the final model for this analysis. The final LMM demonstrated significant fixed effects for condition ($F = 9.35$, df = 1, $P = 0.02$), day ($F = 4.54$, df = 2, $P = 0.01$) and time ($F = 984.72$, df = 1, $P < 0.001$) on log cortisol levels, along with significant interaction effects for condition by day ($F = 3.68$, df = 2, $P = 0.03$), condition by time ($F = 8.20$, df = 1, $P = 0.01$) and day by time ($F = 5.59$, df = 2, $P = 0.01$).

Pairwise comparison of predicted REML means for log cortisol levels indicated that there was no difference between conditions on day 1, before the shortened sleep had been imposed on participants in the SR condition (Figure 4.2a). However, log cortisol significantly differed between SR and CON conditions on day 2 (Figure 4.2b) and day 3 in the late afternoon and evening (Figure 4.2c). Specifically, participants in the SR condition had higher log cortisol levels than the CON condition at 17:30 (+ 0.62, SED = 0.19, $P = 0.02$) and 19:30 (+ 0.50, SED = 0.19, $P = 0.04$) on day 2 and at 15:30 (+ 0.60, SED = 0.19, $P = 0.02$), 17:30 (+ 0.66, SED = 0.19, $P = 0.01$) and 21:30 (+ 0.50, SED = 0.19, $P = 0.04$) on day 3. Furthermore, log cortisol levels in the SR condition on day 3 at 15:30 (+ 0.51, SED = 0.19, $P = 0.03$), 17:30 (+ 0.52, SED = 0.19, $P = 0.03$), 19:30 (+ 0.57, SED = 0.19, $P = 0.02$) and 21:30 (+ 0.67, SED = 0.19, $P = 0.01$) were higher than at these time points on day 1, when firefighters had not been exposed

to any sleep restriction (Figure 4.3b). Meanwhile, there were no significant differences in diurnal cortisol levels between days among subjects in the CON condition (Figure 4.3a).

Area under the Daily Cortisol Curve

The LMM fitted with an unstructured covariance structure was determined to have the best fit for log AUC cortisol data after inspection of the AIC and ΔAIC values. The LMM showed that day (F = 7.35, df = 2, P = 0.01), condition (F = 5.54, df = 1, P = 0.02) and the interaction between day and condition (F = 8.22, df = 2, P = 0.01) all had statistically significant effects on log cortisol AUC.

Pairwise comparison of predicted REML means indicated that the log cortisol AUC was greater in the SR condition on day 2 (+ 4.75, SED = 1.95, P = 0.02) and day 3 (+ 6.70, SED = 1.75, P < 0.001) compared to the CON condition on their respective days (Figure 4.4). Furthermore, participants in the SR condition had a higher log cortisol AUC on day 2 (+ 4.51, SED = 0.94, P < 0.001) and day 3 (+ 6.68, SED = 0.18, P < 0.001) compared to day 1 and a higher AUC on day 3 (+ 2.17, SED = 0.67, P < 0.01) compared to day 2 (Figure 4.4). Meanwhile there were no significant differences in log cortisol AUC between days in the CON condition (Figure 4.4).

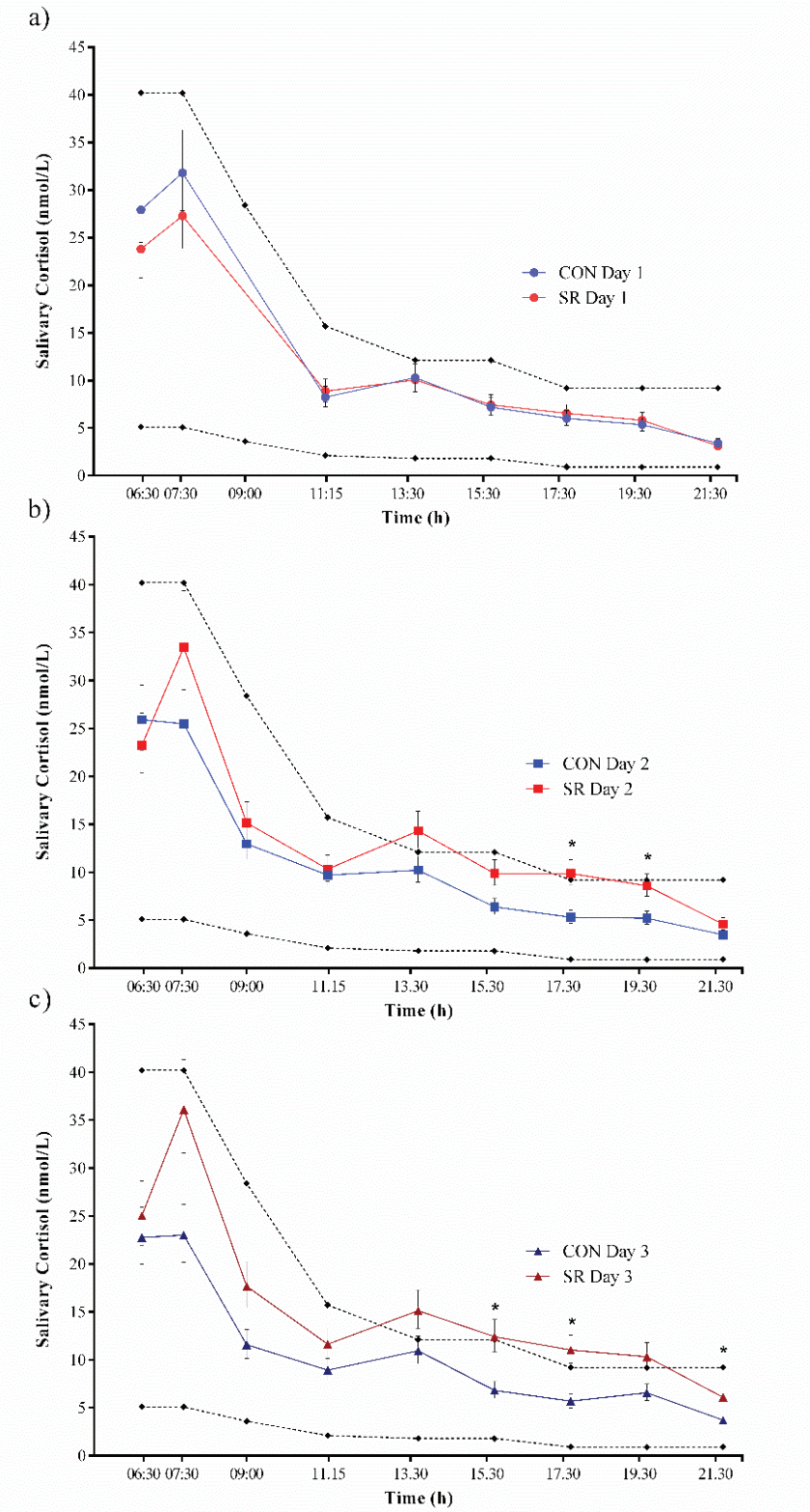

Figure 4.2 Predicted REML means for cortisol profiles in CON and SR condition on day 1 (a; no sleep restriction prior to this day), day 2 (b) and day 3 (c). Data was log-transformed prior to analysis. For ease of interpretation, back-transformations of the means to nmol/L ± standard errors are presented. Dotted lines represent normal cortisol levels of healthy adults (Westermann et al. 2004). Significant comparisons between conditions are indicated by * ($P < 0.05$).

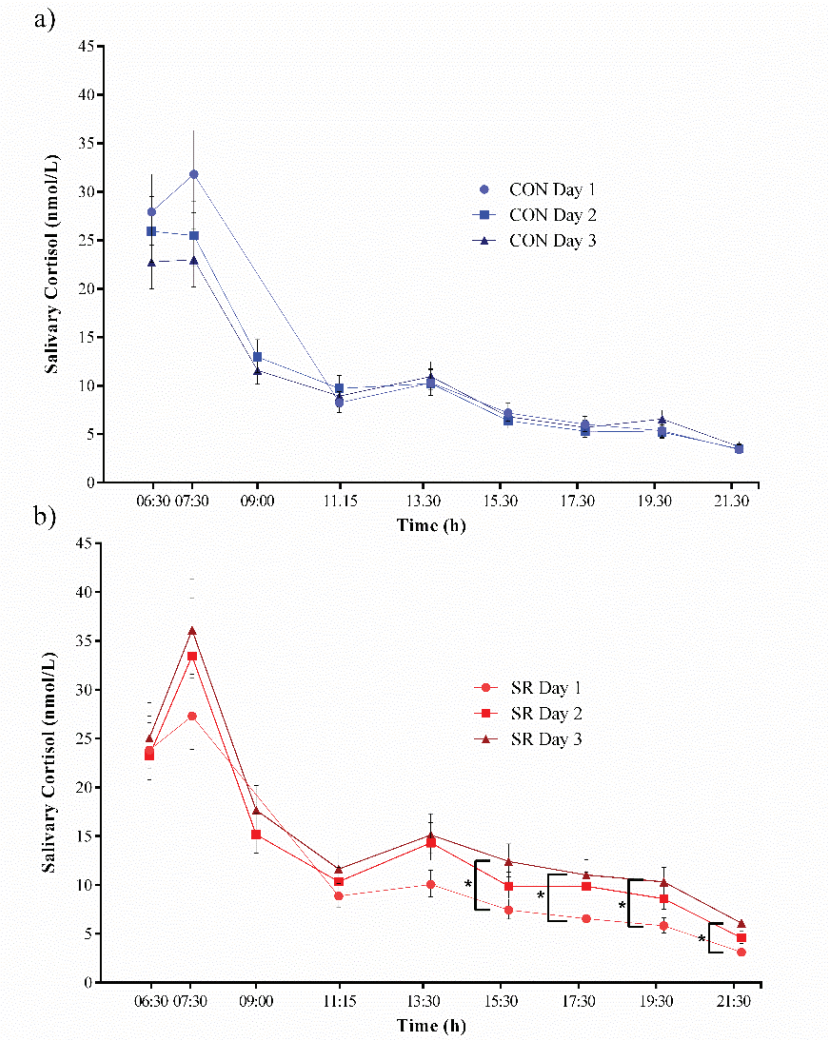

Figure 4.3 Predicted REML means for cortisol profiles across days in CON (a) and SR (b; no sleep restriction prior to day 1). Data was log-transformed prior to analysis. For ease of interpretation, back-transformations of the means to nmol/L ± standard errors are presented. Significant comparisons are indicated by * ($P < 0.05$).

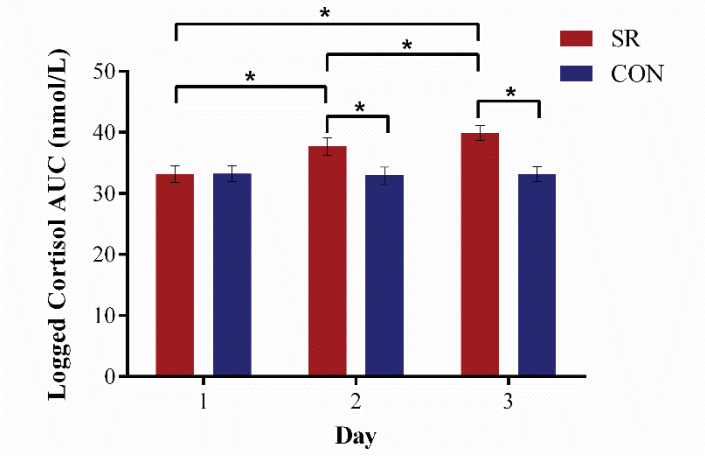

Figure 4.4 Predicted REML means for logged cortisol AUC ± standard errors. Significant comparisons between conditions and between days (i.e., day 1 v day 2, day 2 v day 3 and day 1 v day 3) are indicated by * ($P < 0.05$).

Heart Rate

To analyze daily HR profile, several LMM were fitted to 5-minute averaged HR data. The model with the lowest AIC was the AR1 model and after inspection of the ΔAIC values, it was considered to have the best fit. When time was catered for as a continuous covariate, there was evidence of separate nonlinear trends between days, but no evidence of separate nonlinear behaviour for the conditions or the combinations of condition and days. As a result, the AR1 model with a separate spline term fitted for each day was identified as the final model. A fixed effect for time ($F = 19.96$, df = 1, $P < 0.001$) and interaction effect for day by time ($F = 5.86$, df = 2, $P < 0.01$) on 5-minute average HR were demonstrated. Using least significant differences (1.96 × max SED (2.5) = 4.9 beats/min; Figure 4.5), comparisons of HR between days at specific time points indicate that HR decreased across days, but there were no significant differences found between conditions.

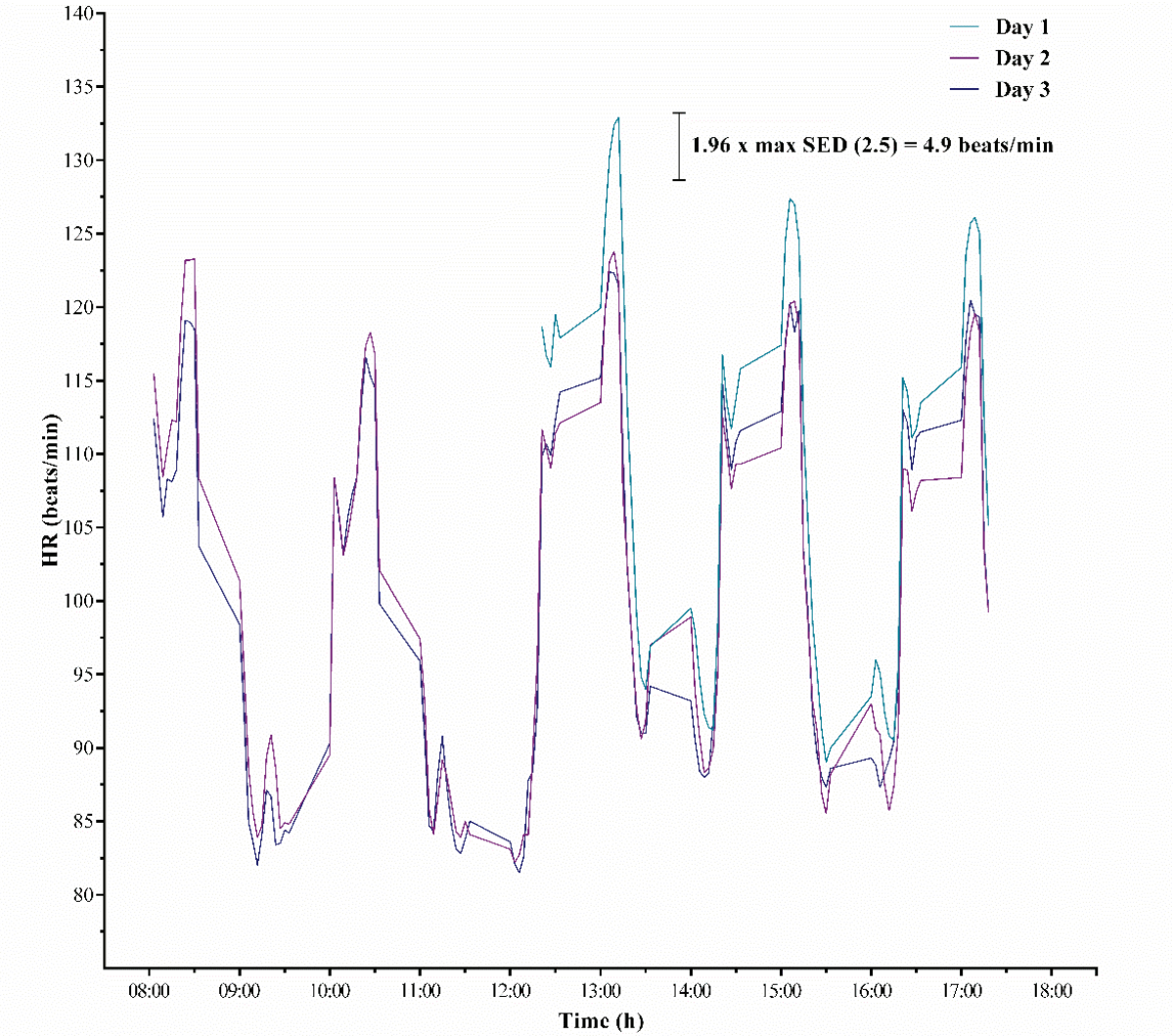

Figure 4.5 Predicted REML means for day by time interaction of 5-minute average HR. Significant comparisons of HR between days within the same time are indicated by least significant difference (1.96 × max SED) error bar.

Discussion

Firefighters in this study were exposed to 3 days of simulated physical firefighting work separated by either an 8-h (i.e., CON condition) or restricted 4-h sleep opportunity (i.e., SR condition) each night in mild temperatures. Findings demonstrated that prior to participants in the SR condition being exposed to shortened sleep, there were no differences in cortisol between conditions on day 1 (Figure 4.2a). However, on day 2 (Figure 4.2b) and day 3 (Figure 4.2c) of the study, firefighters in the SR condition exhibited an elevated diurnal cortisol profile and AUC (Figure 4.4),

compared with the CON condition. Meanwhile, firefighters' HR decreased across the 3 days of testing, yet there were no differences in the decline of HR between the CON and SR conditions (Figure 4.5).

In the absence of a stressor, the release of cortisol follows a normal diurnal pattern, characterised by a morning rise, followed by a gradual decline during the afternoon, through to a nadir in the evening (Miller et al. 2007). Cortisol levels in both conditions on day 1 of this study reflected this normal diurnal change. However, following the shortened sleep period, participants in the SR condition demonstrated higher afternoon and evening cortisol levels than the CON condition (Figure 4.2b, Figure 4.2c). These levels in the SR condition reached the upper limit of the adult normal reference range in the afternoon on day 2 of the simulation, and exceeded the normal range on day 3 at these time points (Westermann et al. 2004). If prolonged, a cumulative trend for elevated cortisol in the latter part of the day has been associated with age-related insulin resistance which can increase metabolic and cardiovascular risk (Dallman et al. 1993; Kern et al. 1996) and highlights a possible pathogenic process by which the combination of sleep loss and physical firefighting work may play a role in the development of metabolic and CVD.

Although elevated, early afternoon (i.e., 13:30) cortisol levels in the SR condition on day 2 (+ 39%; Figure 4.2b) and day 3 (+ 38%; Figure 4.2c) were not significantly different to the CON condition and less than that reported for military personnel exposed to 40-h of sleep deprivation (+150%; Goh et al. 2001) in the absence of physical activity. Leproult et al (1997) also found a greater increase in cortisol levels later in the day following complete sleep deprivation (47%) compared to partial sleep restriction (37%; 3-h). This could suggest that the length of acute sleep restriction

influences the degree to which cortisol levels change the following day, with more extreme periods of sleep restriction eliciting a greater increase in cortisol. One mechanism that may explain the relationship between shortened sleep and cortisol release, is the inhibiting effect slow wave sleep (SWS) is known to have on this hormone (Bierwolf et al. 1997; Vgontzas et al. 1999). For instance, more extreme periods of sleep loss would result in less total SWS, causing less inhibition of cortisol and therefore, a greater overall increase in cortisol the following day. Conversely, some sleep investigations (that did not examine combined physical work and completely restricted physical activity) failed to find an association between SWS and cortisol (Vgontzas et al. 1999; Wu et al. 2008), indicating that further research is required to understand the mechanisms underlying sleep-associated changes in cortisol levels and how this relationship is moderated by additional stressors such as physical work.

Firefighting work and sleep restriction resulted in a significant increase in AUC from day 1 to day 2 of the simulation (14%), followed by a further increase on day 3 (6%; Figure 4.4). Acutely elevated AUC has been positively associated with increased markers of inflammation, including IL-6 (Desantis et al. 2012), and increased negative affect (Piazza et al. 2013). In addition to the link between negative affect and chronic health outcomes (i.e., depression; Peeters et al. 2006), distinct short-term increases in negative affective states have been associated with acute reductions in working memory (Spies et al. 1996) and task motivation (Brose et al. 2012), which for firefighters, could have adverse implications on job performance and safety. An elevation in IL-6, if chronic, can also signal systematic inflammation and have detrimental consequences on cardiovascular health (Willerson and Ridker 2004), which is an issue for wildland and urban firefighting populations in the USA (Kales et

al. 2007; United States Fire Administration 2014). Conversely, there is no evidence of an increase in cardiovascular-related mortality (Glass et al. 2014) or a higher prevalence of CVD risk factors for Australian wildland firefighters when compared to the general population (Wolkow et al. 2014). Therefore, research is needed to establish if there is a link between increased cortisol AUC observed in response to wildland firefighting demands in the current study and the long-term cardiovascular health of Australian firefighters. Furthermore, the direct relationship between AUC and other health outcomes (e.g., atherosclerosis, metabolic syndrome) is less clear (Austin-Ketch et al. 2010; Hajat et al. 2013; Matthews et al. 2006).

The current study is the first to examine daily cortisol AUC among civilian emergency service personnel exposed to physical work and/or sleep restriction. The more commonly used daily measure of HPA-axis activity is mean daily cortisol. For example, soldiers' mean daily cortisol levels have been reported to increase 130% to 150% above baseline following exposure to multiple days of work with limited sleep (1 to 3 h total sleep; Opstad 1994; Opstad and Aakvaag 1981) and between survival training and free-living conditions (Taylor et al. 2008). Unlike mean cortisol levels, AUC involves the use of multiple samples to assess the overall secretion of cortisol over a specific time period (i.e., each work day; Pruessner et al. 2003), complementing the analysis of the diurnal cortisol profile (Figures 4.2 and 4.3; Adam and Kumari 2009). In comparison to the current study, military-based studies that investigated mean cortisol levels were based on fewer samples per day, suggesting that soldier's daily cortisol levels be interpreted with caution. If the results are comparable, a possible explanation for the elevated cortisol levels among military personnel is the presence of specific psychological stressors and their potential impact on HPA-axis function. For instance, investigations by Taylor et al. (2008) and Opstad and Aakvaag

(1981) involved simulated captivity and irrational punishment respectively, which are psychological stressors unique to military work and not yet documented in firefighting. A further reason for the lower increase in cortisol could be due to the current firefighter sample having, on average, more years of occupational experience than the researched military personnel (Opstad 1994; Taylor et al. 2008). Some research suggests that the HPA-axis can adapt to repetitive prior experience of a stressor (Andersen et al. 2013; McEwen 1998). Therefore, it is possible a greater amount of firefighting experience resulted in a more moderate release of cortisol among personnel in the current study. However, further firefighting specific research is needed to determine the possible attenuating effect of experience in this occupation on cortisol.

Morning cortisol levels in the SR condition on day 3 (Figure 4.2c) showed a clear trend towards being significantly greater than the CON condition at 07:30 ($P = 0.053$) and 09:00 ($P = 0.065$). Serum morning (i.e., 07:00) cortisol levels were also investigated among personnel completing a 7-day military training exercise that comprised semi-continuous physical work and extreme sleep restriction (1 h per 24 h; Gundersen et al. 2006; Lundeland et al. 2012). Similar to the current study, cortisol levels increased from baseline to day 4 (Gundersen et al. 2006) and 5 (Lundeland et al. 2012) of training, but returned towards baseline by the last day of training. Elevated morning cortisol levels have been associated with several adverse psychological health effects including the onset of depression, as well as low positive affect (Harris et al. 2000; Steptoe et al. 2007). However the return towards baseline could indicate that soldiers' endocrine systems were able to adapt to the sleep restriction and physical work demands by the final day, potentially mitigating adverse outcomes. In the present study, the 3-day work protocol was chosen to reflect the period firefighters are typically deployed to fight campaign fires (Cater et al. 2007; Ferguson et al. 2011).

However, firefighters can be deployed to fight wildfires that exceed 3 days (Ruby et al. 2002). Therefore, a possible trend for increased morning cortisol levels by day 3 in the SR condition, which may adversely affect psychological health (Harris et al. 2000; Steptoe et al. 2007), warrants future research; specifically the impact of longer periods (i.e., 1 week) of physical work and sleep restriction on morning cortisol levels among personnel. Understanding how firefighters' stress systems respond (and potentially adapt) when exposed to occupational demands over longer durations will provide agencies with important knowledge for managing fatigue related risks among personnel combating extended wildfires. In addition, examining how re-deployments (to the fire-ground), or in the case of volunteer personnel, returning to outside employment, impact cortisol levels would be valuable to further understand the effect of extended and repeated exposure to these demands on firefighters' health.

A prolonged cortisol response is a form of allostatic load that indicates an inability in HPA-axis to shut off following a stressor (McEwen and Seeman 1999). Short sleep and chronic, inappropriate activation of the HPA-axis have been associated with abdominal obesity (Brunner et al. 2002; Van Cauter et al. 2008). Although there were no significant differences in BMI between conditions, the SR condition had an average BMI of 29.6 ± 5.5 kg/m^2 which is almost obese (i.e., > 30.0 kg/m^2), and therefore, may have contributed to the higher cortisol levels found in this condition. However, future research employing the use of more reliable body composition measures (e.g., abdominal height and waist circumference) are needed. Chronically elevated cortisol can also lead to altered glucose regulation, which together with abdominal obesity, are medical conditions that comprise the metabolic syndrome (Brunner et al. 2002; Van Cauter et al. 2008). Assessing if activation of the HPA-axis is chronic can be achieved by measuring the time it takes cortisol to recover to pre-stressor levels. To date,

military- and sleep-based research indicates that the total time (including sleep) it takes cortisol to return to baseline once stressors are removed can vary (Opstad 1994; Pejovic et al. 2013; Wu et al. 2008). Accordingly, examining the duration and/or number of recovery sleep(s) required for cortisol levels to lower to baseline following the 3-day firefighting deployment would be valuable for fire agencies to determine the minimum recovery time required prior to a return to the fire-ground. This is an important area for future research, given that premature return to work with already elevated cortisol levels may exacerbate any subsequent cortisol responses and result in chronic HPA-axis activation, which increases the risk of adverse long-term health outcomes. Specifically, future research should first examine the impact single recovery sleeps of varying durations (i.e., 5-h, 6-h, 7-h, 8-h, 9-h and 10-h sleep opportunities) have on cortisol levels the following day. Comparing this data to cortisol levels from day 1 in the current study as well as the adult normal reference range will establish if a single sleep is capable of restoring cortisol to baseline, and if so, will identify to agencies the minimum amount of recovery sleep needed to safely return personnel to the fire-ground. If a single recovery sleep provides insufficient time to reverse the impact of sleep restriction on cortisol, then multiple nights of recovery sleep should be trialed.

While cortisol levels increased over the duration of the study among participants in the SR condition, the change in HR between conditions was not different. Instead, the HR profile decreased across each of the days (Figure 4.5), yet there were no differences between conditions. The current study is the first to investigate how both the HPA-axis and SAM system respond to the combination of physical work and sleep restriction in firefighters. To date, only two studies have investigated how both these stress systems respond to multiple demands among other emergency personnel (Samel

et al. 2004) and the general population (Konishi et al. 2013). Findings from the available literature have been mixed (Konishi et al. 2013; Samel et al. 2004) and in contrast to the increased cortisol, but lowered HR observed in the current study. For instance, Samel et al. (2004) found that 7 days of helicopter operations separated by moderate sleep restriction each night (i.e., ≤ 6 h) triggered pilots' mean daily cortisol levels to increase 50 to 80% above baseline (i.e., off-duty days), but HR response did not exceed 120 beats/minute and there was no change in HR across successive days of work. Furthermore, Konishi et al. (2013) found that HR decreased during an exercise tolerance test following 34 h of complete sleep deprivation, yet there was no change in cortisol.

Several military and exercise-based studies have investigated the combined effect of prolonged physical work and extreme sleep restriction on HR, independently of measuring cortisol (Dabrowski et al. 2012; Myles 1987; Neylan 2008; Scott and McNaughton 2004). Findings indicate that long periods of intermittent exercise (e.g., cycle ergometer, treadmill) or physical military-based tasks (e.g., marching, combat engineer operations) in combination with 24 to 70 h of complete sleep deprivation resulted in an unchanged (Myles 1987; Scott and McNaughton 2004) or decreased exercise HR (Dabrowski et al. 2012; Neylan 2008). Research by Dettoni et al. (2012) and O'Leary (2014) also found resting HR remained unchanged in response to a 4-h period of sleep restriction investigated over separate 5- and 1-night protocols, respectively. Similar to Dettoni et al. (2012) and O'Leary et al. (2014), sleep restriction in the current study did not appear to accentuate the change in HR response among firefighters. This may indicate that the period and/or length of sleep restriction investigated may not be a significant enough stressor to affect HR over and above the influence of simulated physical work in mild temperatures.

The decrease in HR over the 3 days of firefighting work may have been due to participants becoming increasingly more economical in executing the actions required to complete the physical tasks. Practice-related effects in response to repeated exercise performance may reduce the internal mechanical work needed to coordinate the limbs, resulting in reduced metabolic energy expenditure (Sparrow et al. 1999). Reduced HR could have also resulted from attenuated sympathetic nervous system activation (Dabrowski et al. 2012; Konishi et al. 2013), suggesting an adaptive SAM system response to the demands. However, activation of the HPA-axis can trigger the sympathetic nervous system (Tsigos and Chrousos 2002). Therefore, to better understand the mechanisms contributing to a lowered HR response (e.g., altered epinephrine and norepinephrine levels), research adopting direct measures of the SAM system is needed, such as the measurement of catecholamines. While not assessing catecholamine levels is a limitation of the current study, it is important future research takes into consideration that an accurate assessment of this biomarker requires frequent 24-h urine or blood testing (Chandola et al. 2010; Weinkove 1991), in which smoking, caffeine and certain foods are restricted, limiting the practicality of this biomarker in a high fidelity field environment (Hjemdahl 1993; Lundberg 2008). Furthermore, metabolic stress caused by glycogen depletion has been associated with elevations in cortisol and HR (Branth 2006). Therefore, the restricted energy intake and sleep restriction (Opstad 1994) may have contributed to the large rise in cortisol among military personnel (Baty et al. 2007; Gleeson et al. 1998). Conversely, other studies have found no change in cortisol or HR responses to restricted CHO and/or energy intake during physical work (Glickman-Weiss et al. 1995; Thyfault et al. 2004). Adhering to fire-ground practices, food and drink intake in the current study was *ad libitum*. Therefore, not controlling for firefighters diet in the current study may explain the lower CHO and energy intake on day 3 in the SR condition. Although this is a

limitation, CHO and energy intakes remained within normal adult levels (Food and Agricultural Organization 2004; National Health and Medical Research Council 2013) and were higher than those reported among military personnel (Opstad 1994). An *ad libitum* intake of fluid also resulted in higher caffeine consumption for the SR condition on day 1 and day 2 when compared to the CON, which is a further limitation of this study. The cortisol response to exercise (Cook et al. 2012; Lovallo et al. 2006) can increase with high doses of caffeine (e.g., > 250 mg), but this response is reduced in regular caffeine users (Lovallo et al. 2005). Caffeine intakes in the SR group were not different to habitual caffeine consumption, nor were they as high as those examined in previous studies (Cook et al. 2012; Lovallo et al. 2006). Therefore, it is less likely lower energy and/or CHO intakes and higher caffeine consumption among the SR condition contributed to increased cortisol levels observed in the current study.

Firefighters' diurnal cortisol levels increased outside of the normal range reported for adults (Westermann et al. 2004). Growing evidence suggests that chronically high diurnal cortisol levels both near, and above the normal levels are positively associated with increased CVD risk (Lippi et al. 2008; Prodam et al. 2013; Rosmond et al. 2003). Furthermore, heart disease is the leading cause of on-duty death among firefighters (Kales et al. 2007). Given the association between chronically elevated cortisol and CVD (Lippi et al. 2008; Rosmond et al. 2003) and other adverse health effects (e.g., mood disorders; Mackin and Young 2004), these findings draw further attention to the need for research on the effects of longer and chronic exposure to wildland firefighting work and restricted sleep on long-term cortisol levels. Future research will also help to better understand the mechanisms through which restricted sleep and firefighting work affects abnormal HPA-axis function and potentially contributes to the

pathogenesis of CVD and mood disorders among firefighting personnel (An et al. 2015; Carey et al. 2011; Cook and Mitchell 2013; Kales et al. 2007).

Conclusion

This is the first study to investigate the combined effect of simulated physical firefighting work and sleep restriction on firefighters' cortisol and HR responses. Findings indicate that 3 days of physical firefighting work separated by 2 nights of restricted sleep resulted in an increased cortisol response over successive days of simulated work. Meanwhile, firefighters' HR levels decreased across each day of simulated work, but this change was not different between conditions. These findings could demonstrate that the SAM system responds to these simulated demands in mild temperatures by lowing HR levels, but further research should, where possible, directly measure SAM system activity (i.e., catecholamine levels) to further understand the possible mechanisms contributing to a lowered HR response. Furthermore, the results highlight the protective role an 8-h sleep opportunity between shifts of simulated firefighting work has on preserving normal cortisol levels when compared to a 4-h sleep opportunity. Given the number of adverse health outcomes associated with chronically high cortisol levels (Lippi et al. 2008; Mackin and Young 2004; Rosmond et al. 2003; Silverman and Sternberg 2012), future research should examine how prolonged exposure to firefighting work and restricted sleep (e.g., long or multiple deployments over a fire season) affects firefighters' cortisol levels in the medium to longer term. In addition, examining the amount and/or number of recovery sleep(s) required to restore cortisol levels to baseline following a firefighting deployment is important to fully understand how these occupational demands impact on the functioning of the HPA-axis while determining the minimum recovery time required for firefighters to safely return to the fire-ground. These findings also provide a basis

for further investigation among other occupations with similar periods of physical work and sleep restriction to firefighting (e.g., mining, rescue workers).

References

Adam EK, Kumari M (2009) Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 34(10):1423-1436

Aisbett B, Phillips M, Raines J, Nichols D Work patterns of tanker-based bushfire suppression by Australian volunteer firefighters in south-east Australia. In: Human Dimensions of Wildfire Conference, Fort Collins, Colorado, 2007.

Aisbett B, Wolkow A, Sprajcer M, Ferguson SA (2012) "Awake, smoky, and hot": Providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters. Applied Ergonomics 43(5):916-925

An SJ, Chung YK, Kim BH, Kwak KM, Son JS, Koo JW, Ju YS, Kwon YJ (2015) The effect of organisational system on self-rated depression in a panel of male municipal firefighters. Annals Of Occupational And Environmental Medicine 27:1-7 doi:10.1186/s40557-014-0044-x

Andersen JP, Silver RC, Stewart B, Koperwas B, Kirschbaum C (2013) Psychological and physiological responses following repeated peer death. Plos One 8(9) doi:10.1371/journal.pone.0075881

Austin-Ketch TL, Violanti J, Fekedulegn D, Andrew ME, Burchfiel C, Hartley T, Vena JE (2010) Metabolic syndrome and salivary cortisol: Is there dysregulation among a group of active duty urban police officers? Diabetes and Metabolic Syndrome: Clinical Research and Reviews 4(2):82-88

Baty JJ, Hyonson H, Zhenping D, Bernard JR, Bei W, Bongan K, Ivy JL (2007) The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response and muscle damage. Journal of Strength and Conditioning Research 21(2):321-329

Bierwolf C, Struve K, Marshall L, Born J, Fehm HL (1997) Slow wave sleep drives inhibition of pituitary-adrenal secretion in humans. Journal of Neuroendocrinology 9:479-484

Biopharmaceutics Coordinating Committee (2001) Bioanalytical Method Validation. In: U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER) - Guidance for Industry. Center for Veterinary Medicine (CVM), United States Department of Health and Human Services Food and Drug Administration.

Branth S (2006) Energy Metabolic Stress Syndrome: Impact of Physical Activity of Different Intensity and Duration. PhD, Uppsala University

Brose A, Schmiedek F, Lövdén M, Lindenberger U (2012) Daily variability in working memory is coupled with negative affect: The role of attention and motivation. Emotion 12(3):605-617 doi:10.1037/a0024436

Brunner EJ, Hemingway H, Walker BR, Page M, Clarke P, Juneja M, Shipley MJ, Kumari M, Andrew R, Seckl JR, Papadopoulos A, Checkley S, Rumley A, Lowe GDO, Stansfeld SA, Marmot MG (2002) Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. Circulation 106(21):2659-2665

Budd GM, Brotherhood JR, Hendrie AL, Jeffery SE, Beasley FA, Costin BP, Wu Z, Baker MM, Cheney NP, Dawson MP (1997) Project aquarius 7. Physiological and subjective responses of men supressing wildland fire. International Journal of Wildland Fire 7(133-144)

Burnham KP, Anderson DR (2002) Model selection and multi-model inference : a practical information-theoretic approach / Kenneth P. Burnham, David R. Anderson, 2nd edn. Springer, London, United Kingdom

Buxton OM, Pavlova M, Reid EW, Wei W, Simonson DC, Adler GK (2010) Sleep Restriction for 1 Week Reduces Insulin Sensitivity in Healthy Men. Diabetes 59(9):2126-2133 doi:10.2337/db09-0699

Carey MG, Al-Zaiti SS, Dean GE, Sessanna L, Finnell DS (2011) Sleep problems, depression, substance use, social bonding, and quality of life in professional firefighters. Journal Of Occupational And Environmental Medicine/American College Of Occupational And Environmental Medicine 53(8):928-933 doi:10.1097/JOM.0b013e318225898f

Cater H, Clancy D, Duffy K, Holgate A, Wilison B, Wood J Fatigue on the fireground: the DPI experience. In: Thornton R (ed) Bushfire Cooperative Research Centre/Australasian Fire Authorities Council Conference Research Forum, Hobart, Tasmania, 2007. Bushfire Cooperative Research Centre,

Chandola T, Britton A, Brunner1 E, Hemingway H, Malik M, Kumari M, Badrick E, Kivimaki M, Marmot M (2008) Work stress and coronary heart disease: what are the mechanisms? European Heart Journal 29(5):640-648 doi:10.1093/eurheartj/ehm584

Chandola T, Heraclides A, Kumari M (2010) Psychophysiological biomarkers of workplace stressors. Neuroscience and Biobehavioral Reviews 35(1):51-57

Cook B, Mitchell W (2013) Occupational health effects for firefighters: The extent and implications of physical and psychological injuries. Centre of Full Employment and Equity

Cook C, Beaven CM, Kilduff LP, Drawer S (2012) Acute Caffeine Ingestion's Increase of Voluntarily Chosen Resistance-Training Load After Limited Sleep. International Journal of Sport Nutrition and Exercise Metabolism 22(3):157-164

Cuddy J, Gaskill S, Sharkey B, Harger S, Ruby B (2007) Supplemental feedings increase self-selected work output during wildfire suppression. Medicine and Science in Sports and Exercise 39(6):1004-1012

Cullen T, Thomas AW, Webb R, Hughes MG (2015) The relationship between interleukin-6 in saliva, venous and capillary plasma, at rest and in response to exercise. Cytokine(2):397 doi:10.1016/j.cyto.2014.10.011

Dabrowski J, Ziemba A, Tomczak A, Mikulski T (2012) Physical performance of healthy men exposed to long exercise and sleep deprivation. Medicina Sportiva 16:6-11

Dallman M, Strack A, Akana S, Bradbury M, Hanson E (1993) Feast and Famine: Critical role of glucorticoids with insulin in daily energy flow. Frontiers in Neuroendocrinology 14:303-347

Desantis AS, Diezroux AV, Hajat A, Aiello AE, Golden SH, Jenny NS, Seeman TE, Shea S (2012) Associations of salivary cortisol levels with inflammatory markers: The Multi-Ethnic Study of Atherosclerosis. Psychoneuroendocrinology 37:1009-1018

Dettoni JL, Consolim-Colombo FM, Drager LF, Rubira MC, Souza SBPCd, Irigoyen MC, Mostarda C, Borile S, Krieger EM, Moreno H, Jr., Lorenzi-Filho G (2012) Cardiovascular effects of partial sleep deprivation in healthy volunteers. Journal Of Applied Physiology (Bethesda, Md: 1985) 113(2):232-236 doi:10.1152/japplphysiol.01604.2011

Fahs CA, Huimin Y, Ranadive S, Rossow LM, Agiovlasitis S, Echols G, Smith D, Horn GP, Rowland T, Lane A, Fernhall B (2011) Acute effects of firefighting on arterial stiffness and blood flow. Vascular Medicine 16(2):113-118 doi:10.1177/1358863x11404940

Faulkner SH, Spilsbury KL, Harvey J, Jackson A, Huang J, Platt M, Tok A, Nimmo MA (2014) The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise. European Journal of Applied Physiology 114(6):1207-1216 doi:10.1007/s00421-014-2851-8

Fellmann N, Bedu M, Boudet G, Mage M, Sagnol M, Pequignot JM, Claustrat B, Brun J, Peyrin L, Coudert J (1992) Inter-relationships between pituitary-adrenal hormones and catecholamines during a 6-day Nordic ski race. European journal of applied physiology and occupational physiology 64(3):258-265

Ferguson SA, Aisbett B, Jay SM, Onus K, Lord C, Sprajcer M, Thomas MJW (2011) Design of a valid simulation for researching physical, physiological and cognitive performance in volunteer firefighters during bushfire deployment. Paper presented at the Bushfire Cooperative Research Centre/ Australasian Fire and Emergency Service Authorities Council Conference Research Forum, Sydney, New South Wales,

Field A (2009) Discovering Statistics Using SPSS. Sage, London

Food and Agricultural Organization (2004) Report on human energy requirements. In Food and Agricultural Organization/World Health Organization/United Nations University Expert Consultation (ed). FAO, Rome

Glass D, Sim M, Pircher S, Del Monaco A, Dimitriadis C, Miosge J, Vander Hoorn S, Gordon I (2014) Australian Firefighters' Health Study. vol Full Report. Monash Centre for Occupational and Environmental Health, Melbourne, Victoria.

Gleeson M, Blannin AK, Walsh NP, Bishop NC, Clark AM (1998) Effect of low- and high-carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. International Journal of Sport Nutrition 8(1):49-59

Glickman-Weiss EL, Hegsted M, Nelson AG, Hearon CM, Dunbar CC, Tulley R (1995) A comparison of a carbohydrate-electrolyte beverage versus a placebo beverage in maintaining thermoregulatory and blood homeostasis during the training of fire fighters. Wilderness and Environmental Medicine 6(4):377-384

Goh VH, Tong TY, Lim C, Low EC, Lee LK (2001) Effects of one night of sleep deprivation on hormone profiles and performance efficiency. Military Medicine 166(5):427-431

Golden SH, Wand GS, Malhotra S, Kamel I, Horton K, . (2011) Reliability of hypothalamic–pituitary–adrenal axis assessment methods for use in population-based studies. European Journal of Epidemiology 26(511-525)

Gundersen Y, Opstad PK, Reistad T, Thrane I, Vaagenes P (2006) Seven days' around the clock exhaustive physical exertion combined with energy depletion and sleep deprivation primes circulating leukocytes. European Journal of Applied Physiology 97(2):151-157

Gurrin LC, Scurrah KJ, Hazelton ML (2005) Statistics in Medicine 24(21):3361-3381

Hajat A, Diez-Roux AV, Sanchez BN, Holvoet P, Lima JA, Merkin SS, Polak JF, Seeman TE, Wu MH (2013) Examining the association between salivary cortisol levels and subclinical measures of atherosclerosis: The Multi-Ethnic Study of Atherosclerosis. Psychoneuroendocrinology 38(7):1036—1046

Harris TO, Borsanyi S, Messari S, Stanford K, Cleary SE, Shiers HM, Brown GW, Herbert J (2000) Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. The British Journal of Psychiatry 177:505-510

Hjemdahl P (1993) Plasma catecholamines--analytical challenges and physiological limitations. Baillière's Clinical Endocrinology And Metabolism 7(2):307-353

Hsu JC (1996) Multiple Comparisons Theory and Methods. Chapman and Hall, London, United Kingdom

Kales SN, Soteriades ES, Christophi CA, Christiani DC (2007) Emergency duties and deaths from heart disease among firefighters in the United States. The New England Journal Of Medicine 356(12):1207-1215

Kern W, Dodt C, Born J, Fehm HL (1996) Changes in cortisol and growth hormone secretion during nocturnal sleep in the course of aging. The Journals Of Gerontology Series A, Biological Sciences And Medical Sciences 51(1):3-9

Kirschbaum C, Hellhammer DH (1994) Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology 19(4):313-333

Konishi M, Takahashi M, Endo N, Numao S, Takagi S, Miyashita M, Midorikawa T, Suzuki K, Sakamoto S (2013) Effects of sleep deprivation on autonomic and endocrine functions throughout the day and on exercise tolerance in the evening. Journal of Sports Sciences 31(3):248-255 doi:10.1080/02640414.2012.733824

Leproult R, Copinschi G, Buxton O, Van Cauter E (1997) Sleep loss results in an elevation of cortisol levels the next evening. Sleep 20(10):865-870

Lippi G, Franchini M, Salvagno G, Montagnana M, Guidi G (2008) Higher morning serum cortisol level predicts increased fibrinogen but not shortened APTT. Journal of Thrombosis and Thrombolysis 26(2):103-105

Lovallo WR, Farag NH, Vincent AS, Thomas TL, Wilson MF (2006) Cortisol responses to mental stress, exercise, and meals following caffeine intake in men and women. Pharmacology Biochemistry And Behavior 83(3)

Lovallo WR, Whitsett TL, Al'Absi M, Sung BH, Vincent AS, Wilson MF (2005) Caffeine stimulation of cortisol secretion across the waking hours in relation to caffeine intake levels. Psychosomatic Medicine 67(5):734-739

Lundberg U (2008) Catecholamines and Environmental Stress. In: Allostatic Load notebook. Department of Psychology and Centre for Health Equity Studies (CHESS), Stockholm University, retrieved 6 July 2014, <www.macses.ucsf.edu/research/allostatic/catecholamine.php#assessment>.

Lundeland B, Gundersen Y, Opstad PK, Thrane I, Zhang Y, Olaussen RW, Vaagenes P (2012) One week of multifactorial high-stress military ranger training affects Gram-negative signalling. Scandinavian Journal of Clinical and Laboratory Investigation 72(7):547-554 doi:10.3109/00365513.2012.705017

Mackin P, Young AH (2004) The role of cortisol and depression: exploring new opportunities for treatments. Psychiatric Times 21(5):92-95

Matthews K, Schwartz J, Cohen S, Seeman T (2006) Diurnal cortisol decline is related to coronary calcification: CARDIA study. Psychosomatic Medicine 68(5):657-661

McEwen BS (1998) Stress, adaptation, and disease - Allostasis and allostatic load. Annals of the New York Academy of Sciences 840:33-44

McEwen BS, Seeman T (1999) Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. Annals of the New York Academy of Sciences 896:30-47

Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF, Mullington JM (2004) Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. Journal of the American College of Cardiology 43(4):678-683

Miller GE, Chen E, Zhou ES (2007) If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychological Bulletin 133(1):25-45

Myles WS (1987) Self-paced work in sleep deprived subjects. Ergonomics 30(8):1175-1184

National Health and Medical Research Council (2013) Eat for health - Australian Dietary Guidelines. In: Australian Goverment DoHaA (ed). Canberra, Australian Capital Territory

Neylan TC (2008) Intensity and physiological strain of competitive ultra-endurance exercise in humans. Journal of Sports Sciences 26(5):477-489

Neylan TC, Metzler TJ, Best SR, Weiss DS, Fagan JA, Liberman A, Rogers C, Vedantham K, Brunet A, Lipsey TL, Marmar CR (2002) Critical incident exposure and sleep quality in police officers. Psychosomatic Medicine 64(2):345-352

Nicolson NA (2008) Measurement of cortisol. In: Luecken LJ, Gallo LC, (eds) Handbook of physiological research methods in health psychology. Sage Publications, Thousand Oaks, CA, p 37-74

O'Leary ÉD (2014) Effects of Acute Sleep Restriction on Laboratory and Ambulatory Physiological Reactivity in Young Adults. PhD, National University of Ireland

Opstad PK (1994) Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. European Journal of Endocrinology 131(1):56-66

Opstad PK, Aakvaag A (1981) The effect of a high calory diet on hormonal changes in young men during prolonged physical strain and sleep deprivation. European Journal of Applied Physiology 46(1):31-39

Payne R, Welham S, Harding S (2011) A Guide to REML in GenStat (16th Edition)

Peeters F, Berkhof J, Delespaul P, Rottenberg J, Nicolson NA (2006) Diurnal mood variation in major depressive disorder. Emotion 6(3):383-391 doi:10.1037/1528-3542.6.3.383

Pejovic S, Basta M, Vgontzas AN, Kritikou I, Shaffer ML, Tsaoussoglou M, Stiffler D, Stefanakis Z, Bixler EO, Chrousos GP (2013) Effects of recovery sleep after one work week of mild sleep restriction on interleukin-6 and cortisol secretion and daytime sleepiness and performance. American Journal of Physiology - Endocrinology and Metabolism 305(7):890-896

Perroni F, Tessitore A, Cibelli G, Lupo C, D'Artibale E, Cortis C, Cignitti L, Rosas MD, Capranica L (2009) Effects of simulated firefighting on the responses of salivary cortisol, alpha-amylase and psychological variables. Ergonomics 52(4):484-491

Phillips M, Netto K, Payne W, Nichols D, Lord C, Brooksbank N, Onus K, Jefferies S, Aisbett B (2011) Frequency, intensity and duration of physical tasks performed by Australian rural firefighters during bushfire suppression. Paper presented at the Bushfire Cooperative Research Center/Australasian Fire Authorities Council Conference Research Forum, Sydney, New South Wales,

Phillips M, Netto K, Payne WR, Nichols D, Lord C, Brooksbank N, Aisbett B (2015a) Frequency, intensity, time and type of tasks performed during wildfire suppression. Occupational Medicine and Health Affairs In Press

Phillips M, Payne W, Lord C, Netto K, Nichols D, Aisbett B (2012) Identification of physically demanding tasks performed during bushfire suppression by Australian rural firefighters. Applied Ergonomics 43(2):435-441 doi:10.1016/j.apergo.2011.06.018

Phillips M, Payne WR, Netto K, Cramer S, Nichols D, McConell GK, Lord C, Aisbett B (2015b) Oxygen uptake and heart rate during simulated wildfire suppression tasks performed by Australian rural firefighters. Occupational Medicine and Health Affairs 3(3)

Piazza JR, Charles ST, Stawski RS, Almeida DM (2013) Age and the association between negative affective states and diurnal cortisol. Psychology and Aging 28(1):47-56 doi:10.1037/a0029983

Prodam F, Ricotti R, Agarla V, Parlamento S, Genoni G, Balossini C, Walker GE, Aimaretti G, Bona G, Bellone S (2013) High-end normal adrenocorticotropic hormone and cortisol levels are associated with specific cardiovascular risk factors in pediatric obesity: a cross-sectional study. BioMed Central Medicine 11(1):1-11 doi:10.1186/1741-7015-11-44

Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003) Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28(7):916-931

Raines J, Snow R, Petersen A, Harvey J, Nichols D, Aisbett B (2013) The effect of prescribed fluid consumption on physiology and work behavior of wildfire fighters. Applied Ergonomics 44(3):404-413

Reynolds AC, Dorrian J, Liu P, Van Dongen H, Wittert GA, Harmer L, Banks S (2012) Impact of Five Nights of Sleep Restriction on Glucose Metabolism, Leptin and Testosterone in Young Adult Men. Plos One 7(7):1-10 doi:10.1371/journal.pone.0041218

Rodríguez-Marroyo JA, López-Satue J, Pernía R, Carballo B, García-López J, Foster C, Villa JG (2012) Physiological work demands of Spanish wildland firefighters during wildfire suppression. International Archives of Occupational and Environmental Health 85(2):221-228 doi:10.1007/s00420-011-0661-4

Rosmond R, Wallerius S, Wanger P, Martin L, Holm C, Björntorp P (2003) A 5-year follow-up study of disease incidence in men with an abnormal hormone pattern. Journal of Internal Medicine 254:386-390

Ruby BC, Shriver TC, Zderic TW, Sharkey BJ, Burks C, Tysk S, . (2002) Total energy expenditure during arduous wildfire suppression. Medicine and Science in Sports and Exercise 34(6):1048-1054

Samel A, Vejvoda M, Maass H (2004) Sleep Deficit and Stress Hormones in Helicopter Pilots on 7-Day Duty for Emergency Medical Services. Aviation, Space, and Environmental Medicine 75(11):935-940

Scott JP, McNaughton LR (2004) Sleep Deprivation, Energy Expenditure and Cardiorespiratory Function. International Journal of Sports Medicine 25(6):421-426

Silverman MN, Sternberg EM (2012) Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Annals of the New York Academy of Sciences 1261(1):55-63 doi:10.1111/j.1749-6632.2012.06633.x

Smith DL, Petruzzello SJ, Chludzinski MA, Reed JJ, Woods JA (2005) Selected hormonal and immunological responses to strenuous live-fire firefighting drills. Ergonomics 48(1):55-65

Sparrow WA, Hughes KM, Russell AP, Le Rossignol PF (1999) Effects of practice and preferred rate on perceived exertion, metabolic variables and movement control. Human Movement Science 18(2-3):137-153

Spiegel K, Leproult R, Van Cauter E (1999) Impact of sleep debt on metabolic and endocrine function. The Lancet 354(9188):1435-1439

Spies K, Hesse F, Hummitzsch C (1996) Mood and capacity in Baddeley's model of human memory. Zeitschrift für Psychologie mit Zeitschrift für angewandte Psychologie 204(4):367-381

Steptoe A, Gibson EL, Hamer M, Wardle J (2007) Neuroendocrine and cardiovascular correlates of positive affect measured by ecological momentary assessment and by questionnaire. Psychoneuroendocrinology 32(1):56-64

Tanskanen MM, Kyrolainen H, Uusitalo AL, Huovinen J, Nissila J, Kinnunen H, Atalay M, Hakkinen K (2011) Serum sex hormone-binding globulin and cortisol concentrations are associated with overreaching during strenuous military training. Journal of Strength and Conditioning Research 25(3):787-797 doi:10.1519/JSC.0b013e3181c1fa5d

Taylor MK, Reis JP, Sausen KP, Padilla GA, Markham AE, Potterat EG, Drummond SPA (2008) Trait Anxiety and Salivary Cortisol During Free Living and Military Stress. Aviation, Space and Environmental Medicine 79(2):129-135

Thyfault JP, Carper MJ, Richmond SR, Hulver MW, Potteiger JA (2004) Effects of liquid carbohydrate ingestion on markers of anabolism following high-intensity resistance exercise. Journal of Strength and Conditioning Research 18(1):174-179

Tsigos C, Chrousos GP (2002) Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. Journal of Psychosomatic Research 53:865-871 doi:10.1016/S0022-3999(02)00429-4

United States Fire Administration, National Fire Programs, National Fallen Firefighters Foundation (2014) Firefighter Fatalities in the United States in 2013.

Van Cauter E, Spiegel K, Tasali E, Leproult R (2008) Original article: Metabolic consequences of sleep and sleep loss. Sleep Medicine 9(Supplement 1):S23-S28 doi:10.1016/S1389-9457(08)70013-3

van Leeuwen WMA, Lehto M, Karisola P, Lindholm H, Luukkonen R, Sallinen M, Härmä M, Porkka-Heiskanen T, Alenius H (2009) Sleep Restriction Increases the Risk of Developing Cardiovascular Diseases by Augmenting Proinflammatory Responses through IL-17 and CRP. Plos One 4(2):1-7 doi:10.1371/journal.pone.0004589

Verbyla AP, Cullis BR, Kenward MG, Welham SJ (1999) The Analysis of Designed Experiments and Longitudinal Data by Using Smoothing Splines. Journal of Applied Statistics 48(3):269-311

Vgontzas AN, Mastorakos G, Bixler EO, Kales A, Gold PW, Chrousos GP (1999) Sleep deprivation effects on the activity of the hypothalamic-pituitary-adrenal and growth axes: potential clinical implications. Clinical Endocrinology 51(2):205-215

Vincent G, Ferguson SA, Larsen B, Wolkow A, Tran J, Aisbett B (2015) Sleep restriction during simulated wildfire suppression: effect on physical task performance Plos One 10(1) doi:doi: 10.1371/journal.pone.0115329. eCollection 2015.

Weinkove C (1991) ACP Broadsheet No 127: April 1991. Measurement of catecholamines and their metabolites in urine. Journal Of Clinical Pathology 44(4):269-275

Westermann J, Demir A, Herbst V (2004) Determination of cortisol in saliva and serum by a luminescence-enhanced enzyme immunoassay. Clinical laboratory 50(1-2):11-24

Willerson JT, Ridker PM (2004) Inflammation as a cardiovascular risk factor. Circulation 109(Supplement 1):II2-II10

Wolkow A, Aisbett B, Ferguson SA, Main LC (2015) The effects of work-related sleep restriction on acute physiological and psychological stress responses and their interactions: A review among emergency service personnel. International Journal of Occupational Medicine and Environmental Health 28(2):183-208

Wolkow A, Netto K, Langridge P, Green J, Nichols D, Sergeant M, Aisbett B (2014) Coronary Heart Disease Risk in Volunteer Firefighters in Victoria, Australia. Archives of Environmental and Occupational Health 69(2):112-120

Wu H, Zhao Z, Stone WS, Huang L, Zhuang J, He B, Zhang P, Li Y (2008) Effects of sleep restriction periods on serum cortisol levels in healthy men. Brain Research Bulletin 77(5):241-245 doi:10.1016/j.brainresbull.2008.07.013

Chapter 5: Study 3

Study 3

Relationships between inflammatory cytokine and cortisol responses in firefighters exposed to simulated wildfire suppression work and sleep restriction

Alexander Wolkow BExSpSci (Hons), Brad Aisbett PhD, John Reynolds PhD, Sally A Ferguson PhD, and Luana C Main PhD

Published in

Physiological Reports

doi:10.14814/phy2.12604

Presented here in the journal submission format

Abstract

Purpose: The interplay between inflammatory and cortisol responses modulate an appropriate response to a stressor. Exposure to severe stressors however, may alter the actions and relationships of these responses and contribute to negative health outcomes. Physical work and sleep restriction are two stressors faced by wildland firefighters, yet their influence on the relationship between inflammatory and cortisol responses is unknown. The aim of the present study was to quantify the relationship between cytokine and cortisol responses to sleep restriction while performing simulated physical wildfire suppression work.

Methods: Firefighters completed 3 days of simulated physical firefighting work separated by either an 8-h (Control condition; n=18) or 4-h sleep (Sleep restriction condition; n=17) opportunity on each of the 2 nights. Salivary cortisol and inflammatory cytokines (IL-6, IL-8, IL-1β, TNF-α, IL-4, IL-10) were measured throughout each day.

Results: An increase in morning IL-6 was related to a rise (6.2%; p = 0.043) in evening cortisol among firefighters in the sleep restriction condition. Higher morning IL-6 levels were related to increased (5.5%; p = 0.048) daily cortisol levels, but this relationship was not different between conditions. Less pronounced relationships were demonstrated between TNF-α, IL-10, IL-4 and cortisol independent of the sleep opportunity, but relationships did not persist after adjusting for demographic factors and other cytokines.

Conclusions: These findings quantify the relationship between cytokine and cortisol responses among wildland firefighters exposed to simulated occupational stressors. Potential disturbances to the IL-6 and cortisol relationship among sleep-restricted firefighters' supports further investigations into the negative health effects related to possible imbalances between these systems.

Introduction

A fundamental relationship exists between the immune and endocrine systems to modulate an adequate response to physiological and psychological stressors (Turnbull and Rivier, 1999, McEwen et al., 1997, Elenkov, 2008, Elenkov and Chrousos, 2002). The precise physiological mechanisms underlying this relationship are not fully understood. Evidence does suggest that activation of a bi-directional feedback loop between the end-products, cytokines and cortisol, is central to the appropriate functioning of the hypothalamic-pituitary-adrenal (HPA)-axis, while maintaining homeostasis of the immune system (Petrovsky, 2001, Turnbull and Rivier, 1999). For example, exposure to a stressor and the subsequent release of certain inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α), Interleukin (IL)-10 and IL-6 activate the HPA-axis and cause the release of cortisol (Turnbull and Rivier, 1999, Steensberg et al., 2003). The anti-inflammatory effects of cortisol then feed back and suppress further release of cytokines (Turnbull and Rivier, 1999, Riechlin, 1993, Chrousos, 1995, Elenkov and Chrousos, 1999).

Whilst the effect of cortisol on the immune system has been typically shown to be immuno-suppressive in nature, cortisol can also be immuno-modulatory (Desantis et al., 2012, Elenkov and Chrousos, 2002, Elenkov, 2008, McEwen et al., 1997). For instance, exposure to chronic or severe stressors can cause prolonged activation of the HPA-axis and excessive cortisol release, which is thought to contribute to inflammation by impairing the function of glucocorticoid receptors (e.g., down regulation, reduced expression, nuclear translocation; Mackin and Young, 2004, Kunz-Ebrecht et al., 2004, Silverman and Sternberg, 2012). Glucocorticoid receptor abnormalities reduce the immune system's capacity to respond to cortisol and lower inflammation, resulting in concurrently sustained levels of cytokine and cortisol

release (Miller et al., 2002, Chrousos, 1995, Elenkov and Chrousos, 1999). Altered immune-endocrine interactions have been linked to adverse health outcomes including coronary artery disease (Nijm and Jonasson, 2009) and depression (Lutgendorf et al., 2008, Haddad et al., 2002). It is therefore important to further explore cytokine and cortisol relationships to provide evidence-based recommendations for firefighting populations where there is a high prevalence of mood disorders (An et al., 2015, Carey et al., 2011, Cook and Mitchell, 2013) and cardiovascular disease (CVD)-related events and/or risk factors (Wolkow et al., 2014c, Kales et al., 2007).

Research has demonstrated that the combined stressors of sleep restriction and physical work, which are common in emergency service occupations such as firefighting (Aisbett et al., 2012), can result in either increased cortisol (Wolkow et al., 2014b, Opstad, 1994, Opstad and Aakvaag, 1981) or altered cytokine levels (Abedelmalek et al., 2013, Gundersen et al., 2006, Lundeland et al., 2012, Wolkow et al., 2014a). However the degree to which cytokine and cortisol activity are related in the context of emergency work demands is unknown. Research has focused on a large population of healthy adults (Desantis et al., 2012), clinical samples and/or experimental manipulation, such as exogenous cortisol or acute exercise (DeRijk et al., 1997, Miller et al., 2002, Nijm and Jonasson, 2009, Pledge et al., 2011). Findings in response to physical stressors have been equivocal. For instance, following a graded treadmill exercise test to 100% VO_2max, cortisol appeared to supress IL-1β and TNF-α, but had no effect on IL-6 production (DeRijk et al., 1997). Meanwhile, a more recent study by Pledge et al. (2011) found no association between cortisol and IL-6 in response to a 45-minute resistance exercise protocol performed in the morning and evening. Furthermore, a review by Gómez-González and colleagues (2012) proposed that because sleep loss alters hormone and cytokine release, sleep loss further

compromises the integrity of immune-endocrine interactions, though research examining the relationships between responses is needed. Firefighters exposed to multiple days of physical firefighting work separated by either an 8-h or restricted 4-h sleep exhibited significant changes in inflammatory cytokines (IL-6, TNF-α, IL-8, IL-1β and IL-4) and daily cortisol levels (Wolkow et al., 2014a, Wolkow et al., 2014b). Determining if these changes in cortisol and cytokine levels are related is important in understanding the physiological interactions underlying these responses to physical work and sleep restriction. Further knowledge of immune-endocrine relationships may help identify early indicators of chronic health outcomes associated with a dysregulation between inflammatory and cortisol responses (Haddad et al., 2002, Lutgendorf et al., 2008, Nijm and Jonasson, 2009).

Therefore, the aim of the present study was to examine whether there was a relationship between cytokines and morning, evening and total daily cortisol output and if the observed relationships were altered by sleep restriction. To quantify potential relationships between physiological responses, cytokine and cortisol samples were obtained simultaneously at frequent intervals among firefighters completing a 3-day and 2-night simulated fire-ground deployment, with and without sleep restriction each night. Specifically, we hypothesised that sleep restricted firefighters would have increased IL-6, IL-1β, IL-8 and TNF-α related to higher cortisol levels.

Materials and Methods

Participants

This study was based on data from 30 male and 5 female wildland firefighters (32 volunteer, 3 salaried personnel) who underwent a simulated 3-day fire-ground deployment. Participants were recruited from Australian state and territory-based fire

agencies (Victoria, Tasmania, Australian Capital Territory, South Australia and New South Wales) and included in the study if they had not been diagnosed with any form of respiratory or sleep disorders, heart disease or diabetes. For purposes of analyses, participants were matched for age, sex and body mass index (BMI) and then randomly assigned to either a control (CON) or sleep restriction (SR) condition based on the participant's availability to attend the testing period, assigned at random to the condition. This method of randomization accounted for firefighters availability to volunteer their time for the study (without financial compensation), ensuring an equal chance for each participant being allocated to either condition. There were no significant differences between conditions in BMI ($p = 0.113$), age ($p = 0.913$), pre-simulation physical activity levels ($p = 0.372$) or years of firefighting experience ($p = 0.593$; Table 5.1). Participants completed a short occupational and firefighting history questionnaire and were assessed pre- and post-testing to exclude anyone who sustained an injury or became ill directly prior or during testing that could influence the inflammatory or cortisol levels measured and confound any subsequent comparisons. One firefighter in the SR condition withdrew due to injury and therefore, a final sample of 17 firefighters in the SR condition and 18 firefighters in the CON condition completed this study. Information on injury, illness, and health outcomes were obtained from participants pre- and post-study, but this data was only used for study exclusion purposes and will not be described in any further detail. Participation was voluntary and all firefighters provided written informed consent prior to commencing data collection. This study was approved by the institutions Human Research Ethics Committee and all procedures were in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Table 5.1 Characteristics of firefighters in CON and SR conditions

Characteristic	CON (*n* = 18)	SR (*n* = 17)
Age (years)	39 ± 16	39 ± 15
Men:Women	15:3	15:2
Weight (kg)	84.9 ± 17.8	93.8 ± 20.2
Height (cm)	178.1 ± 7.7	177.8 ± 7.4
BMI (kg/m^2)	26.8 ± 5.0	29.6 ± 5.5
Firefighting experience (years)	6 (min-max 1.0-39.0)	10 (min-max 1.0-20.0)
Pre-simulation (16-h) physical activity (total activity counts)	305233 ± 34369	256726 ± 19609

Note: BMI = Body Mass Index; Age, Weight, Height and BMI are presented as mean ± standard deviation; Firefighting experience is presented as median years and minimum-maximum years; For ease of interpretation, pre-simulation log physical activity data was back-transformed to total activity counts.

On arrival at the testing venue participants completed a familiarization session of the physical work tasks and physiological tests, followed by an adaptation night (8-h sleep opportunity) in the testing environment. Participants then completed a 3-day and 2-night simulated fire-ground deployment. On each night, participants in the CON condition had an 8-h sleep opportunity (i.e., bedtime 22:00-06:00), while participants in the SR condition had a 4-h sleep opportunity (i.e., bedtime 02:00-06:00). Sedentary factors such as extended travel time between the fire front and the staging area (i.e., campsite) or home, difficulty sleeping in an unfamiliar and noisy environment at the staging area and winding down after a shift can contribute to sleep restriction on the fire-ground (Cater et al. 2007). To reflect these conditions, participants in the SR

condition were free to perform sedentary leisure activities (e.g., watching television, reading etc.) until the delayed bedtime. The duration of sleep restriction in this study was based on Australian wildland firefighters' self-reported average sleep per rest period on the fire-ground (Cater et al., 2007). Both conditions received an 8-h recovery sleep opportunity after testing to ensure that all participants were rested before leaving the testing venue. Participants slept on camp beds in the simulated testing environment to replicate sleeping conditions when deployed to the fire-ground (Cater et al., 2007).

All testing procedures were performed in a 9 × 13 metre room that was climate-controlled. Windows were blacked out and ceiling room lights turned on during each wake period (i.e., lights on between 06:00-22:00 in the CON condition and 06:00-02:00 in the SR condition) and turned off each night during the sleep period (i.e., lights off between 22:00-06:00 in the CON condition and 02:00-06:00 in the SR condition). Therefore, participants were not time isolated, as they knew when it was night time and day time, thus preventing the potentially confounding influence a desynchronisation between the external environment and internal physiological rhythms has on cortisol and cytokine responses. Throughout the protocol, the testing environment was maintained at moderate temperatures (18-20^{o}C) in both conditions using split cycle air-conditioners (Daikin Industries Ltd, Japan). Ambient air temperature was monitored using a wireless temperature and humidity logger (HOBO ZW_003, One Temp Pty Ltd, Australia), data receiver (HOBO ZW_RCVR, One Temp Pty Ltd, Australia) and software (HOBO Pro Software, One Temp Pty Ltd, Australia). Adhering to fire-ground practices, food and drink intake during the study was ad libitum and the amount of fluid ingested was recorded. This data was then extracted using the FoodWorks 7 nutrition software (2012 Xyris Software Pty Ltd, Australia).

Although food intake was recorded, the measurement of daily fluid consumption from food and drink combined has only been reported in the results section.

Participants in both conditions were tested in groups of 3 to 5. All participants completed a 2-h testing block, 3 times on day 1 and 5 times on day 2 and day 3 (Figure 5.1). Each testing block consisted of a 55-minute work circuit simulating physical wildland firefighting work (Phillips et al., 2012), followed by physiological (20-25 min) and cognitive (20-25 min; to be reported elsewhere) data collection periods and a 15-20 min rest period. The time allocated to physical work and rest periods described above reflects the physical activity profile observed across a shift on the fire-ground (Phillips et al., 2011, Aisbett et al., 2007, Raines et al., 2013).

*Simulated Physical Firefighting Work Circuit**:*** The physical work circuit comprised 6 simulated wildland firefighting tasks designed to mimic the physical demands involved in Australian wildfire suppression work (Phillips et al., 2012, Ferguson et al., 2011, Vincent et al., 2015). A job task analysis was used to design the firefighting work circuit. Each of the tasks identified for inclusion in the circuit have been verified by panels of firefighter subject matter experts (including senior operational firefighters, occupational health and safety personnel and training officers) as being representative of the movements that encompass key firefighting tasks performed frequently on the fire-ground by each of Australia's state and territory-based fire agencies (Ferguson et al., 2011, Phillips et al., 2012). This maximised the generalisability of potential findings to firefighters across Australia. The firefighting tasks included; lateral repositioning of a hose, rake-hoe work, hose rolling, charged hose advance, black out hose work, and static hold of a hose. The performance of each physical task (i.e., repetitions completed for each task within each work period) was

self-paced and completed in a pre-determined order with work-to-rest ratios designed to mimic the performance of these tasks on the fire-ground (Ferguson et al., 2011, Vincent et al., 2015, Phillips et al., 2015).

Blood Sampling and Cytokine Analysis: Fingertip capillary blood samples were collected to determine IL-6, IL-8, IL-1β, TNF-α, IL-4 and IL-10 cytokine levels in blood plasma at 4 time points each day: a fasting sample in the morning (i.e., 06:15), late morning (i.e., 11:30), evening (i.e., 18:15) and at night (i.e., 21:30; Figure 5.1). To prevent the potential for acute postprandial changes in cytokine levels, samples collected at 11:30 and 18:15 were completed before lunch and dinner respectively. Prior to sample collection, participants held a heat pack in their hand to aid in blood flow to the fingertips. At each time point, a 500-μL sample of whole blood was collected in to a microtainer coated with K_2 EDTA (Becton Dickinson ref: 365974). Whole blood samples were centrifuged for 10 minutes at 5000 revolutions/minute (83 hertz) and the plasma was separated and stored at <-80°C. Although previous emergency service-based studies have used venous blood samples when investigating cytokine levels (Bøyum et al., 1996, Gundersen et al., 2006, Lundeland et al., 2012), capillary blood samples were chosen because it is a minimally invasive method to conveniently obtain multiple daily blood samples from participants wearing personal protective clothing and performing repeated bouts of physical work. Some studies suggest that, due to a small local inflammatory response to the pinprick, capillary blood samples can result in higher cytokine levels (Cullen et al., 2015, Eriksson et al., 2007). However, recent evidence indicates a close correlation between venous and capillary plasma IL-6 responses at rest (Faulkner et al., 2014), as well as during and post-exercise (Cullen et al., 2015, Faulkner et al., 2014). Conversely, other reports have found that venous and capillary concentrations of TNF-α (Eriksson et al., 2007)

and IL-6 (Cullen et al., 2015) differed at rest. However, these studies (Cullen et al., 2015, Eriksson et al., 2007) did not control factors known to impact resting cytokine levels such as the time of day the sample was taken or whether or not the sample was taken under fasting conditions (Zhou et al., 2010). Control over these factors in the current study limits their potentially confounding influence on cytokine levels at rest.

The Milliplex Human MAP Cytokine immunoassay kit (Millipore, Billerica, MD) was used to profile the expression of inflammatory markers in the plasma samples. The assay was performed according to the manufacturer's instructions on the Bioplex 200 array reader (V.5.0, Bio-Rad Laboratories, Hercules, CA). This involved ensuring that quality control samples were run with each cytokine assay. The minimal detectable concentrations were 0.06 pg/mL, 0.42 pg/mL, 0.20 pg/mL, 0.05 pg/mL, 0.48 pg/mL and 0.07 pg/mL for IL-1β, IL-4, IL-6, IL-8, IL-10 and TNF-α, respectively. Cytokines intra- and inter-assay coefficients of variation (CV) were within acceptable ranges (Intra-assay 4.5 – 10.0%; Inter-assay 9.8 – 20.5%) for all analytes (CV <25%; Findlay et al., 2000, Chowdhury et al., 2009) and comparable to CVs reported for cytokines sampled using venous blood in previous exercise-based literature (Abedelmalek et al., 2013, Cullen et al., 2015, Faulkner et al., 2014).

Saliva Sampling and Cortisol Analysis: Salivary samples were collected using a cotton swab (Salivette; Sarstedt, Nümbrecht, Germany) at baseline (i.e., 07:30) and at the completion of each physical work circuit (i.e., 09:00, 11:15, 13:30, 15:30, 17:30). Further daily samples were taken in both conditions after awakening (i.e., 06:30) and in the evening (i.e., 19:30 and 21:30; Figure 5.1). To prevent sample contamination, participants were not allowed to eat or drink 15 minutes prior to saliva collection. All samples were stored at <-80°C. Samples were then thawed and centrifuged for 10

minutes at 5000 revolution/minute (83 hertz) before assessing salivary cortisol concentration using a high sensitivity enzyme immunoassay ELISA kit (IBL International, Hamburg, Germany). The assay was performed according to the manufacturer's directions and read at 450 nm on a luminescence microplate reader (Synergy™ 2 SL, BioTek, Winooski, VT). Analytical sensitivity (lower limit of detection) was 0.14 nmol/L and the intra- and inter-assay CV were 7.2% and 10.7% (both mean 13.8 nmol/L) respectively, which are within the acceptable ranges (i.e., Accuracy <15%; Intra-assay CV <10%; Inter-assay CV <15%; Biopharmaceutics Coordinating Committee, 2001, Nicolson, 2008).

Sleep and Physical Activity Monitoring: Participants' sleep was recorded using the Siesta Portable EEG system (Compumedics E-Series; Melbourne, Victoria, Australia) and standard polysomnographic (PSG) montage. Each night, PSG recording began at 21:00 for both conditions. From each sleep period, participants' total sleep time (minutes) was calculated. In addition, participants wore activity monitors (*Actical* MiniMitter/Respironics, Bend, OR, USA) to measure sleep across the 2 nights prior to the study. Further information on participants' physical activity 16 h prior to the simulation was provided through the use of activity monitors, which were set to sample in 1-minute epochs, with a sensitivity of <40 counts per epoch to distinguish between sleep and wake states (Darwent et al., 2008). Activity data was downloaded using Actical software (version 3.10, MiniMitter/Respironics, Bend, OR) and analysed and expressed using total activity counts.

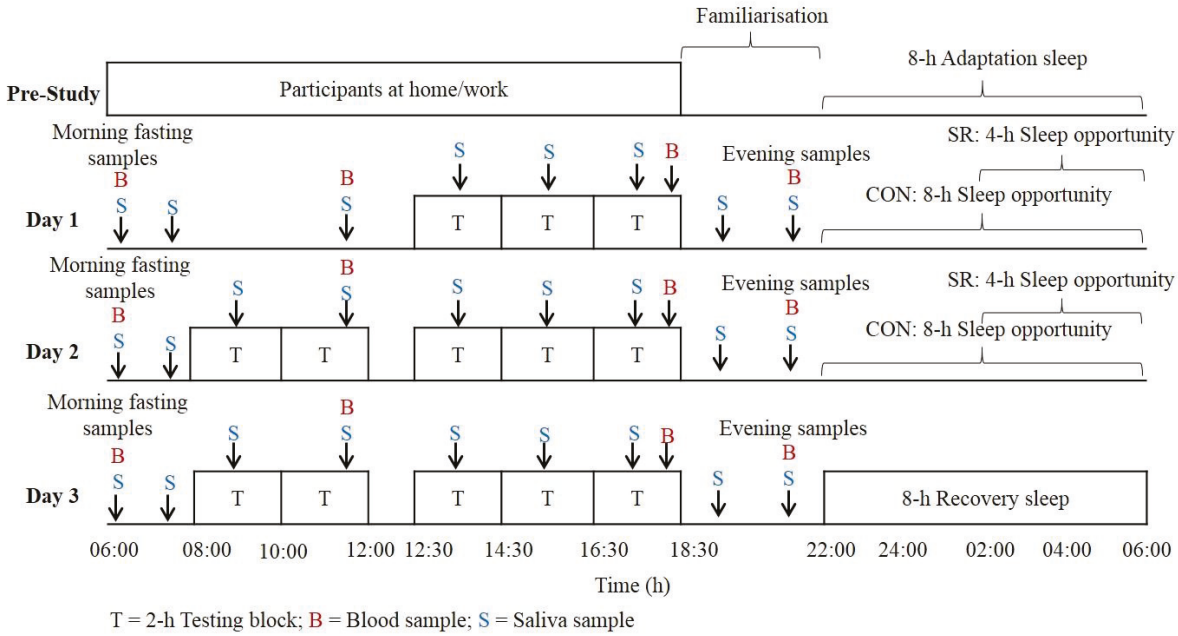

Figure 5.1 Study protocol for the CON and SR condition

Statistical Analyses

Prior to the analysis, cytokine values greater than two standard deviations above (IL-4 = 5% of sample; IL-6 = 6% of sample; IL-1β = 6% of sample; IL-8 = 6% of sample; IL-10 = 5% of sample; TNF-α = 4% of sample) the mean were considered outliers and subsequently excluded (Nguyen et al., 2010, Finnerty et al., 2008). Values below the detectable range of the Milliplex Human MAP Cytokine immunoassay kit (IL-6 = 0.9% of sample; IL-1β = 0.2% of sample; IL-8 = 0.7% of sample; IL-10 = 1.4% of sample; TNF-α = 0.2% of sample, IL-4 = 18% of sample) were replaced with the minimal detectable concentration as advised in the protocol (Liberati et al., 2013, Weiskopf et al., 2009). With the exception of TNF-α (for which raw values achieved normality and homogeneity of variance), all cytokine, cortisol and activity count measurements were adjusted using a natural log-transformation to achieve normality, assessed using the Shapiro-Wilk test ($p > 0.05$). Normality and homogeneity of variance of the residuals were further assessed by inspection of the resulting mixed model analysis (Field, 2009). The resultant diagnostic plots revealed no departures from these required assumptions. Due to the sampling design, a measurement for cortisol at 09:00 was missing for all participants on day 1. Consequently, missing value codes were appended to the data set, but this did not affect the statistical analyses of the data.

Sleep duration, physical activity and demographic characteristics were analysed with the Analysis of Variance (ANOVA) method using GenStat software (GenStat *for Windows* 16.1 Edition. VSN International, Hemel Hempstead, UK). To assess the possible relationships between inflammatory and cortisol responses, cytokine levels were analysed in relation to cortisol parameters which included morning (i.e., 06:30) and evening cortisol levels (i.e., 21:30) and total daily cortisol output. Total daily

cortisol was determined using the area under the curve (AUC) with respect to ground, which was calculated for each participant on each day using the trapezoidal method (Pruessner et al., 2003). To investigate possible relationships, the cortisol parameters were modelled as a function of morning fasting cytokine levels (i.e., 06:15) and across daily cytokine levels (i.e., 06:15, 11:30, 18:15 and 21:30) in separate models. For repeated cortisol and cytokine measurements nested within days, subjects and groups, linear mixed models (LMM) were fitted by the restricted maximum likelihood (REML) method (Payne et al., 2011) using GenStat software (GenStat *for Windows* 16.1 Edition. VSN International, Hemel Hempstead, UK). This method allows for the possibility of autocorrelation in the repeated cytokine or cortisol measurements (i.e., days) on each individual by including a model for the covariance structure.

The final 'full' model fitted to morning, evening and AUC cortisol parameters included potential fixed and interaction effects critical to the design of the study, which included condition and day, along with a two-way interaction of condition by day. To investigate possible relationships between the cytokine measures and cortisol parameters, this final model fitted potential fixed effects of each cytokine along with two-way interactions of condition by cytokine, day by cytokine and potential three-way interactions of condition by day by cytokine. For each model, random effects of group, profile (or participant) and a group by profile interaction were investigated both without (i.e., Independence model) and with an Unstructured covariance model for the within-subject autocorrelation.

Models fitted to the cortisol parameters investigated each of the cytokines separately and all in one model to examine their independent relationships with each of the cortisol variables. All models were fit with and without controlling for the

demographic factors of sex, age and BMI. Model fit was assessed by Akaike Information Criterion (AIC) and small differences (ΔAIC) in this criterion compared to other candidate models were used to identify parsimonious models (Burnham and Anderson, 2002). Statistical significance was set at $p < 0.05$ and slopes of potential interactions are represented using regression (unstandardized) coefficients (b) with mean percentage changes presented.

Results

Sleep, Pre-study Physical Activity and Daily Fluid Consumption

Sleep duration in the 2 nights prior to the study was not significantly different to the adaptation night or between conditions ($p > 0.05$; Table 5.2). The average total sleep time measured for both conditions was similar on the adaptation night ($p > 0.05$; Table 5.2). During nights 2 and 3 of the simulation, total sleep time was as expected, given the sleep opportunity provided in the CON and SR conditions ($p < 0.001$; Table 5.2). Furthermore, there were no differences between conditions in participants' physical activity levels in the 16-h prior to beginning the study (total physical activity counts $p > 0.05$; Table 5.1). The firefighting history questionnaire also revealed that no firefighters attended a firefighting emergency in the 24 h prior to beginning the study. No between-condition differences in pre-simulation sleep and physical activity/work ensures that on entering the study, both groups had experienced a similar level of acute stressors, minimising the impact prior exposure could have on subsequent cortisol and cytokine measures. In addition, participants' mean daily fluid intake was similar between conditions on each of the testing days ($p > 0.05$).

Table 5.2 Total sleep time (mean ± standard deviation) for each night in both conditions (h)

Night	CON	SR
Pre-study 1	7.3 ± 1.4	6.7 ± 0.9
Pre-study 2	6.7 ± 1.3	6.2 ± 1.4
1 (adaptation)	6.3 ± 0.9	6.4 ± 0.7
2	6.9 ± 0.4	3.6 ± 0.2*
3	6.9 ± 0.5	3.7 ± 0.2*

* = $p < 0.001$ between conditions

Cortisol and Cytokine Relationships

The LMM with the lowest AIC for each of the cytokines was the full fixed effects Independence model which, after inspection of the ΔAIC, had the best fit to model the relationship between cytokine and cortisol parameters. While there were no significant relationships between any of the cytokines investigated and morning cortisol levels ($p > 0.05$), significant relationships between morning cytokine levels and cortisol AUC were demonstrated. A main effect for IL-6 indicated that higher morning levels of this cytokine were positively related with greater cortisol AUC levels independent of condition ($p < 0.001$; Table 5.3). For this relationship, a one Standard Error unit increase in morning IL-6 was related to a 5.3% rise in AUC, and a 5.5% increase after controlling for demographic factors ($p = 0.04$; Table 5.3). The relationship between morning IL-6 and cortisol AUC remained significant after controlling for the other cytokines and demographic factors ($p = 0.048$; b = 0.4923; SE = 5.921), demonstrating that the association is independent of IL-8, IL-1β, TNF-α, IL-4 and IL-10, and BMI, sex and age. A further two-way interaction of condition by morning IL-6 for cortisol AUC indicated a steeper rise in AUC for the SR condition when IL-6 levels increased

(p = 0.033; SR b = 4.324, SE = 0.816; CON b = 1.889, SE = 1.122). However, this interaction was no longer significant after controlling for demographic factors (p > 0.05).

Significant relationships between morning TNF-α and IL-10 with cortisol AUC were also demonstrated. For instance, positive (increasing) cortisol AUC levels were related to a rise in morning TNF-α (p = 0.049; 0.3% increase in AUC; Table 5.3). Conversely, there was a negative (decreasing) association for cortisol AUC when morning IL-10 levels increased (p = 0.029; 2.7% decrease in AUC; Table 5.3). But when controlling for other cytokines and demographic factors, the relationships for morning IL-10 and TNF-α were no longer significant (p > 0.05). No significant interactions involving condition and/or day were found when investigating the relationship between these cytokines and cortisol AUC, nor were there relationships between morning levels of IL-4, IL-1β and IL-8 with cortisol AUC.

Investigation of possible relationships between cytokine profiles measured across the day (i.e., 06:15, 11:30, 18:15 and 21:30) and cortisol AUC revealed significant relationships involving the IL-6 and IL-10 daily profiles, independent of the sleep opportunity. A main effect indicated positive (increasing) cortisol AUC levels when IL-6 increased across the day (p = 0.047; b = 0.240; 1% increase in cortisol AUC), but this interaction did not persist after controlling for demographic factors and other inflammatory cytokines. When demographic factors were adjusted for, a significant interaction between day and daily IL-10 measurements (p = 0.023) indicated that higher levels of IL-10 were related to an increase in cortisol AUC on day 2 (b = 0.892; 2.3% increase in cortisol AUC), while slight negative and positive relationships were demonstrated for this parameter on day 1 (b = -0.103; -0.4% decrease in cortisol AUC)

and day 3 (b = 0.152; 0.4% increase in cortisol AUC) respectively. But this relationship did not persist after controlling for other cytokines ($p = 0.07$).

Interactions between condition and morning IL-6 levels were demonstrated for evening cortisol. In the SR condition, this interaction showed that a rise in morning IL-6 was related to an elevation in cortisol that evening, after controlling for demographic characteristics ($p = 0.043$; b = 0.300, SE = 0.154). In the CON condition however, this interaction indicated that when morning IL-6 levels increased, evening cortisol levels decreased slightly (b = -0.136, SE = 0.210). A one Standard Error unit increase in morning IL-6 resulted in a 6.2% rise in evening cortisol for the SR condition and 2.5% decrease in this parameter for the CON condition. The relationships were significant but partially attenuated after controlling for the other cytokines (5.7% increase in SR, b = 0.876, SE = 0.288; 0.5% decrease in CON, b = -0.914, SE = 0.332; $p = 0.039$). A further two-way interaction indicated that a rise in IL-4 levels was related to positive (increasing) evening cortisol levels in the CON condition (b = 0.110), while in the SR condition this interaction demonstrated negative (decreasing) evening cortisol levels when IL-4 increased (b = -0.081; $p = 0.045$). However, this relationship was no longer significant after adjustment for demographic factors ($p > 0.05$).

Table 5.3 Main effects of each cytokine with cortisol AUC with and without controlling for demographic factors

Parameter		Morning Fasting Sample					
		IL-6	TNF-a	IL-8	IL-1β	IL-10	IL-4
Models uncontrolled for demographic factors (i.e., age, BMI and sex)							
Cortisol AUC	b	1.525	0.386	0.149	0.207	-0.676	-0.571
	(SE)	(1.104)	(0.238)	(1.296)	(0.700)	(1.412)	(0.589)
	F	15.42	4.01	0.11	0.27	4.94	0.80
	p	<0.001	0.049	0.738	0.602	0.029	0.372
	%	5.3	0.3	0.6	0.4	-2.7	-1.2
Models controlled for demographic factors (i.e., age, BMI and sex)							
Cortisol AUC	B	1.337	0.270	0.905	1.275	-3.539	-0.847
	(SE)	(1.244)	(0.291)	(1.295)	(0.856)	(1.575)	(0.666)
	F	4.41	1.00	0.49	2.76	0.44	0.06
	p	0.040	0.321	0.486	0.103	0.509	0.801
	%	5.5	0.3	4.3	4.4	-14.3	-2.3

Note: AUC = Area Under the Curve; BMI = Body Mass Index; b = regression (unstandardized) coefficients

Discussion

Findings from the current study quantify the relationship between cytokine and cortisol levels among firefighters exposed to simulated occupational demands. In response to the combined stressors of shortened sleep and physical work, an increase in morning IL-6 was related to a rise in evening cortisol among firefighters in the SR condition and decreased evening cortisol in the CON condition. This relationship remained significant when controlling for other cytokines and demographic factors. A positive association was also demonstrated in the SR condition between the daily IL-6 profile

and cortisol AUC, but this did not persist when demographic factors were included in the analyses. After controlling for demographic factors, a rise in morning IL-6 was found to further relate to increased cortisol AUC, independent of condition. Less pronounced relationships were also demonstrated between TNF-α, IL-10, IL-4 and cortisol.

When the firefighters were sleep restricted, the detected relationship between IL-6 and cortisol may be reflective of how elevated IL-6 stimulates increased corticotropin-releasing hormone secretion as well as arginine vasopressin and other corticotropin secretagogues (Chrousos, 1995, Petrovsky, 2001), which lead to the more pronounced increase in evening cortisol. However, an 8-h sleep opportunity between shifts alters this relationship so that a rise in IL-6 no longer relates to increased evening cortisol. It is possible the interactions revealed here between IL-6 and cortisol may explain how restricted sleep and physical work in our previous study resulted in an elevated cortisol profile in the afternoon and evening when compared to physical work and an 8-h sleep (Wolkow et al., 2014b). Elevated evening (and afternoon) cortisol has been linked to insulin-resistance and impaired memory (Spiegel et al., 1999, Dallman et al., 1993, McEwen, 1998). The current study therefore suggests that despite a rise in morning IL-6, an 8-h sleep opportunity between firefighting shifts mitigates subsequent increases in evening cortisol, offering a protective buffer against adverse health effects.

A positive relationship between IL-6 and evening cortisol in the sleep restricted condition and cortisol AUC independent of condition, appears in contrast to the anti-inflammatory effects of this hormone (Chrousos, 1995). However, this finding is consistent with research suggesting that IL-6 is more resistant to the effects of cortisol

under physical stress (i.e., exercise test to 100% VO_2max; DeRijk et al., 1997). In contrast, no relationships were found between IL-6 and cortisol among healthy males following acute resistance training (Pledge et al., 2011). Meanwhile, to our knowledge, this is the first study to have investigated how sleep influences cytokine and cortisol relationships. The limited and equivocal findings highlight the need for further research conducted among larger samples of healthy adults that examines the relationship between cytokine and cortisol responses to physical work and sleep restriction. Findings in older adults and clinical populations indicate that disturbances to the normal actions of cortisol and cytokines result in elevated IL-6 and evening cortisol and cortisol AUC (Nijm and Jonasson, 2009, Nijm et al., 2007, Lutgendorf et al., 2008), similar to that demonstrated among the current group of firefighters.

Excessive long-term exposure to cortisol may down regulate hormonal receptors and thereby impair the immune systems response to cortisol's anti-inflammatory actions (Desantis et al., 2012, Miller et al., 2002, Chrousos, 1995, Elenkov and Chrousos, 1999). Accordingly, repeated firefighting deployments across a fire season which expose personnel to sleep restriction and physical work could prolong the observed alterations in IL-6 and evening cortisol, resulting in negative physical (e.g., CVD; Nijm et al., 2007) and mental health outcomes (e.g., depression; Lutgendorf et al., 2008, Haddad et al., 2002). Therefore, acute immune-endocrine relationships observed in the current study could be an early indicator of chronic, firefighter-relevant health outcomes associated with dysregulation to these systems. For instance, high levels of depression have been reported among firefighters (Carey et al., 2011, An et al., 2015, Cook and Mitchell, 2013), while fire suppression activities were associated with an increased risk of death from coronary heart disease (Kales et al., 2007).

Short-term elevations in HPA-axis and immune activity have also been related to acute changes in mood (Vgontzas et al., 2008, Kemeny, 2007). Mood has been shown to impact factors important to occupational settings such as worker helpfulness (Carlson et al., 1988), job satisfaction (Fisher, 2000) and the probability of making an error (Appel et al., 1980). For example, Christoforou et al. (2013) found that compared to firefighters who had an 8-h sleep, those who had a 4-h sleep opportunity demonstrated reduced attention over the multiday firefighting simulation. Therefore, it is important to determine how, in response to sleep restriction and physical work, acute changes in mood may relate to disturbances between cytokine and cortisol interactions in the short-term. However, based on the bi-directional relationship between cytokines and cortisol (Petrovsky, 2001, Turnbull and Rivier, 1999), further research including experimental alterations to cortisol or cytokine levels is also needed to understand the causal direction of the observed relationships.

The positive association between morning IL-6 and cortisol AUC (5.5% increase) independent of sleep, is consistent with DeSantis and colleagues (2012) who reported a similar increase (6.5%) in AUC with elevated levels of IL-6 among healthy adults examined under naturalistic settings. Adjustment for levels of other cytokines and demographic factors preserved the positive association between IL-6 and cortisol AUC for firefighters. Similar increases in cortisol AUC have been associated with elevated levels of daily negative affect (i.e., 11%; Piazza et al., 2013), but it is currently unclear how magnitude changes in AUC relate to other health outcomes (e.g., atherosclerosis, metabolic syndrome). The detected rise in firefighters' cortisol AUC after controlling for covariates was greater than that reported (7.4% in AUC) by DeSantis et al. (2012) who controlled for TNF-α and IL-10. However, compared to the naturalistic setting investigated by DeSantis (2012), it is possible the simulated firefighting stressors in

the current study contributed to the larger rise in cortisol AUC. In particular, the 4-h sleep restriction period did not appear to influence the association between morning IL-6 and cortisol AUC, which may highlight that exposure to the physical work demands was the major stressor impacting this relationship. Furthermore, DeSantis and colleagues (2012) controlled for additional confounders (e.g., race/ethnicity, income/wealth, cynical distrust and smoking) that were not measured among the current population of firefighters. Failure to record and control for these behavioural and sociodemographic factors reported to influence the HPA-axis (Ranjit et al., 2009, Hajat et al., 2010) is a limitation of the current study that could also explain the greater rise in firefighters' cortisol AUC.

Altered hydration levels lead to variations in plasma volume, which can further contribute to changes in cytokines and cortisol (Hill et al., 2008, Steptoe et al., 2007). Unfortunately plasma volume was not measured among firefighters and therefore unaccounted for in the current study. Despite this limitation, there were no differences in daily fluid consumption between conditions and fluid intake was similar to levels demonstrated among firefighters completing multiple days of physical work in the heat, reported to be hydrated as determined using urine specific gravity (Larsen et al., 2015). Moreover, daily water intake exceed recommended levels for adult males performing exercise in mild conditions (Kenefick and Sawka, 2007, Gunga et al., 1993). It is therefore unlikely that perturbations in hydration in the current study impacted cytokine and/or cortisol levels. Further understanding the relationships between these physiological responses among firefighters or other occupational groups exposed to physical work and sleep restriction requires additional hydration measures (i.e., plasma volume) and larger samples with sufficient power to examine a range of sleep durations and other potential sociodemographic and behavioural factors.

In the present study, relationships between TNF-α, IL-10, IL-4 and cortisol were less pronounced than for IL-6 and no longer significant after adjusting for demographic factors and/or other cytokines. Despite this, these findings are the first to show how occupational stressors influence the relationship between TNF-α, IL-10, IL-4 and cortisol, and therefore, should still be considered, but with caution. For instance, higher morning levels of TNF-α were related to an increase in cortisol AUC independent of the sleep opportunity across the simulation. While cortisol has been found to interact with TNF-α, findings are mixed with reports indicating that exogenous cortisol can potentiate TNF-α levels in rats (Frank et al., 2010) or suppress TNF-α among humans (DeRijk et al., 1997). Stressor-induced cortisol levels, attained through maximal exercise (i.e., 100% VO_2max), can also suppress TNF-α production (DeRijk et al., 1997). Furthermore, across the simulation an increase in morning IL-10 was related to a decrease in cortisol AUC independent of the sleep opportunity. Higher daily levels of IL-10 were also related to an increase in cortisol AUC on day 2 of the simulation, while smaller negative and positive relationships were demonstrated for this parameter on day 1 and day 3 respectively. Between day differences provide further evidence for the role IL-10 plays in up- and down-regulating the cortisol response to acute stressors (Smith et al., 1999, Elenkov and Chrousos, 1999). Furthermore, the observed associations between, IL-10, TNF-α and cortisol were independent of the sleep opportunity, highlighting that exposure to the physical work demands was the major stressor influencing these relationships in the current study. Consistent with our results that higher IL-4 is related to increased and decreased evening cortisol in the CON and SR conditions respectively, evidence indicates that IL-4 affects cortisol release (Woods and Judd, 2008) by inducing the expression of enzymes involved in regulating this hormone (Thieringer et al., 2001). However, because the relationship between firefighters' IL-4, TNF-α and IL-10 and cortisol AUC did not persist after adjusting

for demographic factors and/or other cytokines, further research should include larger samples with the statistical power to include covariates such as race/ethnicity and smoking (Desantis et al., 2012) in to the analyses.

While further relationships may exist between IL-8 and IL-1β, and cortisol (DeRijk et al., 1997, Corsini et al., 2014), research involving IL-8 and IL-1β, and to an extent IL-4, has been centred on acute immune responses to more intense experimental stressors such as maximal exercise and large doses of exogenous cortisol and cytokines (Corsini et al., 2014, DeRijk et al., 1997, Thieringer et al., 2001)., Moreover, research investigating relationships between IL-8, IL-1β and cortisol has, to date, been conducted in animal models or *in vitro* experiments. Therefore, in response to the moderate stressors studied, immune-endocrine interactions between these cytokines (i.e., IL-8, IL-1β and IL-4) and cortisol are unlikely.

The current study matched firefighters in the CON and SR conditions for age, BMI and gender. However, because a crossover design was not utilised, there is a possibility that intra-group differences may explain the divergent relationships found for IL-6 and cortisol between conditions. For example, firefighters in the SR condition may have had less experience performing the type of physical work tested when compared to the CON condition, thus resulting in the positive immune-endocrine relationship observed for the sleep restricted firefighters. However, employing mixed models for the analyses, factor in unique responses of participants to an intervention (Van Dongen et al. 2004), and therefore, should account for the effects of individual subject variability.

By closely replicating many aspects of a fire-ground deployment, the simulated environment further permitted the quantification of physiological stress responses during periods of controlled physical work and sleep restriction. For instance, the physical work tasks chosen simulate preparatory or post-fire clean-up work that comprises a large component of firefighting (Rodríguez-Marroyo et al. 2012), but is performed in cooler conditions (Raines et al., 2012, Budd et al., 1997). For instance, consecutive days of wildfire suppression work in temperatures ranging between 15.8 to 26.4°C have been reported during large campaign wildfires in parts of south eastern Australia (Raines et al., 2012). Moreover, the sleep durations, bedding and beds in the current study mimic observed fire-ground conditions (Cater et al., 2007, Ferguson et al., 2011). Ensuring this high level of ecological validity for certain phases of a wildfire deployment make the detected findings applicable to the physical work (i.e., blacking out, carrying and dragging hoses and rake hoe work) and sleep involved during wildland firefighting in mild temperatures. However, the artificial setting limits the extrapolation of findings to other fire-ground demands which were not replicated, such as exposure to high ambient and radiant heat and smoke (or its constituent elements; Aisbett et al., 2012). External heat sources while performing physical work may influence inflammatory markers and cortisol (Hailes et al., 2011, Lieberman et al., 2005, Walsh and Whitham, 2006). Wood smoke exposure has also been associated with increases IL-6, IL-8 (Swiston et al., 2008) and decreases in IL-10 and cortisol (Al-Malki et al., 2008, Burgess et al., 2002). Therefore, investigating possible relationships between cytokine and cortisol levels among sleep restricted firefighters performing live physical work in a hot and smoky environment represents a logical next step for firefighting-based research in this area.

Conclusion

When firefighters had restricted sleep while performing physical work, an increase in morning IL-6 levels were positively related to a rise in evening cortisol. Conversely, a rise in IL-6 was associated with a decline in evening cortisol when firefighters had an 8-h sleep between shifts. Given how elevated evening cortisol can have adverse consequences to health (Dallman et al., 1993, McEwen, 1998, Spiegel et al., 1999), a rise in IL-6 in the morning, but decreased evening cortisol may reflect normal 'non-damaging' immune-endocrine function. Therefore, relationships described here highlight to fire agencies the important role an 8-h sleep opportunity between shifts has in preventing elevated evening cortisol. In addition, evidence of altered immune-endocrine function among sleep restricted firefighters and those receiving a normal sleep while performing physical work supports additional investigation into the short- (e.g., changes in mood; Vgontzas et al., 2008, Kemeny, 2007) and long-term (e.g., coronary artery disease and depression; Nijm and Jonasson, 2009, Nijm et al., 2007, Lutgendorf et al., 2008, Karlovic et al., 2012) health effects associated with imbalances between these systems. Using larger sample sizes, further research should also determine the direction of relationships between cytokines and cortisol and the potential impact other factors (e.g., sociodemographic and behavioural) have on these systems.

References

Abedelmalek, S., Souissi, N., Chtourou, H., Denguezli, M., Aouichaoui, C., Ajina, M., Aloui, A., Dogui, M., Haddouk, S. and Tabka, Z. 2013. Effects of Partial Sleep Deprivation on Proinflammatory Cytokines, Growth Hormone, and Steroid Hormone Concentrations During Repeated Brief Sprint Interval Exercise. *Chronobiology International: The Journal of Biological and Medical Rhythm Research,* 30**,** 502-509.

Aisbett, B., Phillips, M., Raines, J. and Nichols, D. Work patterns of tanker-based bushfire suppression by Australian volunteer firefighters in south-east Australia. Human Dimensions of Wildfire Conference, 2007 Fort Collins, Colorado.

Aisbett, B., Wolkow, A., Sprajcer, M. and Ferguson, S. A. 2012. "Awake, smoky, and hot": Providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters. *Applied Ergonomics,* 43**,** 916-925.

Al-Malki, A. L., Rezq, A. M. and Al-Saedy, M. H. 2008. Effect of fire smoke on some biochemical parameters in firefighters of Saudi Arabia. *Journal of Occupational Medicine and Toxicology,* 3**,**1-8.

An, S. J., Chung, Y. K., Kim, B. H., Kwak, K. M., Son, J. S., Koo, J. W., Ju, Y. S. and Kwon, Y. J. 2015. The effect of organisational system on self-rated depression in a panel of male municipal firefighters. *Annals Of Occupational And Environmental Medicine,* 27**,** 1-7.

Appel, C. P., Blomkvist, A. C., Persson, L. O. and Sjöberg, L. 1980. Mood and achievement in a difficult driving task. *Ergonomics,* 23**,** 605-612.

Biopharmaceutics Coordinating Committee 2001. Bioanalytical Method Validation. *In:* U.S. Department Of Health And Human Services Food And Drug Administration and Center For Drug Evaluation And Research (CDER) (eds.) *Guidance for Industry.* Center for Veterinary Medicine (CVM), United States Department of Health and Human Services Food and Drug Administration.

Bøyum, A., Wiik, P., Gustavsson, E., Veiby, O. P., Reseland, J., Haugen, A. H. and Opstad, P. K. 1996. The effect of strenuous exercise, calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines. *Scandinavian Journal Of Immunology,* 43**,** 228-235.

Budd, G. M., Brotherhood, J. R., Hendrie, A. L., Jeffery, S. E., Beasley, F. A., Costin, B. P., Wu, Z., Baker, M. M., Cheney, N. P. and Dawson, M. P. 1997. Project Aquarius 4. Experimental bushfires, suppression procedures, and measurements. *International Journal of Wildland Fire,* 7**,** 99-104.

Burgess, J. L., Nanson, C. J., Hysong, T. A., Gerkin, R., Witten, M. L. and Lantz, R. C. 2002. Rapid decline in sputum IL-10 concentration following occupational smoke exposure. *Inhalation Toxicology,* 14**,** 133-140.

Burnham, K. P. and Anderson, D. R. 2002. *Model selection and multi-model inference : a practical information-theoretic approach,* London, United Kingdom, Springer.

Carey, M. G., Al-Zaiti, S. S., Dean, G. E., Sessanna, L. and Finnell, D. S. 2011. Sleep problems, depression, substance use, social bonding, and quality of life in professional firefighters. *Journal Of Occupational And Environmental Medicine/American College Of Occupational And Environmental Medicine,* 53**,** 928-933.

Carlson, M., Charlin, V. and Miller, N. 1988. Positive Mood and Helping Behavior: A Test of Six Hypotheses. *Journal of Personality and Social Psychology,* 55**,** 211-229.

Cater, H., Clancy, D., Duffy, K., Holgate, A., Wilison, B. and Wood, J. Fatigue on the fireground: the DPI experience. Bushfire Cooperative Research Centre/Australasian Fire Authorities Council Conference Research Forum, 2007 Hobart, Tasmania.

Chowdhury, F., Williams, A. and Johnson, P. 2009. Validation and comparison of two multiplex technologies, Luminex and Mesoscale Discovery, for human cytokine profiling. *Journal Of Immunological Methods,* 340**,** 55-64.

Christoforou, T., Cvirn, M., Ferguson, S., Armstrong, T. & Smith, B. The effect of sleep restriction and exposure to physical activity on the cognitive ability of volunteer firefighters across a 3-day simulated fire-ground tour. *In:* Sargent C & Zhou, X., eds. Sleep, Performance and Wellbeing in Adults and Adolescents - Australasian Chronobiology Society, 2013 Adelaide. 13-17.

Chrousos, G. P. 1995. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *The New England Journal Of Medicine,* 332**,** 1351-1362.

Cook, B. and Mitchell, W. 2013. Occupational health effects for firefighters: The extent and implications of physical and psychological injuries. Centre of Full Employment and Equity.

Corsini, E., Pinto, A., Galbiati, V., Viviani, B., Galli, C. L., Marinovich, M. and Racchi, M. 2014. Corticosteroids modulate the expression of the PKC-anchoring protein RACK-1 and cytokine release in THP-1 cells. *Pharmacological Research: The Official Journal Of The Italian Pharmacological Society,* 81**,** 10-16.

Cullen, T., Thomas, A. W., Webb, R. and Hughes, M. G. 2015. The relationship between interleukin-6 in saliva, venous and capillary plasma, at rest and in response to exercise. *Cytokine,* 71**,** 397-400.

Dallman, M., Strack, A., Akana, S., Bradbury, M. and Hanson, E. 1993. Feast and Famine: Critical role of glucorticoids with insulin in daily energy flow. *Frontiers in Neuroendocrinology,* 14**,** 303-347.

Darwent, D., Lamond, N. and Dawson, D. 2008. The sleep and performance of train drivers during an extended freight-haul operation. *Applied Ergonomics,* 39**,** 614-622.

Derijk, R., Michelson, D., Karp, B., Petrides, J., Galliven, E., Deuster, P., Paciotti, G., Gold, P. W. and Sternberg, E. M. 1997. Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 beta (IL-1 beta), IL-6, and tumor necrosis factor-alpha (TNF alpha) production in humans: high sensitivity of TNF alpha and resistance of IL-6. *The Journal Of Clinical Endocrinology And Metabolism,* 82**,** 2182-2191.

Desantis, A. S., Diezroux, A. V., Hajat, A., Aiello, A. E., Golden, S. H., Jenny, N. S., Seeman, T. E. and Shea, S. 2012. Associations of salivary cortisol levels with inflammatory markers: The Multi-Ethnic Study of Atherosclerosis. *Psychoneuroendocrinology,* 37**,** 1009-1018.

Elenkov, I. J. 2008. Neurohormonal-cytokine interactions: Implications for inflammation, common human diseases and well-being. *Neurochemistry International,* 52**,** 40-51.

Elenkov, I. J. and Chrousos, G. P. 1999. Stress Hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease. *Trends in Endocrinology and Metabolism,* 10**,** 359-368.

Elenkov, I. J. and Chrousos, G. P. 2002. Stress Hormones, Proinflammatory and Antiinflammatory Cytokines, and Autoimmunity. *Annals of the New York Academy of Sciences,* 966**,** 290-303.

Eriksson, M., Sartono, E., Martins, C. L., Bale, C., Garly, M. L., Whittle, H., Aaby, P., Pedersen, B. K., Yazdanbakhsh, M., Erikstrup, C. and Benn, C. S. 2007. A comparison of ex vivo cytokine production in venous and capillary blood. *Clinical and Experimental Immunology,* 150**,** 469-476.

Faulkner, S. H., Spilsbury, K. L., Harvey, J., Jackson, A., Huang, J., Platt, M., Tok, A. and Nimmo, M. A. 2014. The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise. *European Journal of Applied Physiology,* 114**,** 1207-1216.

Ferguson, S. A., Aisbett, B., Jay, S. M., Onus, K., Lord, C., Sprajcer, M. and Thomas, M. J. W. 2011. Design of a valid simulation for researching physical, physiological and cognitive performance in volunteer firefighters during bushfire deployment. *In:* Thornton, R. (ed.) *Bushfire Cooperative Research Centre/ Australasian Fire and Emergency Service Authorities Council Conference Research Forum.* Sydney, New South Wales.

Field, A. 2009. *Discovering Statistics Using SPSS,* London, Sage.

Findlay, J. W., Smith, W. C., Lee, J. W., Nordblom, G. D., Das, I., Desilva, B. S., Khan, M. N. and Bowsher, R. R. 2000. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. *Journal Of Pharmaceutical And Biomedical Analysis,* 21**,** 1249-1273.

Finnerty, C. C., Jeschke, M. G., Herndon, D. N., Gamelli, R., Gibran, N., Klein, M., Silver, G., Arnoldo, B., Remick, D. and Tompkins, R. G. 2008. Temporal cytokine profiles in severely burned patients: A comparison of adults and children. *Molecular Medicine*.

Fisher, C. D. 2000. Mood and emotions while working: Missing pieces of job satisfaction? *Journal of Organizational Behavior,* 21**,** 185-202.

Frank, M., Miguel, Z., Watkins, L. and Maier, S. 2010. Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to E. coli lipopolysaccharide. *Brain, Behavior, and Immunity,* 24**,** 19-30.

Gómez-González, B., Domínguez-Salazar, E., Hurtado-Alvarado, G., Esqueda-Leon, E., Santana-Miranda, R., Rojas-Zamorano, J. A. and Velázquez-Moctezuma, J. 2012. Role of sleep in the regulation of the immune system and the pituitary hormones. *Annals of the New York Academy of Sciences,* 1261**,** 97-106.

Gundersen, Y., Opstad, P. K., Reistad, T., Thrane, I. and Vaagenes, P. 2006. Seven days' around the clock exhaustive physical exertion combined with energy depletion and sleep deprivation primes circulating leukocytes. *European Journal of Applied Physiology,* 97**,** 151-157.

Gunga, H. C., Maillet, A., Kirsch, K., Röcker, L., Gharib, C. & Vaernes, R. 1993. European isolation and confinement study. Water and salt turnover. *Advances In Space Biology And Medicine,* 3**,** 185-200.

Haddad, J. J., Saadé, N. E. and Safieh-Garabedian, B. 2002. Cytokines and neuro-immune-endocrine interactions: A role for the hypothalamic-pituitary-adrenal revolving axis. *Journal of Neuroimmunology,* 133**,** 1-19.

Hailes, W. S., Slivka, D., Cuddy, J. and Ruby, B., . 2011. Human plasma inflammatory response during 5 days of exercise training in the heat. *Journal of Thermal Biology,* 36**,** 277-282.

Hajat, A., Diez-Roux, A., Franklin, T. G., Seeman, T., Shrager, S., Ranjit, N., Castro, C., Watson, K., Sanchez, B. and Kirschbaum, C. 2010. Socioeconomic and race/ethnic differences in daily salivary cortisol profiles: the multi-ethnic study of atherosclerosis. *Psychoneuroendocrinology,* 35**,** 932-943.

Hill, E. E., Zack, E., Battaglini, C., Viru, M., Viru, A. & Hackney, A. C. 2008. Exercise and circulating cortisol levels: the intensity threshold effect. *Journal Of Endocrinological Investigation,* 31**,** 587-591.

Kales, S. N., Soteriades, E. S., Christophi, C. A. and Christiani, D. C., . 2007. Emergency duties and deaths from heart disease among firefighters in the United States. *The New England Journal Of Medicine,* 356**,** 1207-1215.

Karlovic, D., Serretti, A., Vrkic, N., Martinac, M. and Marcinko, D. 2012. Serum concentrations of CRP, IL-6, TNF-alpha and cortisol in major depressive disorder with melancholic or atypical features. *Psychiatry Research,* 198**,** 74-80.

Kemeny, M. E. 2007. Emotions and the Immune System. *In:* Ader, R. (ed.) *Psychoneuroimmunology, Vol 1*. Elsevier.

Kenefick, R. W. & Sawka, M. N. 2007. Hydration at the work site. *Journal of the American College of Nutrition,* 26**,** 597S-603S.

Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M. and Steptoe, A. 2004. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology,* 29**,** 516-528.

Larsen, B., Snow, R., Vincent, G., Tran, J., Wolkow, A. & Aisbett, B. 2015. Multiple Days of Heat Exposure on Firefighters' Work Performance and Physiology. *PLoS One,* 10**,** e0136413.

Liberati, T. A., Trammell, R. A., Randle, M., Barrett, S. and Toth, L. A. 2013. Cytokine and chemokine responses of lung exposed to surrogate viral and bacterial infections. *Comparative Medicine,* 63**,** 114-126.

Lieberman, H. R., Bathalon, G. P., Falco, C. M., Kramer, M. F., Morgan, C. A. and Niro, P. 2005. Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat. *Biological Psychiatry,* 57**,** 422-429.

Lundeland, B., Gundersen, Y., Opstad, P. K., Thrane, I., Zhang, Y., Olaussen, R. W. and Vaagenes, P. 2012. One week of multifactorial high-stress military ranger training affects Gram-negative signalling. *Scandinavian Journal of Clinical and Laboratory Investigation,* 72**,** 547-554.

Lutgendorf, S. K., Weinrib, A. Z., Penedo, F., Russell, D., Degeest, K., Costanzo, E. S., Henderson, P. J., Sephton, S. E., Rohleder, N., Lucci, J. A. 3rd, Cole, S., Sood, A. K. and Lubaroff, D. M. 2008. Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients. *Journal Of Clinical Oncology: Official Journal Of The American Society Of Clinical Oncology,* 26**,** 4820-4827.

Mackin, P. and Young, A. H. 2004. The role of cortisol and depression: exploring new opportunities for treatments. *Psychiatric Times,* 21**,** 92-95.

Mcewen, B. S. 1998. Protective and Damaging Effects of Stress Mediators. *New England Journal of Medicine,* 338**,** 171-179.

Mcewen, B. S., Biron, C. A., Brunson, K. W., Bulloch, K., Chambers, W. H., Dhabhar, F. S., Goldfarb, R. H., Kitson, R. P., Miller, A. H., Spencer, R. L. and Weiss, J. M. 1997. The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions. *Brain Research Reviews,* 23.

Miller, G. E., Cohen, S. and Ritchey, A. K. 2002. Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. *Health Psychology,* 21**,** 531-541.

Nguyen, H. P., Björkqvist, M., Bode, F. J., Stephan, M. and Von Hörsten, S. 2010. Serum levels of a subset of cytokines show high interindividual variability and are not altered in rats transgenic for Huntington´s disease. *Plos Currents Huntington Disease,* 2**,** 1-5.

Nicolson, N. A. 2008. Measurement of cortisol. *In:* Luecken, L. J. and Gallo, L. C. (eds.) *Handbook of physiological research methods in health psychology*. Thousand Oaks, CA: Sage.

Nijm, J. and Jonasson, L. 2009. Inflammation and cortisol response in coronary artery disease. *Annals of Medicine,* 41**,** 224-233.

Nijm, J., Kristenson, M., Olsson, A. G. and Jonasson, L. 2007. Impaired cortisol response to acute stressors in patients with coronary disease. Implications for inflammatory activity. *Journal of Internal Medicine,* 262**,** 375-384.

Opstad, P. K. 1994. Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. *European Journal of Endocrinology,* 131**,** 56-66.

Opstad, P. K. and Aakvaag, A. 1981. The effect of a high calory diet on hormonal changes in young men during prolonged physical strain and sleep deprivation. *European Journal of Applied Physiology,* 46**,** 31-39.

Payne, R., Welham, S. and Harding, S. 2011. A Guide to REML in GenStat (16th Edition). Hempstead, Hertfordshire, UK: VSN International.

Petrovsky, N. 2001. Towards a unified model of neuroendocrine–immune interaction. *Immunology and Cell Biology,* 79**,** 350-357.

Phillips, M., Netto, K., Payne, W., Nichols, D., Lord, C., Brooksbank, N., Onus, K., Jefferies, S. and Aisbett, B. 2011. Frequency, intensity and duration of physical tasks performed by Australian rural firefighters during bushfire suppression. *In:* Thornton, R. P. (ed.) *Bushfire Cooperative Research Center/Australasian Fire Authorities Council Conference Research Forum.* Sydney, New South Wales.

Phillips, M., Payne, W., Lord, C., Netto, K., Nichols, D. and Aisbett, B. 2012. Identification of physically demanding tasks performed during bushfire suppression by Australian rural firefighters. *Applied Ergonomics,* 43**,** 435-441.

Phillips, M., Payne, W. R., Netto, K., Cramer, S., Nichols, D., Mcconell, G. K., Lord, C. and Aisbett, B. 2015. Oxygen uptake and heart rate during simulated wildfire suppression tasks performed by Australian rural firefighters. *Occupational Medicine and Health Affairs,* 3, 1-8.

Piazza, J. R., Charles, S. T., Stawski, R. S. and Almeida, D. M. 2013. Age and the association between negative affective states and diurnal cortisol. *Psychology and Aging,* 28**,** 47-56.

Pledge, D., Grosset, J.-F. and Onambélé-Pearson, G. L. 2011. Is there a morning-to-evening difference in the acute IL-6 and cortisol responses to resistance exercise? *Cytokine,* 55**,** 318-323.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G. and Hellhammer, D. H. 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology,* 28**,** 916-931.

Raines, J., Snow, R., Petersen, A., Harvey, J., Nichols, D. and Aisbett, B. 2012. Pre-shift fluid intake: Effect on physiology, work and drinking during emergency wildfire fighting. *Applied Ergonomics,* 43**,** 532-540.

Raines, J., Snow, R., Petersen, A., Harvey, J., Nichols, D. and Aisbett, B. 2013. The effect of prescribed fluid consumption on physiology and work behavior of wildfire fighters. *Applied Ergonomics,* 44**,** 404-413.

Ranjit, N., Diez-Roux, A. V., Sanchez, B., Seeman, T., Shea, S., Shrager, S. and Watson, K. 2009. Association of salivary cortisol circadian pattern with cynical hostility: multi-ethnic study of atherosclerosis. *Psychosomatic Medicine,* 71**,** 748-755.

Riechlin, S. 1993. Neuroendocrine-immune interactions. *The New England Journal Of Medicine,* 329**,** 1246-1253.

Rodríguez-Marroyo, J. A, López-Satue, J, Pernía, R, Carballo, B, García-López, J, Foster, C and Villa, J. G 2012, Physiological work demands of Spanish wildland firefighters during wildfire suppression. *International Archives Of Occupational And Environmental Health*, 85, 221-228.

Silverman, M. N. and Sternberg, E. M. 2012. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. *Annals of the New York Academy of Sciences,* 1261**,** 55-63.

Smith, E. M., Cadet, P., Stefano, G. B., Opp, M. R. and Hughes, J. T. K. 1999. IL-10 as a mediator in the HPA axis and brain. *Journal of Neuroimmunology,* 100**,** 140-148.

Spiegel, K., Leproult, R. and Van Cauter, E. 1999. Impact of sleep debt on metabolic and endocrine function. *The Lancet,* 354**,** 1435-1439.

Steensberg, A., Fischer, C. P., Keller, C., Moller, K. and Pedersen, B. K. 2003. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology - Endocrinology and Metabolism,* 285**,** 433-437.

Steptoe, A., Hamer, M. & Chida, Y. 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. *Brain, Behavior, and Immunity,* 21**,** 901-912.

Swiston, J. R., Davidson, W., Attridge, S., Li, G. T., Brauer, M. and Eeden, S. F. V. 2008. Wood smoke exposure induces a pulmonary and systemic inflammatory response in firefighters. *European Respiratory Journal.* Sheffield; UK: European Respiratory Society.

Thieringer, R., Le Grand, C. B., Carbin, L., Cai, T. Q., Wong, B., Wright, S. D. and Hermanowski-Vosatka, A. 2001. 11 Beta-hydroxysteroid dehydrogenase type 1 is induced in human monocytes upon differentiation to macrophages. *The Journal of Immunology,* 167**,** 30-35.

Turnbull, A. V. and Rivier, C. L. 1999. Regulation of the Hypothalamic-Pituitary-Adrenal Axis by Cytokines: Actions and Mechanisms of Action. *Physiological Reviews,* 79**,** 1-71.

Van Dongen, H. P., Olofsen, E., Dinges, D. F. & Maislin, G. 2004. Mixed-model regression analysis and dealing with interindividual differences. *Essential Numerical Computer Methods,* 225.

Vgontzas, A. N., Bixler, E. O., Chrousos, G. P. and Pejovic, S. 2008. Obesity and sleep disturbances: Meaningful sub-typing of obesity. *Archives of Physiology and Biochemistry,* 114**,** 224-236.

Vincent, G., Ferguson, S. A., Larsen, B., Wolkow, A., Tran, J. and Aisbett, B. 2015. Sleep restriction during simulated wildfire suppression: effect on physical task performance *Plos One,* 10.

Walsh, N. P. and Whitham, M. 2006. Exercising in environmental extremes : a greater threat to immune function? *Sports Medicine,* 36**,** 941-976.

Weiskopf, R. B., Yau, R., Sanchez, R., Lowell, C. and Toy, P. 2009. Microarray kit analysis of cytokines in blood product units and segments. *Transfusion,* 49**,** 2269-2275.

Wolkow, A., Aisbett, B., Ferguson, S. A. and Main, L. C. 2014a. The impact of sleep restriction on acute inflammatory stress responses to simulated physical firefighting work. Sleep DownUnder 2014 ASM: Sleep Frontiers, 9-11 October 2014, Perth. Wiley.

Wolkow, A., Aisbett, B., Ferguson, S. A., Reynolds, J. and Main, L. C. 2014b. Effects of sleep restriction on cortisol during simulated physical firefighting work. Fourth International Conference on Health Wellness, and Society, 14-15 March 2014 Vancouver, Canada.

Wolkow, A., Netto, K., Langridge, P., Green, J., Nichols, D., Sergeant, M. and Aisbett, B. 2014c. Coronary Heart Disease Risk in Volunteer Firefighters in Victoria, Australia. *Archives of Environmental and Occupational Health,* 69**,** 112-120.

Woods, A. M. and Judd, A. M. 2008. Interleukin-4 increases cortisol release and decreases adrenal androgen release from bovine adrenal cells. *Domestic Animal Endocrinology,* 34**,** 372-382.

Zhou, X., Fragala, M. S., Mcelhaney, J. E. and Kuchel, G. A. 2010. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Current Opinion in Clinical Nutrition and Metabolic Care,* 13**,** 541-547.

Chapter 6: Study 4

Study 4

Acute psychophysiological relationships between mood, inflammatory and cortisol changes in response to simulated physical firefighting work and sleep restriction

Alexander Wolkow BExSc (Hons), Brad Aisbett PhD, John Reynolds PhD, Sally A Ferguson PhD and Luana C Main PhD

Article under second review in

Applied Psychophysiology and Biofeedback

Presented here in the journal submission format

Abstract

This study examined how changes in wildland firefighters' mood relate to cytokine and cortisol levels in response to simulated physical firefighting work and sleep restriction. Firefighters completed 3 days of simulated wildfire suppression work separated by an 8-h (Control condition; *n*=18) or 4-h sleep opportunity (Sleep restriction condition; *n*=17) each night. Firefighters' mood was assessed daily using the Mood Scale II and Samn-Perelli fatigue scale. Participants also provided samples for the determination of salivary cortisol and pro- (IL-6, IL-8, IL-1β, TNF-α) and anti-inflammatory (IL-4, IL-10) cytokine levels. An increase in positive mood dimension Happiness was related to a rise in IL-8 and TNF-α in the sleep restriction condition. A rise in the positive mood dimension Activation among sleep restricted firefighters was also related to higher IL-6 levels. An increase in the negative mood dimension Fatigue in the sleep restriction condition was associated with increased IL-6, TNF-α, IL-10 and cortisol levels. In addition, an increase in Fear among sleep restricted firefighters was associated with a rise in TNF-α. Elevated positive mood and immune activation may reflect an appropriate response by the firefighters to these stressors. To further understand this relationship, subsequent firefighting-based research is needed that investigate whether immune changes are a function of affective arousal linked to the expression of positive moods. Positive associations between negative mood and inflammatory and cortisol levels to physical work and restricted sleep could be used to underpin workplace monitoring where subjective mood indicates physiological changes on the fire-ground.

Introduction

Exposure to a stressor, either physical or psychological, triggers the immune system to produce cytokines (Maier and Watkins 1998), which in turn, activate the hypothalamic-pituitary-adrenal (HPA)-axis causing the release of cortisol (Lundberg 1999). Changes in the immune and endocrine system relate to affective states such as mood and emotion (Mittwoch-Jaffe et al. 1995; Kemeny 2007), indicating that activation of these physiological systems in response to a stressor and the subsequent release of their end products cortisol and cytokines form a bi-directional communication network with the brain (Maier 2003; Maier and Watkins 1998). Evidence indicates that certain emotions are associated with distinct patterns of activation in the central (Canli et al. 2001; Damasio et al. 2000) and autonomic nervous systems (Ekman et al. 1983; Herrald and Tomaka 2002). Given that activation of these neural systems can impact differently on immune and endocrine responses, it has been proposed that cortisol and cytokines respond in different patterns depending on the affective state (Kemeny 2007). Moods are affective states that can be categorized as positive or negative. While fewer studies have demonstrated relationships between positive mood on physiological changes (Marsland et al. 2007; Barak 2006), the literature linking negative mood to indicators of immune, and to a lesser extent endocrine function, is more substantial (Kemeny 2007; Vgontzas et al. 2008; von Känel et al. 2008; Wright et al. 2005; McEwen 2005). If cytokines and cortisol are moderated by mood than any true assessment of the stress response needs to examine how positive and negative mood may relate to changes in cytokines and cortisol.

The stress-induced changes in psychological responses may coordinate physiological processes that form important adaptations necessary for maintaining homeostasis (Maier and Watkins 1998; Dhabhar and McEwen 2006). However, prolonged or

severe stressors may result in inappropriate responses, including increased negative moods, maladaptation of the inflammatory responses and a dysregulated cortisol release, which over time, have been implicated in cardiovascular disease, insulin resistance and depression (Heinz et al. 2003; Zunszain et al. 2011; Lundberg 1999; McEwen and Seeman 1999; Pradhan et al. 2001; Ridker et al. 2000; Vgontzas et al. 2000). Relationships between psychological and physiological responses support a psychophysiological approach to understanding the impact of acute stress on the body in certain occupations.

Two common stressors for firefighters performing wildfire suppression are physical work and sleep restriction (Aisbett et al. 2012; Cater et al. 2007; Phillips et al. 2007). For instance, firefighters often work consecutive long shifts (i.e., 12 to 16-h) involving high-intensity, intermittent physical work (Aisbett et al. 2007) separated by shortened sleep opportunities (i.e., 3 to 6 h; Cater et al. 2007). Research demonstrates that consecutive days of physical firefighting work combined with a 4-h sleep opportunity each night results in increased cortisol (Reported in Chapter 4; Wolkow et al. 2014b). Elevated IL-6 and IL-8 cytokine levels have also been found following multiple days of physical work, but restricted sleep between days did not exacerbate cytokine levels (Repoted in Chapter 3; Wolkow et al. 2014a). To the authors' knowledge however, no firefighting-based research has examined if mood responses to the combination of physical work and sleep restriction influence the cortisol and cytokine levels previously demonstrated.

Exercise- and sleep-based studies investigating periods of sleep loss and physical work, similar to those experienced during wildland firefighting (Aisbett et al. 2012), have demonstrated acute psychophysiological relationships (Jürimäe et al. 2002;

Robson-Ansley et al. 2009; Wolkow et al. 2015). For instance, increases in cortisol and IL-6 receptor levels following 6-days of rowing (i.e., total of 21.5 ± 2.2-h of exercise; Jürimäe et al. 2002) and cycling training (i.e., 160 ± 43 to 391 ± 30 mins of exercise per day; Robson-Ansley et al. 2009) were respectively, strongly and moderately related to increased subjective fatigue levels. Elevated IL-6 has also been strongly associated with increased subjective pain ratings following 12 nights of restricted sleep (4 h; Haack et al. 2007). However, research is limited and inconsistent, with investigations reporting no association between negative mood and hormonal changes to physical work (Filaire et al. 2004) or sleep loss (Bouhuys et al. 1990). Meanwhile, following a 15-km run at high intensity (completed in 1.5 h), positive affect correlated strongly with increases in corticotropin-releasing hormone (CRH), which is a neuropeptide in the HPA-axis (Harte et al. 1995). Dysregulation of the HPA-axis, such as changes in CRH release, have been related to altered immune function (Pressman and Cohen 2005; Haddad et al. 2002). Positive mood provoked hormonal and inflammatory responses can represent a potential disease risk if severe, prolonged or result in disruption to the diurnal rhythm of responses (Pressman and Cohen 2005; Marsland et al. 2007).

Despite varying protocols, restricted sleep (Haack et al. 2007) and physical work (Jürimäe et al. 2002; Robson-Ansley et al. 2009) have been reported to affect mood, cytokine and HPA-axis responses. Though the relationship is likely to be bi-directional, attention has focused on how both positive and negative mood may modulate these parameters of the immune and endocrine system (Kemeny 2007; Marsland et al. 2007). While it has been established that physical work and sleep loss can effect firefighters' cytokine (see Chapter 3: Study 1 for details) and cortisol levels (see Chapter 4: Study 2 for details), it is yet to be determined what impact these

demands have on acute psychophysiological responses. Therefore, we aimed to determine what moderating effect (if any) changes in wildland firefighters' mood are having on firefighters' cytokine and cortisol levels in response to simulated physical firefighting work and sleep restriction. We hypothesised that when sleep restricted, firefighters will display alterations in mood that moderate an elevation in cytokines and cortisol. Investigating this potential psychophysiological relationship in the context of firefighting presents new information for psychoneuroimmunology research, while providing the first steps in understanding how changes in mood on the fire-ground influence physiological responses. Potential insights may have significance for monitoring firefighters' health.

Materials and Methods

Prior work from this sample has examined cytokine (Reported in Chapter 3; Wolkow et al. 2014a) and cortisol (Reported in Chapter 4; Wolkow et al. 2014b) responses separately and the relationship between these markers (Reported in Chapter 5). This study focuses on the relationship between the observed cortisol and cytokine activity with subjective mood responses from this same sample of firefighters.

Participants

Volunteer and salaried firefighters (n = 35) from Australian state and territory fire agencies (Victoria, South Australia, New South Wales, Australian Capital Territory and Tasmania) were recruited for this study using flyers and presentations to fire agencies. Interested firefighters contacted researchers directly and provided written informed consent. Participants were then screened to exclude anyone with diagnosed heart disease, diabetes, respiratory and/or sleep disorders. Although health outcome data was obtained from participants, this information was only used for study

exclusion purposes, and therefore will not be described in any further detail in the current study. For purposes of analysis, participants were matched for age, sex and body mass index (BMI) and randomly assigned to either a control (CON) or sleep restriction (SR) condition based on the participant's availability to attend the testing session, allocated at random, to the condition. Use of this randomization method took into account differences in firefighters' availability to volunteer their time for the study, ensuring an equal chance for each participant of being allocated to either condition. There were no differences between conditions in BMI, age or firefighting experience (Table 6.1). Firefighters participating in this study were not compensated financially for their time. This study was approved by the institutions' Human Research Ethics Committee and all procedures were performed in accordance with the 1964 Helsinki declaration and its latter amendments.

Table 6.1 Demographic characteristics of participants in each condition (mean ± standard deviation)

Characteristic	CON (n = 18)	SR (n = 17)
Age (years)	39 ± 16	39 ± 15
Male:Female (n)	15:3	15:2
Body mass (kg)	85.1 ± 17.7	93.8 ± 20.2
Height (cm)	178.1 ± 7.7	177.8 ± 7.4
BMI (kg/m^2)	26.8 ± 5.0	29.6 ± 5.5
Firefighting experience (years)	8.7 ± 9.3	10.2 ± 6.4

Note: BMI = Body Mass Index

Protocol

Participants arrived at the testing venue and informed of what condition they were in. Both conditions then completed a familiarization session of the physiological measures, psychological questionnaires and physical work tasks. This was followed by an 8-h sleep adaptation night in the testing environment. Participants in both conditions were then tested over a 3-day and 2-night simulated fire-ground tour that mimicked the typical length of a fire-ground deployment. On each of the 2 nights, participants in the CON condition had an 8-h sleep opportunity (i.e., 22:00-06:00), while participants in the SR condition had a restricted 4-h sleep opportunity (i.e., 02:00-06:00). The sleep restriction duration and timing was based on observational studies of Australian wildland firefighting (Cater et al. 2007). After the testing period, all participants had an 8-h recovery sleep (in which no measures were taken) to ensure participants' were rested prior to leaving the venue. The testing environment was maintained in moderate temperatures (18-20^{o}C) throughout the simulation.

Experimental Procedures

Participants in both conditions were tested in groups of 3 to 5 over the firefighting simulation. Participants completed a 2-h testing block 3 times on day 1 and 5 times on day 2 and day 3 (Figure 6.1). Each testing block consisted of a 55-min simulated physical firefighting work circuit, followed by 20-25 min of physiological data collection, 20-25 min of cognitive testing and 15-20 min rest period. The physical work and non-work periods described here match work-to-rest activity patterns on the fire-ground (Aisbett et al. 2007; Phillips et al. 2011; Raines et al. 2013). Participants' physiological (i.e., cytokine and cortisol) and psychological (i.e., mood questionnaires) measures were recorded at pre-determined time points throughout the

wake periods (Figure 6.1). Cognitive and other physiological measures were part of a larger study and therefore, will not be described.

Simulated Physical Firefighting Work Circuit: The physical work circuit was developed using a job task analysis of wildfire suppression work (Phillips et al. 2012). The circuit was verified by industry experts and incumbent firefighters as being representative of fitness components, actions and movements frequently performed on the fire-ground by each of Australia's state and territory fire agencies (Ferguson et al. 2011). This increased the generalisability of potential findings to firefighters across Australia. The 6 physical tasks included; team rake, charged hose advance, black out hose work, hose rolling, lateral repositioning and static hold of a hose. The tasks were chosen because they were the most physically demanding (Phillips et al. 2012), had the highest operational importance and were the longest, most intense, or most frequently occurring tasks during wildfire suppression work (Phillips et al. 2015). Performance of each physical task was self-paced (i.e., repetitions completed within each work period) and completed in a pre-devised circuit consisting of work-to-rest ratios designed in accordance with the performance of the tasks on the fire-ground (Ferguson et al. 2011; Vincent et al. 2015).

Sleep: All participants wore activity monitors (*Actical* MiniMitter/Respironics, USA) to measure sleep across the 2 nights prior to the study. Sleep was recorded on the adaptation night and testing nights using portable polysomnographic (PSG) equipment (Siesta, Compumedics E-Series, Australia). Each night, PSG wire up and recording began at 21:00 for both conditions. From each sleep recording, participants' total sleep time (minutes) was calculated. Sleep duration in the 2 nights prior to testing did not differ to the adaptation night or between conditions ($p > 0.05$; data not presented).

Average total sleep time for both conditions was similar on the adaptation night (CON 6.3 ± 0.9 h; SR 6.4 ± 0.7 h; $p > 0.05$), while on the 2 experimental nights, sleep time was as expected given the sleep opportunity in each condition (CON 6.9 ± 0.4 h; SR 3.6 ± 0.3 h; $p < 0.001$). Participants completed the adaptation night to ensure their sleep and possible stress responses during the study were not influenced by a lack of familiarity with the PSG equipment.

Physiological Stress Responses

Cortisol: Saliva samples were collected using Salivette tubes (Sarstedt, Nümbrecht, Germany) each day in both conditions at baseline (i.e., 06:30) and across the day (i.e., 07:30, 09:00, 11:30, 13:30, 15:30, 17:30, 19:30, 21:30; Figure 6.1). To prevent contamination, participants were not allowed to eat or drink 15 minutes prior to saliva collection. Samples were centrifuged for 10 minutes at 5000 revolution/minutes (83 hertz) and stored at ≤ -80°C until analysis. Salivary cortisol concentration was determined using a high sensitivity enzyme immunoassay ELISA kit (IBL International, Hamburg, Germany). The assay was performed according to the manufacturer's directions and read at 450 nm on a luminescence microplate reader (Synergy™ 2 SL, BioTek, Winooski, VT). Analytical sensitivity (lower limit of detection) was 0.14 nmol/L and acceptable intra- and inter-assay coefficients of variation, 7.2% and 10.7% (both mean 13.8 nmol/L), respectively were determined.

Cytokines: Capillary blood plasma samples for the determination of pro- (IL-6, IL-8, IL-1β and TNF-α) and anti-inflammatory (IL-4 and IL-10) cytokine levels were collected from participants each day when fasted at 06:15, then at 11:30, 18:15 and 21:30 (Figure 6.1). In order to prevent acute postprandial changes in cytokine levels, samples collected at 11:30 and 18:15 were completed prior to lunch and dinner

respectively. A 500-µL sample of whole blood was taken from each participant at each time point from the fingertip in to microtainer coated with K_2 EDTA (Becton Dickinson ref: 365974). Samples were centrifuged for 10 minutes at 5000 revolutions/minute (83 hertz) and the plasma was separated and stored at ≤ -80°C until analysis. The Milliplex Human MAP Cytokine immunoassay kit (Millipore, Billerica, MD) was used to profile the expression of inflammatory markers in participants' plasma samples. The assay kits provide a mixture of microbead populations with distinct fluorescent intensities that are pre-coated with capture antibodies specific for each cytokine. The assay was performed using the Bioplex 200 array reader (V.5.0, Bio-Rad Laboratories, Hercules, CA). The minimal detectable concentrations were 0.06 pg/mL, 0.42 pg/mL, 0.20 pg/mL, 0.05 pg/mL, 0.48 pg/mL and 0.07 pg/mL for IL-1β, IL-4, IL-6, IL-8, IL-10 and TNF-α, respectively. Intra- and inter-assay coefficients of variation (CV) were in acceptable ranges (Intra-assay 4.5 – 10.0%; Inter-assay 9.8 – 20.5%) for all analytes (CV <25%; Findlay et al. 2000; Chowdhury et al. 2009) and comparable to CVs reported for cytokines sampled using venous blood in previous exercise-based literature (Abedelmalek et al. 2013; Cullen et al. 2015; Faulkner et al. 2014).

Psychological Stress Responses

Mood Scale II: Participants' mood was measured using the Mood Scale II which is part of the Walter Reed Performance Assessment Battery (Thorne et al. 1985). The Mood Scale II was designed to assess variations in mood states over time, especially in field-research and during sleep restriction (Paterson et al. 2010; Thorne et al. 1985). The questionnaire is comprised of 36 mood-related adjectives, to which participants were asked to respond on a 3-point Likert scale (1 = not at all, 2 = somewhat/sometimes, 3 = mostly/generally) to indicate their current experience of each item at the specified

time point. Items from this scale load to 6 mood states, which include positive mood dimensions; Activation, Happiness, and negative mood dimensions; Depression, Anger, Fear and Fatigue. Mean scores for each mood dimension were calculated for inclusion in the analyses by determining the average score from the adjectives that correspond to the dimension, giving a possible average score between 1 and 3 for each dimension. The Mood Scale II was developed and validated against a range of performance criteria in Navy recruits where it demonstrated moderate to high validities in a number of situations and moderately high inter-item reliability (Ryman et al. 1974). Furthermore, the Mood Scale II is similar to the Short Form Profile of Mood States which has demonstrated good validity when used in its abbreviated and complete forms in sleep research (Carpenter et al. 2004; Friedmann et al. 1977). Participants completed the Mood Scale II each day at 06:15, 11:30, 18:30-19:30 and at 21:30 (Figure 6.1).

Samn-Perelli Fatigue Scale: Participants' subjective ratings of fatigue were recorded using the Samn-Perelli Fatigue Scale which is a 7-point Likert scale (1 = fully alert, wide awake; 2 = very lively, responsive but not at peak; 3 = ok, somewhat fresh; 4 = a little tired, less than fresh; 5 = moderately tired, let down; 6 = extremely tired, very difficult to concentrate; 7 = completely exhausted, unable to function effectively) that was developed for and validated in occupational settings such as aviation operations where it has shown to be reliable and sensitive to the effects of sleep loss at different times of the day (Samn and Perelli 1982; International Civil Aviation Organization 2011; Powell et al. 2007). Participants' completed the scale each day at 09:30, 10:45-11:15, 14:00, 16:00 and 18:00 (Figure 6.1) where they were asked to respond according to how they felt on the scale at that moment. For the analyses, the Samn-Perelli Fatigue Scale Daily Profile was determined for each participant from their score

on the scale at the 5 daily time points. In addition, the Samn-Perelli Fatigue Scale Daily Mean was determined for each participant by calculating daily averages from the 5 time points. In both instances, possible scores were between 1 and 7.

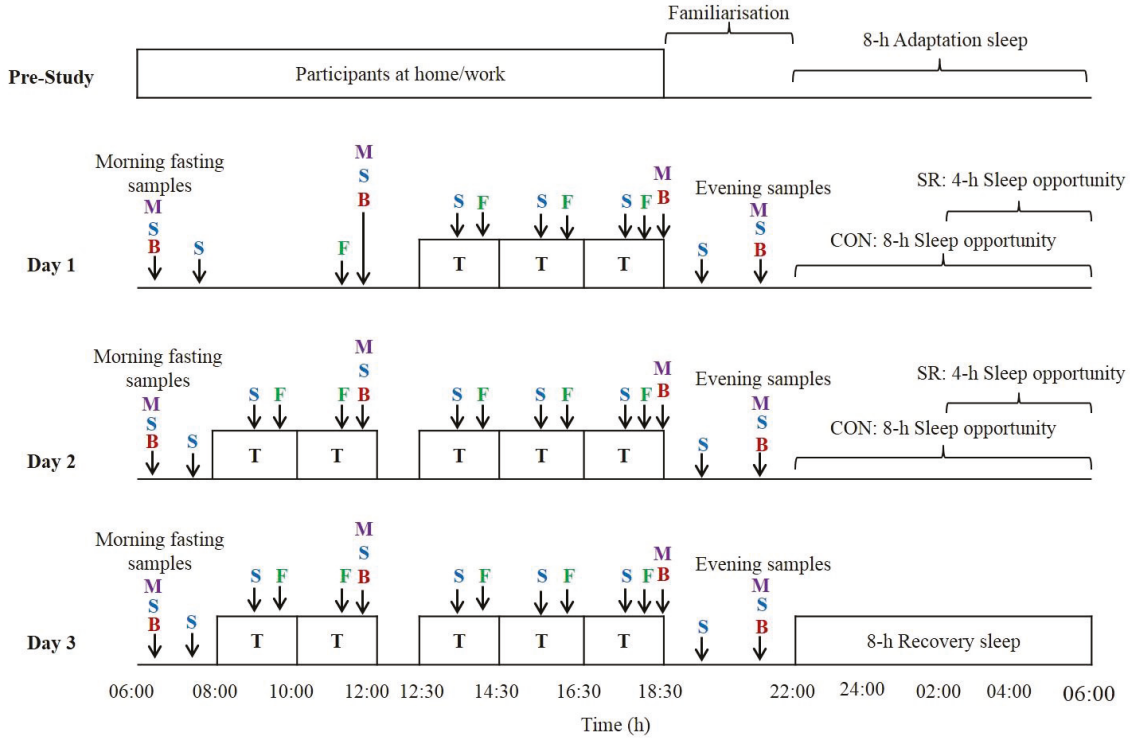

Figure 6.1 Sampling protocol for inflammatory, cortisol and mood measures in the CON and SR condition

Statistical Analyses

Prior to the analysis, cytokine values greater than 2 standard deviations above the mean were considered outliers and subsequently excluded (Nguyen et al. 2010). Values below the detectable range of the Milliplex Human MAP Cytokine immunoassay kit were replaced with the minimal detectable concentration as advised in the protocol (Millipore, Billerica, MD). With the exception of TNF-α (for which raw values achieved normality and homogeneity of variance), all cytokine and cortisol values were adjusted using a natural log-transformation to achieve normality or the raw data, assessed using the Shapiro-Wilk test ($p > 0.05$). Normality and homogeneity of variance of the residuals were further determined by visual inspection of the mixed model analysis (Field 2009) where the resultant diagnostic plots revealed no departures from these required assumptions. Due to the sampling design, a cortisol and Samn-Perelli Fatigue scale measurement at 09:00 and 09:30 respectively, was missing for all participants on day 1. Consequently, the missing combinations were appended to the data, but missing value codes were associated with these additional records. This did not affect the hypothesis tests and estimates for the daily profiles, but it facilitated the fitting of models for auto-correlated errors.

Variables measured just once on an individual, or aggregated over occasions, (e.g., sleep duration and demographic characteristics were analysed with the Analysis of Variance method using GenStat software (GenStat *for Windows* 16.1 Edition. VSN International, Hemel Hempstead, UK). The relationships between stress measures were analyzed using linear mixed models (LMM) fitted by the restricted maximum likelihood (REML; Payne et al. 2011) method in GenStat (GenStat *for Windows* 16.1 Edition, VSN International, Hemel Hempstead, UK). The LMM approach was used to investigate if changes in Mood Scale II and Samn-Perelli Fatigue Scale responses

relate to cytokine and cortisol responses. To investigate these potential associations, cortisol and cytokine measures were modelled as a function of the Mood Scale II and Samn-Perelli Fatigue Scale scores.

Key within-participant outcome variables included each daily cytokine profile (i.e., 4 samples per day), morning fasting cytokine levels, the daily cortisol profile (i.e., 9 samples per day) and the cortisol area under the curve (AUC) with respect to ground. Cortisol AUC was calculated for each participant on each day using the trapezoidal rule (Pruessner et al. 2003). Between-participant predictor variables included the 6 Mood Scale II mood dimensions (i.e., Happiness, Fear, Fatigue, Activation, Vigour, and Depression). In addition, the Samn-Perelli Fatigue Scale Daily Profile (i.e., 5 measurements per day) and Samn-Perelli Fatigue Scale Daily Mean were included as separate predictor variables. LMM allow for the possibility of auto-correlation in the repeated measurements (i.e., samples and days) on each individual by including a model for the covariance structure. Model fit was assessed by the Akaike Information Criterion (AIC) and small differences (ΔAIC) in values of this criterion compared to the minimum observed value in a set of candidate models were used to identify parsimonious models (Burnham and Anderson 2002).

The final 'full' model fitted to each daily cytokine and cortisol profile included potential fixed effects for condition (CON or SR), day (day 1, day 2 or day 3) and time of day along with potential 2- and 3-way interactions of condition by day, condition by time and condition by day by time, critical to the design of the study. For morning fasting cytokines and cortisol AUC, the final 'full' model included potential fixed effects for condition (CON or SR) and day (day 1, day 2 or day 3), along with a potential 2-way interaction of condition by day. To investigate possible associations

between cortisol and cytokine responses with the 6 Mood Scale II mood dimensions, the final model for each dependent variable tested 2-way interactions of condition by mood dimension, day by mood dimension and time by mood dimension. Possible 3-way interactions of condition by day by mood dimension and condition by time by mood dimension were also investigated. To assess potential associations between the Samn-Perelli Fatigue Scale (mean and profile) and cortisol (profile and AUC) and cytokines (profile and morning fasting measures), 2-way interactions of condition and day with Samn-Perelli Fatigue Scale (mean and profile) were investigated. Potential 3-way interactions of condition by day by Samn-Perelli Fatigue Scale (mean and profile), and condition by time by Samn-Perelli Fatigue Scale (only profile) were also examined. For each model, random effects of group, profile (or participant) and a group by profile interaction were investigated both without (i.e., Independence model) and with an Unstructured covariance model for the within-participant auto-correlation. The slopes of potential interactions are represented using (unstandardized) regression coefficients (b) and statistical significance was set at $p < 0.05$.

Significant interactions between cytokines and cortisol (i.e. daily profiles, morning fasting measures and cortisol AUC) with Mood Scale II mood dimensions and the Samn-Perelli Fatigue Scale are presented as diagrams. These diagrams do not quantify the interactions, but rather pictorially represent information on the trend and nature of any significant associations identified between mood or fatigue responses and cytokine or cortisol levels. The y-axis represents either cortisol or cytokine levels, while the x-axis lists the Mood Scale II scores (i.e., 1, 2 or 3) or Samn-Perelli fatigue ratings (i.e., 1 to 7). The regression lines in each of these interaction diagrams represent the combined effect of the estimated main and interaction effects of either the mood

dimension or fatigue and a designated factor on the predicted mean of the cytokine or cortisol measure for an individual.

Results

Mood Scale II and daily cytokine profiles

Several LMM were fitted to each cytokine profile, but the model with the lowest AIC for the majority of cytokines was the full fixed effects Independence model. After inspection of the ΔAIC values, this LMM had the best fit to model the relationship between mood dimensions and log IL-6, IL-8 and IL-1β and raw TNF-α profiles. No significant relationships between any of the Mood Scale II mood dimensions and log IL-4 and IL-10 profiles were found ($p > 0.05$).

IL-6: Interaction effects for condition by Activation ($p = 0.002$), day by Fear ($p = 0.037$) and time by Happiness ($p = 0.050$) on log IL-6 were found. The condition by Activation interaction indicates slightly positive (increased) log IL-6 levels if and when Activation increased in the CON condition (Figure 6.2a). For the SR condition, the association demonstrated positive (increased) log IL-6 levels when Activation increased, but the slope at which log IL-6 increased (b = 0.493) was greater than the CON condition (b = 0.115; Figure 6.2a), indicating that Activation was having a larger moderating effect on IL-6 in the SR condition. The day by Fear interaction for day 1 indicated a slight increase in log IL-6 levels if and when Fear increased, independent of the sleep opportunity (b = 0.385; Figure 6.2b). For day 2, the association suggested no change in log IL-6 with different levels of Fear, while for day 3 the association indicated slightly negative (decreasing) log IL-6 levels if and when Fear increased, independent of the sleep opportunity (b = -0.993; Figure 6.2b). The time by Happiness interaction also predicted negative (decreasing) log IL-6 levels at 06:15, if and when

Happiness increased at this time point, independent of the sleep opportunity (Figure 6.2c). Conversely, at 18:15 the interaction indicated positive (increasing) log IL-6 if and when Happiness increased, while at 11:30 and 21:30 there is little change in log IL-6 levels (Figure 6.2c), but given the interaction was of borderline significance (p = 0.050), the results should be interpreted with caution.

IL-8: Significant 2-way interaction effects for condition by Happiness (p = 0.042) and day by Happiness (p = 0.005) on log IL-8 were found. The condition by Happiness interaction indicated positive (increased) log IL-8 levels when Happiness increased in the CON condition (Figure 6.2d). For the SR condition, an increase in Happiness also predicted positive (increased) log IL-8, but the slope at which log IL-8 increased is steeper (b = 0.841) than that observed in the CON condition (b = 0.237; Figure 6.2d), indicating that Happiness had a relatively larger moderating effect on IL-8 in the SR condition. Furthermore, the day by Happiness interaction suggested positive (increased) log IL-8 levels when Happiness increased on day 1 independent of the sleep opportunity (Figure 6.2e). The interaction was also positive for day 2, but the slope for log IL-8 is flatter in comparison to day 1 (b = 0.232; Figure 6.2e). For day 3, the association indicated little change in log IL-8 with different levels of Happiness (Figure 6.2e). Thus Happiness had the largest moderating effect on IL-8 during day 1.

IL-1β: While there were no differences in associations between conditions for log IL-1β and mood, there were significant 2-way interactions for day by Activation (p = 0.021) and day by Fatigue (p = 0.021). For each day, the interaction between day and Activation predicted positive (increased) log IL-1β levels when Activation increased (Figure 6.2f). However, compared to day 1 (b = 0.552) and day 2 (b = 0.903), the slope for day 3 was steepest (b = 1.803), suggesting that Activation had a larger moderating

effect on IL-1β during day 3 (Figure 6.2f). The day by Fatigue interaction indicated little change in log IL-1β with different levels of Fatigue on day 1 (Figure 6.2g). For day 2 and day 3 however, this interaction indicated positive (increased) log IL-1β levels if and when Fatigue increased (Figure 6.2g).

TNF-α: For TNF-α, 2-way interactions were found involving condition by Happiness (p = 0.003) and day by Happiness (p = 0.040). As demonstrated in Figure 6.2h, the condition by Happiness interaction predicted no change in TNF-α with different levels of Happiness in the CON condition, while for the SR condition, the association suggested positive (increased) TNF-α levels if and when Happiness increased (Figure 6.2h). The day by Happiness interaction further indicated positive (increased) TNF-α levels when Happiness increased on day 1 independent of the sleep opportunity (Figure 6.2i). On day 2, an increase in Happiness predicted positive (increased) TNF-α levels (Figure 6.2i), while for day 3, there was no change in TNF-α with different levels of Happiness (Figure 6.2i). The association with Happiness was steepest for day 1 (b = 2.688), indicating that compared to other days, Happiness had a larger moderating effect on TNF-α on the first day of work (Figure 6.2i).

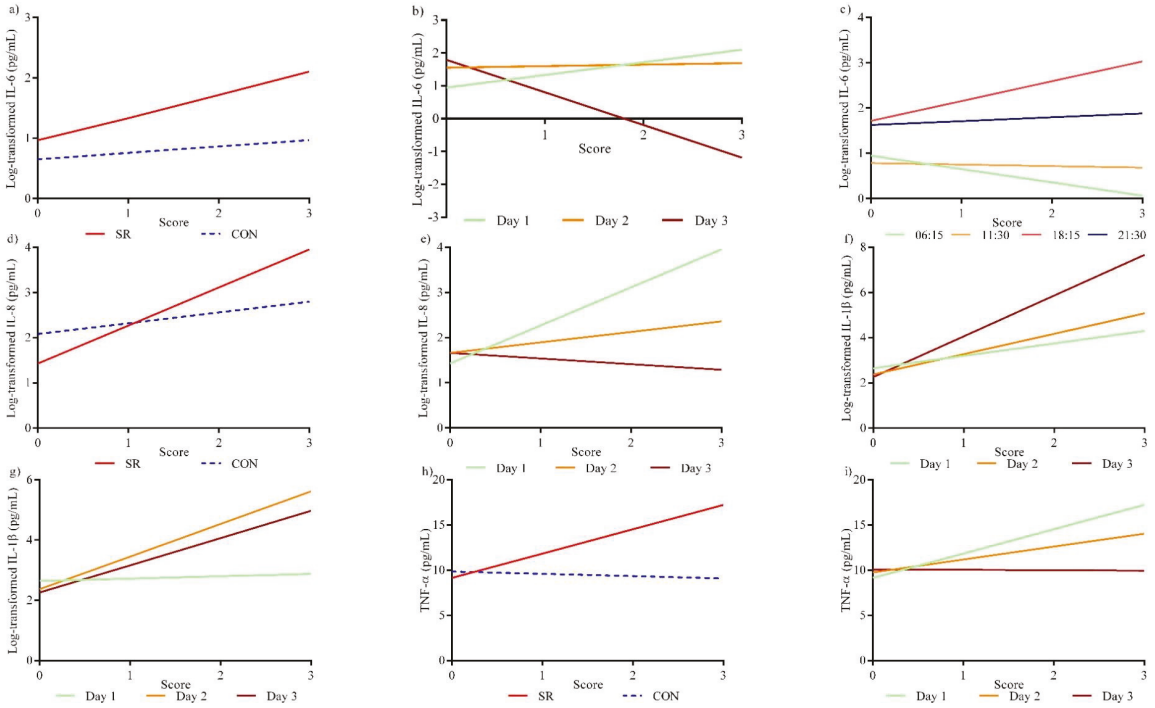

Figure 6.2 Interaction diagrams for Mood Scale II mood dimensions and daily cytokine profiles. (a) Condition by Activation interaction for log IL-6 (p = 0.002). (b) Day by Fear interaction for log IL-6 (p = 0.037). (c) Time by Happiness interaction for log IL-6 (p = 0.050). (d) Condition by Happiness interaction for log IL-8 (p = 0.040). (e) Day by Happiness interaction for log IL-8 (p = 0.005). (f) Day by Activation interaction for log IL-1β (p = 0.021). (g) Day by Fatigue interaction for log IL-1β (p = 0.021). (h) Condition by Happiness interaction for TNF-α (p = 0.003). (i) Day by Happiness interaction for TNF-α (p = 0.040).

Mood Scale II and morning fasting cytokine levels

The LMM with the lowest AIC and ΔAIC for the majority of morning fasting cytokine measures was the full fixed effects Independence model. However, no significant interactions were demonstrated for log IL-6, IL-8, IL-4, IL-10 and IL-1β ($p > 0.05$). For morning TNF-α, the Unstructured model had the lowest AIC and showed a significant 2-way interaction of condition by Fear ($p = 0.036$), which indicated positive (increased) TNF-α levels when Fear increased in the SR condition. Meanwhile, there was little change in TNF-α with different levels of Fear in the CON (Figure 6.3).

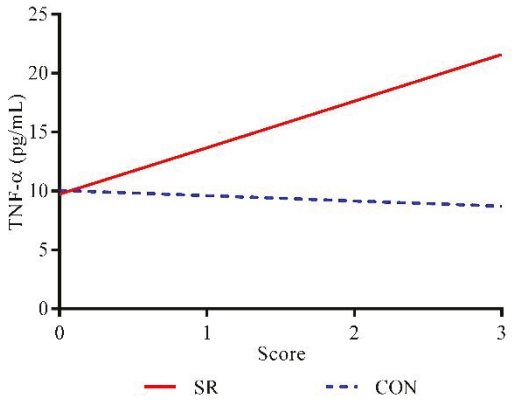

Figure 6.3 Diagram of condition by Fear interaction for morning TNF-α ($p = 0.036$)

Mood Scale II and cortisol AUC

There were no significant relationships between any of the Mood Scale II mood dimensions and cortisol AUC ($p > 0.05$).

Samn-Perelli fatigue scale daily mean and cytokine profiles

After inspecting AIC and ΔAIC values, the Independence LMM had the best fit to model the relationship between log IL-6 and TNF-α profiles and Samn-Perelli Fatigue Scale Daily Mean. The Unstructured model had the lowest AIC and ΔAIC for the Samn-Perelli Fatigue Scale Daily Mean and log IL-10 profile, and therefore had the

best fit to model the relationship. There were no significant relationships between Samn-Perelli Fatigue Scale Daily Mean and log IL-1β and IL-8 profiles ($p > 0.05$).

IL-6: A 3-way interaction of condition by time by Samn-Perelli Fatigue Scale Daily Mean was demonstrated for log IL-6 ($p = 0.044$) which indicated positive (increasing) levels of IL-6 if and when Fatigue increased (Figure 6.4a). In the SR condition, the association with Fatigue was steepest at 11:30 (b = 0.532), while in the CON the slope was greatest at 18:15 (b = 0.237). In comparison to the CON condition, most of the slopes for IL-6 were steeper in the SR condition (Figure 6.4a) suggesting that Fatigue had a larger moderating effect on IL-6 when sleep restricted. While there was no difference in the slopes for this 3-way interaction between days (because day did not interact with Fatigue), the position and intercepts of the plotted values depicted in Figure 6.4a are only relevant for this interaction on day 1.

TNF-α: For TNF-α, 2-way interactions of condition by Samn-Perelli Fatigue Scale Daily Mean ($p = 0.050$) and time by Samn-Perelli Fatigue Scale Daily Mean ($p = 0.023$) were found. For both conditions, the condition by Samn-Perelli Fatigue Scale interaction indicated that TNF-α rose if and when Fatigue increased (Figure 6.4b). For the SR condition however, the slope for TNF-α was steeper (b = 1.710) than the CON condition (b = 0.529; Figure 6.4b), suggesting that Fatigue had a larger moderating effect on TNF-α when sleep restricted. Across each of the daily time points, the time by Samn-Perelli Fatigue Scale Daily Mean interaction indicated positive (increasing) TNF-α levels independent of the sleep opportunity, if and when Fatigue increased (Figure 6.4c). Compared to the other time points (b for; 11:30 = 1.675, 18:15 = 1.092, 21:30 = 1.547), the rise in TNF-α was steepest at the 06:15 time point (b = 1.710;

Figure 6.4c), suggesting that Fatigue had a slightly larger moderating effect on TNF-α at this time.

IL-10: A condition by Samn-Perelli Fatigue Scale Daily Mean ($p = 0.048$) interaction was found for log IL-10. For the CON condition, this interaction indicated negative (decreasing) IL-10 levels if and when Fatigue increased (Figure 6.4d). Whereas for the SR condition, there was a slight increase in log IL-10 with an increase in Fatigue (Figure 6.4d).

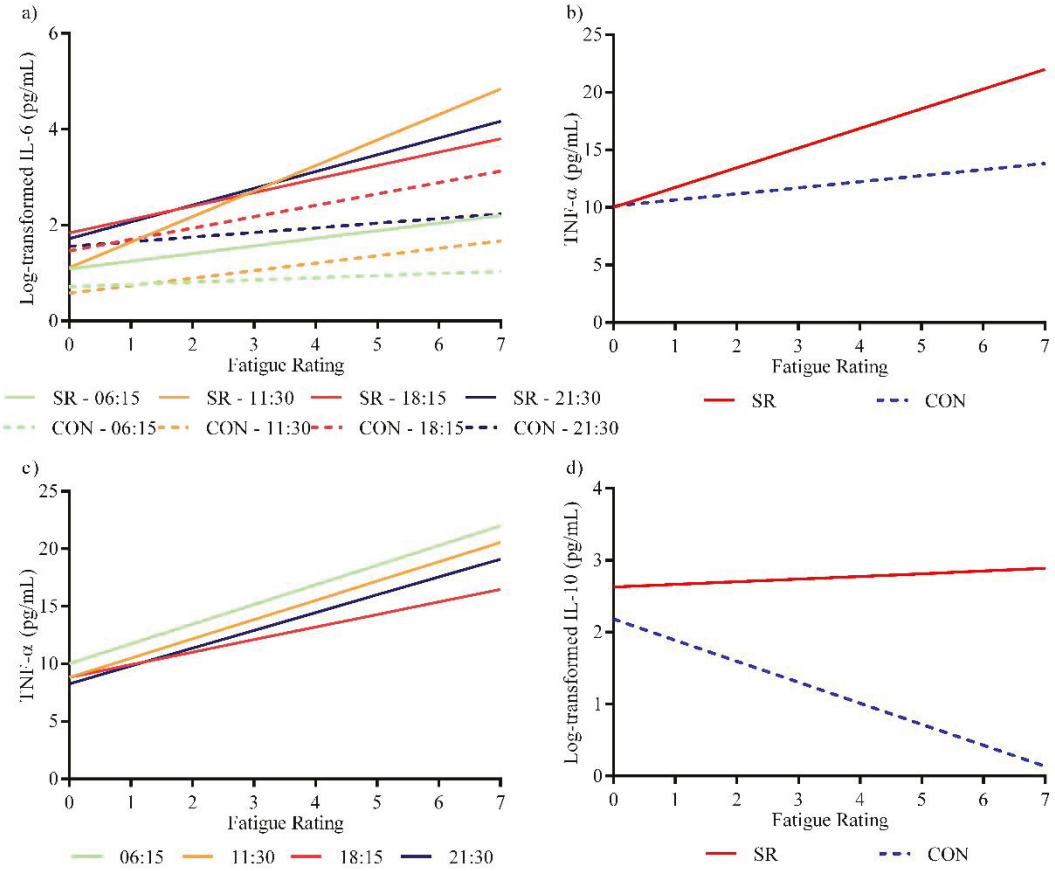

Figure 6.4 Interaction diagrams for Samn-Perelli fatigue scale and daily cytokine profiles. (a) Condition by time by Fatigue Scale mean interaction for log IL-6 ($p = 0.044$). (b) Condition by Fatigue Scale mean interaction for TNF-α ($p = 0.050$). (c) Time by Fatigue Scale mean interaction for TNF-α ($p = 0.023$). (d) Condition by Fatigue Scale mean interaction for log IL-10 ($p = 0.048$).

Samn-Perelli fatigue scale (mean and profile) and morning fasting cytokines and daily cortisol profile

No significant relationships were demonstrated between the Samn-Perelli Fatigue Scale (mean and profile) and morning fasting cytokine measures or the daily cortisol profile (all $p > 0.05$).

Samn-Perelli fatigue scale daily mean and profile and cortisol AUC

The Independence LMM had the lowest AIC and ΔAIC, and therefore the best fit to model relationships between the Samn-Perelli Fatigue Scale Daily Profile and Daily Mean with cortisol AUC. A significant 2-way interaction of condition by Samn-Perelli Fatigue Scale Daily Profile was demonstrated for cortisol AUC ($p = 0.006$; Figure 6.5a). For the SR condition, this interaction indicated positive (increasing) cortisol AUC levels if and when Fatigue increased (Figure 6.5a). Conversely, for the CON condition the interaction was reversed (Figure 6.5a). In addition, a 2-way interaction of condition by Samn-Perelli Fatigue Scale Daily Mean indicated positive (increasing) cortisol AUC levels for the SR condition if and when Fatigue increased ($p = 0.029$; Figure 6.5b). For the CON condition, the association indicated negative (decreasing) cortisol AUC if and when Fatigue increased (Figure 6.5b).

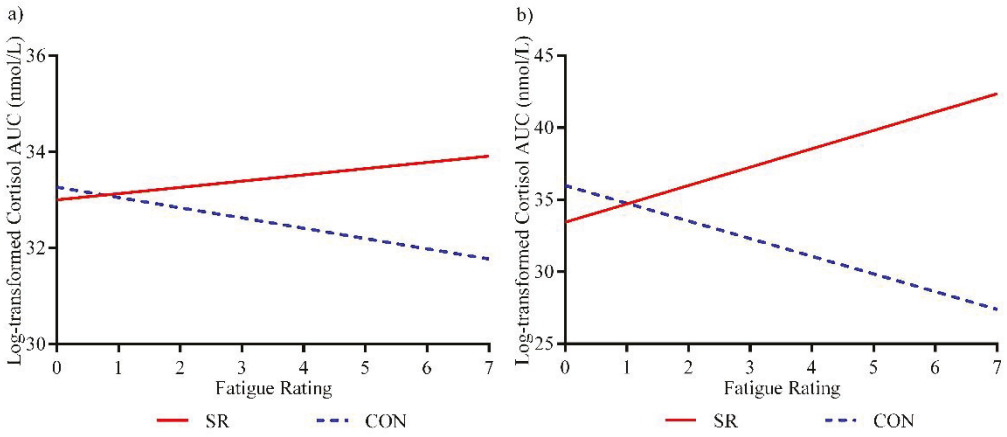

Figure 6.5 Interaction diagrams for Samn-Perelli Fatigue Scale. (a) Condition by Samn-Perelli Fatigue Scale Daily Profile interaction for cortisol AUC ($p = 0.006$). (b) Condition by Samn- Perelli Fatigue Scale Daily Mean interaction for cortisol AUC ($p = 0.029$).

Discussion

The current study examined the relationships between mood, cortisol and inflammatory responses among firefighters performing 3 days of simulated physical firefighting work separated by an 8-h or restricted 4-h sleep on each of the 2 nights. Increases in positive mood dimensions were related to a rise in IL-6, IL-8 and TNF-α in the sleep restriction condition. Meanwhile, increases in negative mood dimensions in the sleep restriction condition were associated with increased IL-6, TNF-α, IL-10 and cortisol. Evidence of psychophysiological relationships in response to physical work and restricted sleep provide insights to fire agencies about subjective fire-ground indicators of physiological changes.

Alterations in positive mood during periods of physical and psychological stress have been linked to immune changes (Kemeny 2007; Marsland et al. 2007; Mittwoch-Jaffe et al. 1995). Findings from the current study are consistent with this and indicate that the mood dimensions Activation and Happiness were associated with changes in IL-6,

IL-8 and TNF-α, and that these relationships were impacted differently by the sleep opportunity. In the SR condition, an increase in Happiness had a greater moderating effect on IL-8 and TNF-α, reflected by a steeper increase for these cytokines. Higher levels of Activation in the SR condition were also associated with increased IL-6. While evidence shows that positive mood is associated with an elevation in Natural Killer cells (Matsunaga et al. 2008) involved in the production of some cytokines (Takahashi et al. 2001; Berk et al. 2001), the current study is the first to observe a positive relationship between positive mood and pro-inflammatory cytokines. The brief up-regulation of innate immune function in response to acute physical work and sleep restriction among the firefighters tested could indicate an adaptive response to these stressors (Sergerstrom and Miller 2004).

While literature investigating the combined impact of physical work and sleep loss on psychophysiological responses is sparse, sleep loss without physical activity has been found to increase cytokine levels (Chennaoui et al. 2011; Vgontzas et al. 2004) and in contrast to our findings, negatively impact positive mood (Paterson et al. 2011). However findings from previous research are reported in response to total sleep deprivation or extended periods of sleep restriction (i.e., ≥ 5 nights), rather than shorter, and potentially less stressful periods of restricted sleep examined in the current study (i.e., 4-h per night for 2 nights). The sleep protocol in the current study was chosen to reflect a typical campaign fire deployment (Cater et al. 2007; Ferguson et al. 2011). Firefighters can however be deployed to fight fires that exceed 3 days (5 to 14 day deployments; Cuddy et al. 2007; Ruby et al. 2002; Ruby et al. 2003). Longer deployments involving complete sleep restriction may result in opposite changes to positive mood (i.e., negative impact), which consequently, may moderate cytokine levels differently. Future research should therefore examine how extended periods of

sleep restriction, during firefighting, impact psychophysiological responses. Laboratory-based studies (Chennaoui et al. 2011; Paterson et al. 2011; Vgontzas et al. 2004) also restricted participants from strenuous physical activity (e.g., exercise), which for active people can increase total mood disturbance, state anxiety, tension and depression (Mondin et al. 1996) and in conjunction with sleep restriction, may contribute to exacerbated mood and inflammatory responses. Firefighters' enhanced immune activation and positive mood may therefore reflect the shorter sleep restriction protocol and less restriction of physical activity, indicating an appropriate response to these demands, rather than a potentially pathogenic psychophysiological relationship (Dhabhar and McEwen 2006). Moreover, short periods of sleep loss have been found to evoke excessive reactivity to reward-relevant stimuli the following day, resulting in an increased number of stimuli considered pleasant (Gujar et al. 2011). Positive mood has also been linked to activation of the immune system (Marsland et al. 2007). Therefore, we may speculate that elevation in positive mood and cytokines in the SR condition resulted from an affective imbalance imposed by sleep loss that triggered firefighters to seek and react excessively to positive reward-relevant stimuli, such as completing firefighting tasks. However, pleasure seeking may also lead to adverse risk taking behaviours (Gujar et al. 2011), which if demonstrated among firefighters on the fire-ground, could compromise the safety of others, highlighting the potential dangers of sleep loss in the field.

Higher levels of Activation were associated with a larger increase in IL-1β on day 3 when compared to day 2 and day 1 in both conditions. Conversely, Happiness was associated with a steep increase in IL-8 and TNF-α on day 1 in both conditions, followed by an attenuation in these cytokines on day 2 and day 3. Within a work day in the current study, higher levels of Happiness were associated with increasing TNF-

α independent of the sleep opportunity. Other studies have found positive mood induction to result in increases in certain cytokines (Mittwoch-Jaffe et al. 1995), suggesting that positive affect may up-regulate innate immunity (Marsland et al. 2007). However, findings are mixed with reports of no change (Ryff et al. 2004) or decreases in cytokines with increased positive mood (Mittwoch-Jaffe et al. 1995; Yoshino and Mukai 2003). Moreover, some investigations demonstrate similar immune changes in response to positive and negative moods (Futterman et al. 1992; Knapp et al. 1992; Marsland et al. 2007). This has led researchers to suggest that the level of affective arousal (i.e., activation or deactivation) rather than valance (i.e., negative or positive), associated with the positive mood is contributing to immune responses (Marsland et al. 2007). Therefore, the variability in cytokine levels across different days and time points during this study could be explained by high levels of arousal linked to the expression of these positive mood dimensions. As research is yet to determine if immune responses depend on affective states or are a function of arousal, subsequent studies examining how interventions that induce high- and low-activation positive mood influence cytokine levels may shed some light on this area. While not taking follow-up measures is a limitation of the study, future research that tracks mood and cytokine interactions post-deployment may also enhance our understanding of the complex relationships between positive mood and immune function.

Relationships were demonstrated between firefighters' TNF-α, IL-6 and IL-1β levels and negative mood dimensions Fear and Fatigue. In the SR condition, increased Fear was associated with a rise in morning fasting TNF-α levels. Fear is quantified in the Mood Scale II using the adjectives 'insecure', 'alarmed', 'afraid', 'uneasy', 'jittery' and 'hopeless' (Thorne et al. 1985). Interpreted this way, an increase in Fear related to

higher TNF-α might reflect firefighters having to contend with the challenge of combined work demands (i.e., sleep restriction and physical work) in the SR condition. Although research supports the role of sleep and cytokine release in regulating various aspects of negative mood (Wright et al. 2005), the authors are unaware of research that has examined how sleep restriction or physical work influence the relationship between Fear and the immune system. Fear conditioning experiments however (i.e., pairing a non-threatening stimulus with a threatening stimulus until a fear response to both stimuli are achieved), suggest that sleep loss can negatively alter brain processes involved in the adaptation to Fear in different situations (Goldstein and Walker 2014). These impairments have been observed alongside sleep loss induced inflammation (Zhu et al. 2012), but research is yet to investigate this interaction in healthy humans. Independent of sleep restriction, increased Fear in the current study was also associated with a decrease in IL-6 on day 3 of the simulation. Previous research is limited, but some comparisons to the current results may be drawn from findings from animal models which have reported that IL-6 deficient mice demonstrate greater fear-related behaviours (Armario et al. 1998; Butterweck et al. 2003). Further research in humans is important in our understanding of how stressors (e.g., sleep restriction and physical work) may impact the relationship between alterations in Fear and the immune system, which could potentially influence or help identify vulnerability to stress-related diseases (e.g., anxiety and depression; McEwen 2006).

Increased Fatigue levels measured on the Mood Scale II were associated with a steep increase in IL-1β on day 2 and day 3 of the simulation in both conditions. Elevated IL-1β levels have also been reported among people experiencing severe fatigue or chronic fatigue syndrome (CFS; Chao et al. 1991; van Zuiden et al. 2012), yet the current study is the first to observe this relationship between subjective fatigue and IL-1β among

healthy people. Further investigation of firefighters in the current study using the Samn-Perelli Fatigue Scale revealed that both IL-6 and TNF-α were positively related to an increase in Fatigue in the SR condition. For IL-6, the SR condition demonstrated steep elevations of this cytokine at all-time points, the largest of which at 12:00. Whereas for TNF-α, a steeper rise in this marker occurred earlier in the day (06:15 and 12:00) across both conditions. Consistent with our findings, sleep loss and physical activity combined have been reported to trigger increases in TNF-α and IL-6 (Abedelmalek et al. 2013; Lundeland et al. 2012; Gundersen et al. 2006), while separate studies have demonstrated the detrimental effect these demands can have on subjective Fatigue (Lieberman et al. 2005; Lieberman et al. 2006; Scott et al. 2006; Wolkow et al. 2015). This is however, the first study to demonstrate a psychophysiological relationship between the experience of Fatigue and an increase in cytokines in response to the stressors physical work and sleep restriction. This link gives insight to early indicators of adverse inflammatory responses and has applications for the use of mood measures to monitor wildfire personnel, which in comparison to biomarkers, offer a less invasive and more practical option. Further establishing thresholds for mood measures that can predict health-related cortisol and inflammatory changes will allow fire agencies to closely monitor personnel on the fire-ground, and if necessary, intervene before their health is impaired.

Fatigue was associated with changes in anti-inflammatory IL-10 levels among wildland firefighters in the current study. For instance, higher Fatigue levels were associated with increases in IL-10 among sleep restricted firefighters, while an inverse relationship was demonstrated in the 8-h sleep condition. To date, clinical based research has reported mixed findings when examining relationships between anti-inflammatory cytokines and fatigue. For instance, CFS patients have been reported to

have elevated IL-10 levels when compared to non-fatigued participants (ter Wolbeek et al. 2007), while similar levels of IL-10 producing cells were demonstrated among participants with CFS and healthy adults (Skowera et al. 2004). Although the impact of physical work and sleep restriction on fatigue and anti-inflammatory cytokines has not been previously investigated, some comparisons to the CFS literature may be useful (ter Wolbeek et al. 2007). In particular, the increased fatigue observed might be related to a shift in regulatory IL-10 cytokine production among the firefighters performing physical work while sleep restricted. However this explanation is speculative and additional research focused on healthy individuals is needed to further elucidate how occupational stressors may influence the relationship between subjective fatigue and the anti-inflammatory profile.

The current study expands on the relationship demonstrated between cytokine and Fatigue by further demonstrating how this negative mood dimension is positively associated with elevated cortisol levels in the SR condition. This supports the work by Vgontzas and colleagues (2008) who proposed that an elevation in cytokine and cortisol is associated with increased fatigue within the following 24-hour cycle. The mechanisms that drive the simultaneous increase in fatigue, inflammation and cortisol among healthy individuals are still largely unknown, but research by Thomas et al. (2011) suggests that a possible reduction in slow wave sleep might be one pathway through which inflammation leads to fatigue. Although findings are mixed, sleep restriction similar in length to the current study has been linked to a small curtailment of slow wave sleep in two studies (Mavanji et al. 2013; Kopasz et al. 2010) which may indicate that a reduction in this sleep phase is underlying the associations in the current study.

To simultaneously assess firefighters over the 3 day simulation required participants to complete the deployment in small groups of 3 to 5, which is different to crew sizes in some parts of the world (e.g., ~ 20 firefighters in rural USA; Cuddy et al. 2007). Interactions between people in small groups when compared to larger groups can impact psychological responses differently. For instance, smaller group size and density may evoke greater hostility and aggressiveness (Doll and Gunderson 1971; Schettino and Borden 1976). Therefore, the psychophysiological relationships observed are most applicable to tanker-based teams that operate in groups of 3 to 5 (Phillips et al. 2012). Additional testing that verifies if the psychological measures examined reflect acute physiological changes among firefighting teams of different sizes will ensure their ecological validity for application to larger groups on the fire-ground. Investigating larger groups will also help determine how sex, and other demographic factors (e.g., age, firefighting experience, BMI) may differently impact firefighters' psychophysiological stress responses to physical work and restricted sleep. An application of this future research may allow fire agencies to monitor firefighters based on certain characteristics such as sex or age. Furthermore, although the brevity and sensitivity of the Mood Scale II (Thorne et al. 1985) and Samn-Perelli Fatigue scale (International Civil Aviation Organization 2011) to mood fluctuations make them well suited to occupational settings, neither measure has been specifically validated in firefighters. Despite this limitation, previous research has investigated their use in occupations with similar demands to firefighting (e.g., aviation, military and nursing; Ryman et al. 1974; Powell et al. 2007; Paterson et al. 2010), which indicates these measures are likely to be sensitive to sleep loss in this population. However, further research should validate their use in firefighters specifically. Together, these next steps (i.e., validating self-report mood measures among larger

groups of firefighters) will help refine the ability of psychological measures to accurately monitor physiological responses among firefighters.

Conclusion

In response to simulated firefighting stressors, changes in mood were related to physiological responses. Increases in positive mood were associated with a larger increase in pro-inflammatory cytokines when firefighters were sleep restricted and performing physical work. Enhanced immune activation and positive mood may reflect an appropriate response by the firefighters to the stressors, yet the available research examining how positive mood affects physiological systems is limited, with no studies having investigated the role of sleep loss and physical work on this psychophysiological relationship. While the current study provides novel insights, future research should employ a more in-depth assessment of positive affect to closely consider the possible influence affective arousal and sleep-loss induced affective imbalances have on physiological responses. Subsequent studies investigating interventions that induce high- and low-activation positive mood on firefighters' cytokine levels, as well as post-deployment tracking of psychophysiological responses will enhance our understanding of the complex relationships between positive mood and immune function. Findings further highlight how, in response to physical wildfire work and restricted sleep, an increase in negative mood dimensions are associated with larger increases in pro-inflammatory cytokines and cortisol. This relationship demonstrates how subjective ratings of negative mood may be used on the fire-ground as indicators of physiological changes. For instance, self-report measures such as the Mood Scale II and Samn-Perelli Fatigue scale provide a practical option for fire-agencies to monitor firefighters' physiological responses on the fire-ground. Additional research is needed that establishes health-related thresholds for these

measures while also exploring the role the observed relationships, if prolonged (e.g., in response to extended or multiple deployments), have in the development of negative health outcomes among personnel.

References

Abedelmalek, S., Souissi, N., Chtourou, H., Denguezli, M., Aouichaoui, C., Ajina, M., et al. (2013). Effects of Partial Sleep Deprivation on Proinflammatory Cytokines, Growth Hormone, and Steroid Hormone Concentrations During Repeated Brief Sprint Interval Exercise. *Chronobiology International: The Journal of Biological and Medical Rhythm Research, 30*(4), 502-509.

Aisbett, B., Phillips, M., Raines, J., and Nichols, D. Work patterns of tanker-based bushfire suppression by Australian volunteer firefighters in south-east Australia. In *Human Dimensions of Wildfire Conference, Fort Collins, Colorado, 2007*

Aisbett, B., Wolkow, A., Sprajcer, M., and Ferguson, S. A. (2012). "Awake, smoky, and hot": Providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters. *Applied Ergonomics, 43*(5), 916-925.

Armario, A., Hernández, J., Hidalgo, J., and Bluethmann, H. (1998). IL-6 deficiency leads to increased emotionality in mice: Evidence in transgenic mice carrying a null mutation for IL-6. [Article]. *Journal Of Neuroimmunology, 92*(1-2), 160-169, doi:10.1016/S0165-5728(98)00199-4.

Barak, Y. (2006). The immune system and happiness. *Autoimmunity Reviews, 5*(8), 523-527.

Berk, L. S., Felten, D. L., Tan, S. A., Bittman, B. B., and Westengard, J. (2001). Modulation of neuroimmune parameters during the eustress of humor-associated mirthful laughter. *Alternative Therapies in Health and Medicine, 7*(2), 62-72.

Bouhuys, A., Flentge, F., and Van den Hoofdakker, R. (1990). Effects of Total Sleep Deprivation on Urinary Cortisol, Self-rated Arousal, and Mood in Depressed Patients. *Psychiatry Research, 34*, 149-162.

Burnham, K. P., and Anderson, D. R. (2002). *Model selection and multi-model inference : a practical information-theoretic approach (2nd ed.).* London, United Kingdom: Springer.

Butterweck, V., Prinz, S., and Schwaninger, M. (2003). Research report: The role of interleukin-6 in stress-induced hyperthermia and emotional behaviour in mice. *Behavioural Brain Research, 144*, 49-56.

Canli, T., Zhao, Z., Desmond, J., Kang, E., Gross, J., and Gabrieli, J. D. E. (2001). An fMRI study of personality influences on brain reactivity to emotional stimuli. *Behavioral Neuroscience, 115*(1), 33-42, doi:10.1037/0735-7044.115.1.33.

Carpenter, J. S., Elam, J. L., Ridner, S. H., Carney, P. H., Cherry, G. J., and Cucullu, H. L. (2004). Sleep, fatigue, and depressive symptoms in breast cancer survivors and matched healthy women experiencing hot flashes. *Oncology Nursing Forum, 31*(3), 591-598, doi:10.1188/04.ONF.591-598.

Cater, H., Clancy, D., Duffy, K., Holgate, A., Wilison, B., and Wood, J. Fatigue on the fireground: the DPI experience. In R. Thornton (Ed.), *Bushfire Cooperative Research Centre/Australasian Fire Authorities Council Conference Research Forum,* Hobart, Tasmania, 2007:

Chao, C. C., Janoff, E. N., Hu, S. X., Thomas, K., Gallagher, M., Tsang, M., et al. (1991). Altered cytokine release in peripheral blood mononuclear cell cultures from patients with the chronic fatigue syndrome. *Cytokine, 3*(4), 292-298.

Chennaoui, M., Sauvet, F., Drogou, C., Van Beers, P., Langrume, C., Guillard, M., et al. (2011). Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-α) levels in healthy men. [Article]. *Cytokine, 56*(2), 318-324, doi:10.1016/j.cyto.2011.06.002.

Chowdhury, F., Williams, A., and Johnson, P. (2009). Validation and comparison of two multiplex technologies, Luminex and Mesoscale Discovery, for human cytokine profiling. *Journal Of Immunological Methods, 340*(1), 55-64, doi:10.1016/j.jim.2008.10.002.

Cuddy, J., Gaskill, S., Sharkey, B., Harger, S., & Ruby, B. (2007). Supplemental feedings increase self-selected work output during wildfire suppression. *Medicine and Science in Sports and Exercise, 39*(6), 1004-1012.

Cullen, T., Thomas, A. W., Webb, R., and Hughes, M. G. (2015). The relationship between interleukin-6 in saliva, venous and capillary plasma, at rest and in response to exercise. *Cytokine*(2), 397, doi:10.1016/j.cyto.2014.10.011.

Damasio, A. R., Grabowski, T. J., Bechara, A., Damasio, H., Ponto, L. L., Parvizi, J., et al. (2000). Subcortical and cortical brain activity during the feeling of self-generated emotions. *Nature Neuroscience, 3*(10), 1049-1056.

Dhabhar, F. S., and McEwen, B. S. (2006). Bidirectional effects of stress on immune function: possible explanations for salubrious as well as harmful effects. In R. Ader (Ed.), *Psychoneuroimmunology* (4 ed.,Vol. 1, pp. 723-760): Elsevier.

Doll, R. E., & Gunderson, E. K. E. (1971). Group size, occupational status and psychological symptomatology in an extreme environment. [Article]. *Journal of Clinical Psychology, 27*(2), 196-198.

Ekman, P., Levenson, R. W., and Friesen, W. V. (1983). Autonomic Nervous System Activity Distinguishes among Emotions. *Science, 221*(4616), 1208-1210.

Faulkner, S. H., Spilsbury, K. L., Harvey, J., Jackson, A., Huang, J., Platt, M., et al. (2014). The detection and measurement of interleukin-6 in venous and capillary blood samples, and in sweat collected at rest and during exercise. *European Journal of Applied Physiology, 114*(6), 1207-1216, doi:10.1007/s00421-014-2851-8.

Ferguson, S. A., Aisbett, B., Jay, S. M., Onus, K., Lord, C., Sprajcer, M., et al. (2011). *Design of a valid simulation for researching physical, physiological and cognitive performance in volunteer firefighters during bushfire deployment.* Paper presented at the Bushfire Cooperative Research Centre/ Australasian Fire and Emergency Service Authorities Council Conference Research Forum, Sydney, New South Wales,

Field, A. (2009). *Discovering Statistics Using SPSS* (Third Edition). London: Sage.

Filaire, E., Legrand, B., Lac, G., and Pequignot, J. M. (2004). Training of elite cyclists: effects on mood state and selected hormonal responses. *Journal of Sports Sciences, 22*(11-12), 1052-1033.

Findlay, J. W., Smith, W. C., Lee, J. W., Nordblom, G. D., Das, I., DeSilva, B. S., et al. (2000). Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. *Journal Of Pharmaceutical And Biomedical Analysis, 21*(6), 1249-1273.

Friedmann, J., Globus, G., Huntley, A., Mullaney, D., Naitoh, P., and Johnson, L. (1977). Performance and Mood During and After Gradual Sleep Reduction. *Psychophysiology, 14*(3), 245-250.

Futterman, A. D., Kemeny, M. E., Shapiro, D., Polonsky, W., and Fahey, J. L. (1992). Immunological variability associated with experimentally-induced positive and negative affective states. *Psychological Medicine, 22*(1), 231-238.

Goldstein, A. N., and Walker, M. P. (2014). The role of sleep in emotional brain function. *Annual Review of Clinical Psychology, 10*, 679-708, doi:10.1146/annurev-clinpsy-032813-153716.

Gujar, N., Yoo, S.-S., Hu, P., and Walker, M. P. (2011). Sleep deprivation amplifies reactivity of brain reward networks, biasing the appraisal of positive emotional experiences. *The Journal of Neuroscience, 31*(12), 4466-4474.

Gundersen, Y., Opstad, P. K., Reistad, T., Thrane, I., and Vaagenes, P. (2006). Seven days' around the clock exhaustive physical exertion combined with energy depletion and sleep deprivation primes circulating leukocytes. *European Journal of Applied Physiology, 97*(2), 151-157.

Haack, M., Sanchez, E., and Mullington, J. M. (2007). Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. *Sleep, 30*(9), 1145-1152.

Haddad, J. J., Saadé, N. E., & Safieh-Garabedian, B. (2002). Cytokines and neuro-immune-endocrine interactions: A role for the hypothalamic-pituitary-adrenal revolving axis. *Journal Of Neuroimmunology, 133*(1-2), 1-19, doi:10.1016/S0165-5728(02)00357-0.

Harte, S. L., Eifert, G. H., & Smith, R. (1995). The effects of running and meditation on beta-endorphin, corticotropin-releasing hormone and cortisol in plasma, and on mood. *Biological Psychology, 40*(3), 251-265.

Heinz, A., Hermann, D., Smolka, M. N., Ricks, M., Graf, K., Pohlau, D., et al. (2003). Effects of acute psychological stress on adhesion molecules, interleukins and sex hormones: implications for coronary heart disease. *Psychopharmacology, 165*(2), 111-117.

Herrald, M. M., and Tomaka, J. (2002). Patterns of emotion-specific appraisal, coping, and cardiovascular reactivity during an ongoing emotional episode. *Journal of Personality and Social Psychology, 83*(2), 434-450.

International Civil Aviation Organization, I. (2011). Fatigue risk management systems: implementation guide for operators.

Jürimäe, J., Mäestu, J., Purge, P., Jürimäe, T., and Sööt, T. (2002). Relations among heavy training stress, mood state and performance for male junior rowers. *Perceptual and Motor Skills, 95*(2), 520-526, doi:10.2466/pms.95.5.520-526.

Kemeny, M. E. (2007). Emotions and the Immune System. In R. Ader (Ed.), *Psychoneuroimmunology* (Vol. 4, pp. 619-629): Elsevier.

Knapp, P. H., Levy, E. M., Giorgi, R. G., Black, P. H., Fox, B. H., and Heeren, T. C. (1992). Short-term immunological effects of induced emotion. *Psychosomatic Medicine, 54*(2), 133-148.

Kopasz, M., Loessl, B., Valerius, G., Koenig, E., Matthaeas, N., Hornyak, M., et al. (2010). No persisting effect of partial sleep curtailment on cognitive performance and declarative memory recall in adolescents. *Journal of Sleep Research, 19*(1), 71-79, doi:10.1111/j.1365-2869.2009.00742.x.

Lieberman, H. R., Bathalon, G. P., Falco, C. M., Kramer, M. F., Morgan, C. A., and Niro, P. (2005). Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat. [Article]. *Biological Psychiatry, 57*, 422-429.

Lieberman, H. R., Niro, P., Tharion, W. J., Nindl, B. C., Castellani, J. W., and Montain, S. J. (2006). Cognition During Sustained Operations: Comparison of a Laboratory Simulation to Field Studies. *Aviation, Space, and Environmental Medicine, 77*(9), 929-935.

Lundberg, U. (1999). Coping with stress: neuroendocrine reactions and implications for health. *Noise and Health, 1*(4), 67-74.

Lundeland, B., Gundersen, Y., Opstad, P. K., Thrane, I., Zhang, Y., Olaussen, R. W., et al. (2012). One week of multifactorial high-stress military ranger training affects Gram-negative signalling. [Article]. *Scandinavian Journal of Clinical and Laboratory Investigation, 72*(7), 547-554.

Maier, S. F. (2003). Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition. *Brain, Behavior, and Immunity, 17*, 69-85.

Maier, S. F., and Watkins, L. R. (1998). Cytokines for Psychologists: Implication of Bidirectional Immune-to-Brain Communication for Understanding Behaviour, Mood, and Cognition. *Psychological Review, 105*(1), 83-107.

Marsland, A. L., Pressman, S., and Cohen, S. (2007). Positive Affect and Immune Function. In R. Ader (Ed.), *Psychoneuroimmunology* (Vol. 4, pp. 761-779): Elsevier.

Matsunaga, M., Isowa, T., Kimura, K., Miyakoshi, M., Kanayama, N., Murakami, H., et al. (2008). Associations among central nervous, endocrine, and immune activities when positive emotions are elicited by looking at a favorite person. *Brain, Behavior, and Immunity, 22*, 408-417, doi:10.1016/j.bbi.2007.09.008.

Mavanji, V., Teske, J. A., Billington, C. J., and Kotz, C. M. (2013). Partial sleep deprivation by environmental noise increases food intake and body weight in obesity-resistant rats. *Obesity, 21*(7), 1396-1405.

McEwen, B. S. (2005). Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism, 54*(Supplement 1), 20-23, doi:10.1016/j.metabol.2005.01.008.

McEwen, B. S. (2006). Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism: Clinical And Experimental, 55*(Supplement 2), S20-S23.

McEwen, B. S., and Seeman, T. (1999). Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences, 896*, 30-47.

Mittwoch-Jaffe, T., Shalit, F., Srendi, B., and Yehuda, S. (1995). Modification of cytokine secretion following mild emotional stimuli. *Neuroreport, 6*(5), 789-792.

Mondin, G. W., Morgan, W. P., Piering, P. N., and Stegner, A. J. (1996). Psychological consequences of exercise deprivation in habitual exercisers. *Medicine and Science in Sports and Exercise, 28*(9), 1199-1203, doi:10.1097/00005768-199609000-00018.

Nguyen, H. P., Björkqvist, M., Bode, F. J., Stephan, M., and von Hörsten, S. (2010). Serum levels of a subset of cytokines show high interindividual variability and are not altered in rats transgenic for Huntington´s disease. *Plos Currents Huntington Disease, 2*, 1-5, doi:10.1371/currents.RRN1190.

Paterson, J. L., Dorrian, J., Ferguson, S. A., Jay, S. M., Lamond, N., Murphy, P. J., et al. (2011). Changes in structural aspects of mood during 39-66 h of sleep loss using matched controls. *Applied Ergonomics, 42*(2), 196-201.

Paterson, J. L., Dorrian, J., PinCombe, J., Grech, C., and Dawson, D. (2010). Mood Change and Perception of Workload in Australian Midwives. *Industrial Health, 48*, 381-389.

Payne, R., Welham, S., and Harding, S. (2011). A Guide to REML in GenStat (16th Edition). Hempstead, Hertfordshire, UK: VSN International.

Phillips, M., Netto, K., Payne, W., Nichols, D., Lord, C., Brooksbank, N., et al. (2011). Frequency, intensity and duration of physical tasks performed by Australian rural firefighters during bushfire suppression. In R. P. Thornton (Ed.), *Bushfire Cooperative Research Center/Australasian Fire Authorities Council Conference Research Forum*, Sydney, New South Wales,

Phillips, M., Netto, K., Payne, W. R., Nichols, D., Lord, C., Brooksbank, N., and Aisbett, B. (2015). Frequency, intensity, time and type of tasks performed during wildfire suppression. *Occupational Medicine and Health Affairs, 3,*199*,* doi: 10.4172/2329-6879.1000199

Phillips, M., Payne, W., Lord, C., Netto, K., Nichols, D., and Aisbett, B. (2012). Identification of physically demanding tasks performed during bushfire suppression by Australian rural firefighters. *Applied Ergonomics, 43*(2), 435-441, doi:10.1016/j.apergo.2011.06.018.

Phillips, M., Raines, J., Nichols, D., and Aisbett, B. Work demands of tanker based bushfire suppression. In R. P. Thornton (Ed.), *Bushfire Cooperative Research Center/Australasian Fire Authorities Conference,* Hobart, Tasmania, 2007.

Powell, D. M. C., Spencer, M. B., Holland, D., Broadbent, E., & Petrie, K. J. (2007). Pilot Fatigue in Short-Haul Operations: Effects of Number of Sectors, Duty Length, and Time of Day. *Aerospace Medicine & Human Performance, 78*(7), 698-701.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., and Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association, 286*(3), 327-334.

Pressman, S. D., & Cohen, S. (2005). Does Positive Affect Influence Health? *Psychological Bulletin, 131*(6), 925-971, doi:10.1037/0033-2909.131.6.925.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., and Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28*(7), 916-931, doi:10.1016/s0306-4530(02)00108-7.

Raines, J., Snow, R., Petersen, A., Harvey, J., Nichols, D., and Aisbett, B. (2013). The effect of prescribed fluid consumption on physiology and work behavior of wildfire fighters. *Applied Ergonomics, 44*(3), 404-413, doi:10.1016/j.apergo.2012.10.002.

Ridker, P. M., Rifai, N., Stampfer M.J, and Hennekens, C. H. (2000). Plasma Concentration of Interleukin-6 and the Risk of Future Myocardial Infarction Among Apparently Healthy Men. *Circulation, 101*, 1767-1772.

Robson-Ansley, P., Barwood, M., Canavan, J., Hack, S., Eglin, C., Davey, S., et al. (2009). The effect of repeated endurance exercise on IL-6 and sIL-6R and their relationship with sensations of fatigue at rest. *Cytokine, 45*, 111-116.

Ruby, B., Schoeller, D., Sharkey, B., Burks, C., & Tysk, S. (2003). Water turnover and changes in body composition during arduous wildfire suppression. *Medicine & Science in Sports & Exercise, 35*(10), 1760-1765.

Ruby, B., Shriver, T., Zderic, T., Sharkey, B., Burks, C., & Tysk, S. (2002). Total energy expenditure during arduous wildfire suppression. *Medicine and Science in Sports and Exercise, 34*(6), 1048-1054.

Ryff, C. D., Singer, B. H., and Dienberg Love, G. (2004). Positive health: connecting well-being with biology. *Philosophical transactions of the Royal Society of London, Series B, Biological sciences, 359*(1449), 1383-1394, doi:10.1098/rstb.2004.1521.

Ryman, D. H., Biersner, R. J., & La Rocco, J. M. (1974). Reliabilities and validities of the mood questionnaire. *Psychological Reports, 35*(1, Pt 2), 479-484, doi:10.2466/pr0.1974.35.1.479.

Samn, S. W., and Perelli, L. P. (1982). Estimating aircrew fatigue: a technique with implications to airlift operations. *Technical Report SAM TR 82 21*. Texas, United States: UASF School of Aerospace Medicine.

Schettino, A. P., & Borden, R. J. (1976). Sex differences in responses to naturalistic crowding: Affective reactions to group size and group density. *Personality and Social Psychology Bulletin, 2*(1), 67-70, doi:10.1177/014616727600200115.

Scott, J. P. R., McNaughton, L. R., and Polman, R. C. J. (2006). Effects of sleep deprivation and exercise on cognitive, motor performance and mood. *Physiology and Behavior, 87*, 396-408.

Sergerstrom, S., and Miller, G. (2004). Psychosocial stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin, 130*(4), 601-630.

Skowera, A., Cleare, A., Blair, D., Bevis, L., Wessely, S. C., and Peakman, M. (2004). High levels of type 2 cytokine-producing cells in chronic fatigue syndrome. *Clinical and Experimental Immunology, 135*(2), 294-302.

Takahashi, K., Iwase, M., Yamashita, K., Tatsumoto, Y., Ue, H., Kuratsune, H., et al. (2001). The elevation of natural killer cell activity induced by laughter in a crossover designed study. *International Journal of Molecular Medicine, 8*(6), 645-650.

ter Wolbeek, M., van Doornen, L. J. P., Kavelaars, A., van de Putte, E. M., Schedlowski, M., and Heijnen, C. J. (2007). Longitudinal analysis of pro- and anti-inflammatory cytokine production in severely fatigued adolescents. *Brain, Behavior, and Immunity, 21*(8), 1063-1074.

Thomas, K. S., Motivala, S., Olmstead, R., and Irwin, M. R. (2011). Sleep depth and fatigue: role of cellular inflammatory activation. *Brain, Behavior, and Immunity, 25*(1), 53-58, doi:10.1016/j.bbi.2010.07.245.

Thorne, D. R., Genser, S. G., Sing, H. C., and Hedge, F. W. (1985). The Walter Reed Performance Assessment Battery. *Neurobehavioral Toxicology and Teratology, 7*, 415-418.

van Zuiden, M., Kavelaars, A., Amarouchi, K., Maas, M., Vermetten, E., Geuze, E., et al. (2012). IL-1beta reactivity and the development of severe fatigue after military deployment: a longitudinal study. *Journal of Neuroinflammation, 9*, 205, doi:10.1186/1742-2094-9-205.

Vgontzas, A. N., Bixler, E. O., Chrousos, G. P., and Pejovic, S. (2008). Obesity and sleep disturbances: Meaningful sub-typing of obesity. *Archives of Physiology and Biochemistry, 114*(4), 224-236, doi:10.1080/13813450802521507.

Vgontzas, A. N., Bixler, E. O., Papanicolaou, D. A., and Chrousos, G. P. (2000). Chronic systemic inflammation in overweight and obese adults. *Journal of the American Medical Association, 283*(17), 2235-2236.

Vgontzas, A. N., Zoumakis, E., Bixler, E. O., Lin, H., Follett, H., Kales, A., et al. (2004). Adverse effects of modest sleep restriction on sleepiness, performance and inflammatory cytokines. *The Journal Of Clinical Endocrinology And Metabolism, 89*(5), 2119-2126.

Vincent, G., Ferguson, S. A., Larsen, B., Wolkow, A., Tran, J., and Aisbett, B. (2015). Sleep restriction during simulated wildfire suppression: effect on physical task performance *PLoS ONE, 10*(1), doi:doi: 10.1371/journal.pone.0115329. eCollection 2015.

von Känel, R., Bellingrath, S., and Kudielka, B. M. (2008). Original article: Association between burnout and circulating levels of pro- and anti-inflammatory cytokines in schoolteachers. *Journal of Psychosomatic Research, 65*, 51-59, doi:10.1016/j.jpsychores.2008.02.007.

Wolkow, A., Aisbett, B., Ferguson, S. A., and Main, L. C. (2014). The impact of sleep restriction on acute inflammatory stress responses to simulated physical firefighting work. In *Sleep DownUnder 2014 ASM: Sleep Frontiers,* Perth, 9-11 October 2014 2014a (Vol. 12, pp. 79): Wiley

Wolkow, A., Aisbett, B., Ferguson, S. A., and Main, L. C. (2015). The effects of work-related sleep restriction on acute physiological and psychological stress responses and their interactions: A review among emergency service personnel. *International Journal of Occupational Medicine and Environmental Health, 28*(2), 183-208.

Wolkow, A., Aisbett, B., Ferguson, S. A., Reynolds, J., and Main, L. C. Effects of sleep restriction on cortisol during simulated physical firefighting work. In *Fourth International Conference on Health Wellness, and Society,* Vancouver, Canada, 14-15 March 2014b

Wright, C. E., Strike, P. C., Brydon, L., and Steptoe, A. (2005). Acute inflammation and negative mood: mediation by cytokine activation. *Brain, Behavior, and Immunity, 19*(4), 345-350, doi:10.1016/j.bbi.2004.10.003.

Yoshino, S., and Mukai, E. (2003). Neuroendocrine-immune system in patients with rheumatoid arthritis. *Modern Rheumatology, 13*(3), 193-198, doi:10.1007/s10165-003-0223-z.

Zhu, B., Dong, Y., Xu, Z., Gompf, H. S., Ward, S. A., Xue, Z., et al. (2012). Sleep disturbance induces neuroinflammation and impairment of learning and memory. *Neurobiol Dis, 48*(3), 348-355, doi:10.1016/j.nbd.2012.06.022.

Zunszain, P. A., Anacker, C., Cattaneo, A., Carvalho, L. A., and Pariante, C. M. (2011). Glucocorticoids, cytokines and brain abnormalities in depression. *Progress In Neuro-Psychopharmacology and Biological Psychiatry, 35*(3), 722-729.

Chapter 7: General Discussion

General Discussion

Wildland firefighting exposes personnel to long periods of physical work with shortened sleep opportunities between shifts. By implementing a 3-day and 2-night simulated wildland firefighting deployment, this dissertation sought to investigate how physical firefighting work and sleep restriction affected firefighters' acute inflammatory, cortisol and heart rate responses, their interactions, and how the firefighters' mood may influence these responses. Specifically, firefighters had either an 8-h or restricted 4-h sleep opportunity each night between days of simulated physical firefighting work. Adopting a multivariate approach to examine stress responses, the current set of studies observed acute changes in several inflammatory cytokines (IL-6, IL-8, TNF-α, IL-1β and IL-4) and alterations to cortisol, heart rate and psychophysiological relationships in response to these demands.

Firefighters completing physical work with restricted sleep exhibited an elevated diurnal release of cortisol that exceeded levels observed among firefighters who had an 8-h sleep, as well as the normal reference range for healthy adults. This draws attention to the role an 8-h sleep on the fire-ground may have in maintaining normal cortisol levels. Meanwhile, the 4-h sleep restriction period did not impact heart rate or cytokine responses in excess of any disturbance caused by physical work alone. A quantifiable immune-endocrine relationship between cytokines and cortisol responses to physical work and sleep restriction was observed. Specifically, the immune-endocrine relationship indicated an increase in morning IL-6 that was related to a rise in evening cortisol among sleep restricted firefighters. Elevated IL-6 levels were further related to increased daily cortisol, but this relationship was not different between firefighters who had an 8-h or 4-h sleep. Exposure to sleep restriction while

performing physical firefighting work was also associated with acute increases in both positive and negative mood that were related to a rise in cortisol and pro- and anti-inflammatory cytokine levels. The interactions between mood, inflammatory and cortisol responses highlight psychophysiological relationships and demonstrate the potential utility of subjective mood as an indicator of physiological stress responses on the fire-ground. In agreement with the concepts of the allostatic load model (McEwen et al. 1999), these findings reveal that exposure to firefighting stressors elicits acute interplay between related stress systems (i.e., immune-endocrine and psychophysiological relationships). The insights provided by this thesis indicate stress response pathways and their underlying interactions through which physical firefighting work, with and without restricted sleep may, over time, impact the health of personnel.

The next section of this chapter provides a detailed summary and discussion of the major findings of this thesis and how they advance the state of knowledge for firefighting and related areas of occupational research. The discussion will then focus on four themes which have emerged from these studies, in which the implications of the findings are considered. The implications relate to firefighting and other physically demanding workplaces and contexts. This section will also address the limitations of this work as they relate to the identified themes before directions for future research are proposed.

7.1 Thesis summary and advances to the state of knowledge

The release of inflammatory cytokines plays an important role in coordinating the body's immune response to a stressor (Elenkov 2008). *The aim of Study 1 was to investigate the effect restricted sleep has on wildland firefighters' pro- and anti-*

inflammatory cytokine levels while performing simulated physical work. Findings revealed that extended periods of simulated wildfire work separated by an 8-h sleep each night elicited an acute IL-8 response that was greater than following the 4-h sleep. The levels of IL-8 in response to the 4-h sleep opportunity are comparable to previous research reporting no significant changes in this cytokine following 2 and 5-h sleep restriction periods over multiple nights (Boudjeltia et al. 2011; Faraut et al. 2011). Meanwhile, increases in IL-8 in response to long duration running (1 to 3 h) performed over 1 to 3 days with no sleep restriction have only been identified in exercise research (Nieman et al. 2003; Nieman et al. 2014; Pedersen et al. 2008) as opposed to occupational settings. These current findings therefore advance our understanding of the effects of multiday periods of physical work (in this case wildfire-specific work) on IL-8.

The impact of physical activity in an occupational setting on IL-8 may have relevance to other jobs in which personnel perform prolonged periods of manual labour and achieve sleep durations greater than 4-h, such as construction workers (Powell et al. 2010). However, reasons for the differences in the IL-8 response between conditions in Study 1 are difficult to explain and could be related to factors that were not controlled for in an effort to simulate fire-ground conditions. For instance, there were no restrictions on physical activity outside of the work circuits and during the rest breaks. The lack of restrictions on physical activity may have allowed firefighters who had an 8-h sleep opportunity to be more physically active during the rest periods than those who had a restricted 4-h sleep opportunity (Vincent et al. 2015). While it is unknown what caused the differences in physical activity between conditions, Vincent and colleagues (2015) propose that the sleep-restricted firefighters may have altered their activity to conserve effort throughout the rest periods, so that they could maintain

their performance during the work circuits. Given that long-duration physical activity is known to increase IL-8 (Pedersen et al. 2008), the greater physical activity recorded among participants who had 8-h sleep may have contributed to the elevated IL-8 levels observed in this condition.

Study 1 findings are in agreement with applied exercise physiology research (Bruunsgaard 2005; Pedersen et al. 2008). The increased release of IL-6, and attenuation of IL-1β and TNF-α observed among firefighters in both conditions, act to mediate regulatory inflammatory responses to aid in maintaining homeostasis of the immune system during periods of physical stress. In addition to pro-inflammatory properties, the anti-inflammatory actions of IL-6 (Pedersen et al. 2008; Pedersen et al. 2007; Starkie et al. 2003), along with IL-4 (Opal et al. 2000), which also increased among the firefighters tested, can down regulate pro-inflammatory TNF-α and IL-1β, resulting in a decrease in these cytokines. This inflammatory cascade exhibited in the current study represents an acute response distinctively different to that associated with severe inflammation (i.e., increases TNF-α, IL-1β and IL-6 in that order; Bruunsgaard 2005; Pedersen et al. 2008), indicative of a non-damaging homeostatic cytokine response to the simulated physical work performed by firefighters in the current study. By advancing our understanding of the acute interplay between cytokines that regulate inflammatory responses to physical work and sleep restriction, this study demonstrates how short-term exposure to these firefighting stressors does not represent an acute inflammatory risk.

In response to physical and psychological stressors, cytokines can activate the hypothalamic-pituitary-adrenal (HPA)-axis causing the release of cortisol (Steensberg et al. 2003; Turnbull et al. 1999). Stressors also stimulate the sympathetic-adrenal-

medullary (SAM) system, triggering an increase in heart rate (Chandola et al. 2010; Lundberg 1999). Like the immune system, HPA-axis and SAM system activation to stressors is expected, but severe or repeated exposure can dysregulate cortisol and heart rate responses (Juster et al. 2010; McEwen 2006; McEwen et al. 1999). *Study 2 aimed to assess the effect restricted sleep has on wildland firefighters' cortisol and heart rate responses while performing simulated physical work*. Implementation of a high resolution sampling protocol in this study allowed for the measurement of cortisol output over a specific time period (i.e., daily cortisol AUC), complemented by changes in cortisol at different time points (i.e., diurnal cortisol profile). Compared to military-based studies that have examined limited sleep totalling 1 to 7-h over 5 to 7 days (Gundersen et al. 2006; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981), the comprehensive assessment of cortisol in Study 2 provides new evidence that a moderate 4-h sleep-restriction period over 2 consecutive nights markedly alters cortisol levels above the upper limit of the adult normal reference range in the afternoon and evening. The controlled periods of work and sleep examined in Study 2 are more ecologically relevant for civilian occupations (e.g., emergency rescue and medical personnel) who experience 5 to 6 h of sleep restriction (Geiger-Brown et al. 2012; Jenkins et al. 2007) when compared to military demands that involve almost complete sleep deprivation and continuous physical work (Gundersen et al. 2006; Lundeland et al. 2012; Opstad 1994; Opstad et al. 1981). Therefore, findings may have implications for people who experience moderate levels of sleep restriction and work jobs that involve extended physical demands (e.g., rescue workers and nurses; Geiger-Brown et al. 2012; Jenkins et al. 2007). Details of the possible short- and long-term health consequences elevated cortisol has for personnel will be presented in the next section.

In addition to cortisol, quantifying diurnal heart rate as a measure of SAM system activity advances the understanding of concurrent HPA-axis and SAM system responses within the neuroendocrine system. The HPA-axis and SAM system responses have seldom been assessed simultaneously in an applied occupational setting. Heart rate decreased across the 3-days, but this change was not different between firefighters who had 8-h or 4-h sleep opportunity, revealing that the SAM system may adapt to the simulated demands in mild temperatures. Alternatively, participants may have become increasingly more economical in executing the actions required to complete the physical tasks (Sparrow et al. 1999). Either way, this indicates a non-damaging SAM system response to the simulated physical work and sleep restriction investigated among the firefighters tested.

In an extension of studies 1 and 2, *Study 3 aimed to quantify the relationship between firefighters' cytokine and cortisol responses to restricted sleep while performing simulated physical work.* This is the first occupational-based study to have investigated this immune-endocrine interaction between cytokines and cortisol which is central to the functioning of the HPA-axis and immune system (Petrovsky 2001; Turnbull et al. 1999). Results indicated that elevated levels of IL-6 may have stimulated a more pronounced rise in evening cortisol among the sleep-restricted firefighters. The positive association between IL-6 and cortisol AUC, despite cortisol's known anti-inflammatory actions (Chrousos 1995) provides the first occupational-based evidence supporting the notion that cortisol may have immuno-modulatory effects in response to stressors rather than being solely immune-suppressive (Desantis et al. 2012; Elenkov 2008; Elenkov et al. 2002; McEwen et al. 1997). This could be represented by the immune system becoming resistant to the inhibitory actions of cortisol, resulting in sustained or upregulated inflammation (Miller et al. 2002). Acute elevations in

HPA-axis and IL-6 activity may be linked to alterations in mood (Vgontzas et al., 2008, Kemeny, 2007).

Changes in immune and cortisol responses discussed above may be related to both negative and positive mood states (Kemeny 2007; Marsland et al. 2007; Mittwoch-Jaffe et al. 1995). *Study 4 examined how changes in wildland firefighters' mood relate to cytokine and cortisol levels in response to restricted sleep while performing simulated physical work.* Although research has linked mood to immune function in response to various stressors in different populations (Chao et al. 1991; Kemeny 2007; van Zuiden et al. 2012; Vgontzas et al. 2008), Study 4 is the first to report a positive relationship between negative mood states and pro-inflammatory cytokines (i.e., TNF-α, IL-6 and IL-1β) among healthy adults exposed to sleep restriction and physical work in an occupational setting. Evidence of a psychophysiological relationship among firefighters provides a basis for further research to understand how, in response to occupational stressors, alterations in negative mood and the immune system may influence or help identify vulnerability to stress-related diseases (e.g., anxiety and depression).

Using a specific 'fatigue' measure (i.e., Samn-Perelli Fatigue Scale), Study 4 further revealed that subjective 'fatigue' had a greater moderating effect on IL-6 and cortisol in the sleep-restricted condition which resulted in a steeper rise for these markers compared to the 8-h sleep condition. This supports previous work (Vgontzas et al. 2008) and highlights how exposure to sleep restriction may cause increases in subjective fatigue which reflects inflammatory and cortisol responses. A bi-directional communication network between the brain (i.e., central nervous system) and the immune system most likely explains the observed relationships between subjective

mood and cytokine responses (Kemeny 2007; Lorton et al. 2006; Maier 2003). Inflammatory changes also lead to HPA-axis activation, and the activity of this axis (i.e., cortisol release) is controlled by the brain (Maier 2003; Turnbull et al. 1999). Although, the specific mechanisms that drive the simultaneous increase in subjective fatigue, IL-6 and cortisol when exposed to sleep restriction are still largely unknown, some research implicates changes in sleep architecture as meditating the relationship between these responses (Späth-Schwalbe et al. 1998; Thomas et al. 2011). For instance, Thomas et al. (2011) showed that reduced slow wave sleep (SWS) mediated the relationship between higher evening IL-6 and fatigue among healthy adults. A low dose of exogenous IL-6 was also found by Späth-Schwalbe et al. (1998) to be positively associated with increased fatigue and reduced SWS in the first part of the night, in which this stage is usually more dominant. Disruption to SWS may have been due to the increased release of cortisol, induced by exogenous IL-6 (Späth-Schwalbe et al. 1998). Sleep restriction similar in length to that investigated in this dissertation has been linked to a small curtailment of SWS (Mavanji et al. 2013; Kopasz et al. 2010). It is possible therefore, that a reduction in this sleep phase is underlying the observed relationship between fatigue, cortisol and cytokine responses among the firefighters' tested. The potential fire-ground implications of these findings will be discussed in detail in the next section.

In contrast to research examining the moderating effects of negative mood (Kemeny 2007; Vgontzas et al. 2008), investigations into relationships between positive mood, and immune and HPA-axis function is limited. To date, experiencing positive mood has been linked to an elevation in Natural Killer cells (Matsunaga et al. 2008) involved in the production of some cytokines (Berk et al. 2001; Takahashi et al. 2001). Study 4 expands on this by revealing a potential link between increased positive moods (i.e.,

'Happiness' and 'Activation') and elevated IL-6, IL-8 and TNF-α levels during simulated physical work. Positive mood and immune function may have a curvilinear relationship, with small to medium changes in mood increasing cytokine release to cope with a given stressor (Barak 2006; Koh 1998; Marsland et al. 2007). However, intense/strong positive mood associated with changes to the arousal dimension (i.e., high arousal/activation and low arousal/de-activation), irrespective of valance (i.e., level of pleasantness to unpleasantness), may have maladaptive effects (Marsland et al. 2007). Therefore, elevated positive mood and immune activation among sleep restricted firefighters exposed to simulated physical work, may either reflect the body's normal defence to these stressors, corroborating conclusions drawn from Study 1, or indicate potentially detrimental effects. In order to understand these complex interactions between positive mood and cytokine response, and their potential implications for health, further research is required.

7.2 Implications and future research directions

7.2.1 Sleep restriction

The detailed assessment of the cortisol response in Study 2 highlights that sleep-restricted firefighters had significantly higher daily AUC cortisol levels and an elevated cortisol profile in the afternoon and evening when compared to participants who had an 8-h sleep opportunity. Therefore, to reduce the risk of reduced sleep disrupting HPA-axis function, I recommend that fire agencies should, where possible, ensure their personnel receive more than 4 h sleep between days of work on the fire-ground. In addition to firm evidence supporting the importance of an 8- to 9-h sleep opportunity in maintaining cognitive function (Belenky et al. 2003; Van Dongen et al. 2003), these findings further demonstrate the protective role an 8-h sleep opportunity between shifts of firefighting work may have on preserving cortisol levels. However,

additional research trialling 7-h, 6-h and 5-h sleep opportunities would help establish the minimum sleep required on the fire-ground to maintain normal cortisol levels. Greater daily cortisol output in the short-term (i.e., across the simulation) were related to higher levels of negative affect among firefighters in Study 4, a finding which was supported by Piazza et al. (2013). This affective state has been linked to reduced working memory (Spies et al. 1996) and task motivation (Brose et al. 2012), which for firefighters, could have critically adverse implications on job performance and safety. Further, confirming the cortisol findings on the fire-ground would inform future industry recommendations regarding the management of risk associated with sleep in the field.

In contrast to cortisol, the 4-h sleep restriction period investigated was not a significant enough stressor to affect heart rate over and above the influence of simulated physical work. Consequently, heart rate decreased across the 3-days which may reflect an improved economy and efficiency of physical work performance (Sparrow et al. 1999). It is also possible that attenuated heart rate stemmed from an adaptation of the SAM system or possibly the exhaustion of this stress response pathway to the demands. Furthermore, it is conceivable that in Study 2, firefighters' SAM system was activated differently (i.e., down-regulated) when compared to experiencing physical work demands in a real world setting. But given the uncertainty, recommendations for industry would be strengthened by future research examining other measures of SAM system activity in the laboratory, and most importantly, in the field. For instance, heart rate variability (i.e., variation in consecutive beat to beat intervals) has been shown to closely reflect diurnal SAM system activity, characterised in healthy individuals by increases during the night and decreases during the day (Marques et al. 2010). Conversely, catecholamine levels offer a more direct measure of SAM system activity,

but several methodological difficulties exist with collecting this measure (e.g., 24-h urine or blood sampling and restriction of nicotine, certain drugs and foods containing vanilla or amines). In addition, catecholamines have distinct diurnal rhythms (Lundberg 2010), which means that the assessment of these markers would require firefighters to refrain from smoking, caffeine and certain foods while multiple daily blood or urine samples are collected. In comparison to plasma catecholamines which provide variable time-point readings and must be rapidly separated and stored frozen, urinary epinephrine and norepinephrine reflect mean stress response levels over longer periods (e.g., a day; Weinkove 1991) making them a preferred method in the field when assessing occupational stressors.

Evidence of acute inflammatory and cortisol relationships which underlie these stress systems was demonstrated among firefighters in Study 3. Specifically, an 8-h sleep opportunity separating shifts of physical work influenced immune-endocrine interactions so that a rise in morning IL-6 does not relate to increased evening cortisol. Conversely, when firefighters had a restricted 4-h sleep opportunity between shifts, increased morning IL-6 levels were positively related to a rise in evening cortisol. This supports the above recommendation that fire agencies should, where possible, ensure their personnel receive more than 4-h sleep between days of work on the fire-ground to reduce the risk of shortened sleep disrupting interactions between the HPA-axis and the immune system, specifically IL-6. Findings also provide a basis from which to compare and forecast how future workplace interventions (e.g., trialling new shift schedules) that influence sleep duration alter immune-endocrine interactions. Like Study 3, it is important a comprehensive sampling protocol (i.e., multiple daily cortisol and cytokine samples) that captures potential imbalances between responses is implemented when investigating such interventions. However, considering the bi-

directional relationship between cytokines and cortisol (Petrovsky 2001; Turnbull et al. 1999), future research that experimentally alters cortisol or cytokine levels (e.g., administering exogenous cortisol or cytokines) may be needed to confirm our understanding of the casual direction of the observed associations.

The duration and timing of sleep in this series of studies was based on fire-ground research (Cater et al. 2007) and constrained the examined sleep restriction period to a late night phase (i.e., 02:00-06:00). Emergency workers, in addition to personnel from other occupations (e.g., air crew, rail and truck drivers) may experience restricted sleep at different times (Åkerstedt 1990; Åkerstedt et al. 2010). This emphasises the need to further determine how an early-phase sleep restriction period (e.g., bedtime 22:00-02:00 due to the need to go to bed early prior to an early morning shift/emergency call-out) affects firefighters' HPA-axis and inflammatory responses. In particular, advanced timing of the sleep-phase may result in a larger reduction in rapid-eye-movement sleep (Wu et al. 2010), a stage of sleep known to have a positive association with IL-6 and TNF-α (Pandey et al. 2011; Yehuda et al. 2009). This may explain why, in contrast to our findings, research investigating a 4.5-h earlier-phase sleep opportunity (22:30-03:00) reported a rise in IL-6 post-physical work and sleep restriction compared to an 8.5-h sleep opportunity (22:30-07:00; Abedelmalek et al. 2013). In addition, it is possible that personnel may also experience a very late-phase sleep restriction period (e.g., bedtime 04:00-08:00, due to difficulties initiating sleep after a late shift/emergency call-out). However, emergency service- or sleep-based research is yet to investigate how this phase of sleep restriction impacts inflammatory or HPA-axis function, potentially an important area for further investigation.

Acute inflammatory and cortisol changes in response to different phases and durations of sleep restriction are thought to be, at least in part, the result of alterations in sleep architecture (Bierwolf et al. 1997; Pandey et al. 2011; Vgontzas et al. 1999; Yehuda et al. 2009). Furthermore, a recent review by Irwin et al. (2015) concluded that acute sleep disturbances (e.g., sleep quality, insomnia etc.) rather than sleep restriction, contribute more to increases in pro-inflammatory cytokine levels. Not analysing sleep quality or sleep architecture among firefighters was a limitation of this thesis. Therefore, it is important future research employ measures to assess sleep quality, such as the Pittsburgh Sleep Quality Index (Buysse et al. 1989), and sleep architecture through the use of PSG. Although PSG was only used to measure total sleep time in this thesis, our laboratory plans to use this data to further examine the mechanistic pathways relating to slow wave sleep and rapid-eye-movement sleep that may impact acute changes in HPA-axis and inflammatory responses.

7.2.2 Recovery between, longer and repeated firefighting deployments

Having established acute increases in cortisol, several cytokines (i.e., IL-6, IL-8 and IL-4) and immune-endocrine interactions which demonstrate how a rise in IL-6 is linked to elevated cortisol, the introduction of follow-up measures in the days after an deployment represents an important next step for this area of firefighting research. For instance, such measures may provide useful information on the ability of inflammatory and HPA-axis responses to recover. If recovery time is prolonged, this could lead to the development of adverse health outcomes (Geurts et al. 2006). Although not assessing follow-up measures is an acknowledged limitation of this work, cumulative trends for increasing cortisol and pro-inflammatory cytokines (i.e., IL-6, IL-8 and IL-4) support further research to establish the amount and/or number of recovery sleep(s) necessary to restore cortisol and cytokine levels to baseline following a deployment.

Of priority, is to now measure cortisol and cytokine levels after the 8-h recovery sleep that all participants had prior to leaving the testing venue in the current series of studies. Accordingly, our research team plans to examine these follow-up measures along with samples taken after an extra work circuit on the day following the simulated deployment. This would aid fire-agencies in determining the minimum recovery time needed for the same personnel to safely return to the same or another fire-ground or other demanding emergency events and hazards (e.g., rescues, floods etc.). Premature return may exacerbate inflammatory and cortisol responses and increase the risk of adverse long-term health outcomes.

The ratio between physical work and recovery time (i.e., rest and sleep opportunities) in wildland fire operations can vary depending on a range of factors related to the fire. Often, sedentary factors such as extended travel time between the fire line and camp, difficulty sleeping in an unfamiliar and noisy environment at the camp site and winding down after a shift can contribute to sleep restriction on the fire-ground (Cater et al., 2007). To reflect these conditions, sleep restricted firefighters were free to perform sedentary leisure activities (e.g., watching television, reading, playing cards etc.) until the delayed bedtime (i.e., 02:00). The study design enabled the same amount of simulated physical work to be studied in both conditions, which allowed for an easier interpretation of observed stress responses. However, at times firefighters need to control the spread of wildfire without relief from other personnel (e.g., wildfires in remote locations). In these instances, firefighters may have their shifts lengthened (Cater et al., 2007) resulting in less time for sleep. Consequently, the combined impact prolonged periods of physical work which extend to the onset of shortened sleep opportunities have on cortisol, inflammatory and psycho-physiological responses could be greater than that found in the current thesis. Accordingly, further research

should examine how different ratios of physical work to sleep time impact acute physiological and psychological stress responses.

The 3-day and 2-night protocol examined in the present series of studies was chosen to reflect the period firefighters are typically deployed to fight campaign fires in Australia (Cater et al. 2007; Ferguson et al. 2011). However, firefighters can be deployed to fight wildfires that exceed 3 days (e.g., 5 to 14 day wildfire deployments; Cuddy et al. 2007; Ruby et al. 2003; Ruby et al. 2002). Therefore the acute increases in cytokine (i.e., IL-6, IL-8 and IL-4) and cortisol levels, immune-endocrine interactions and psychophysiological responses observed in the current work are not necessarily generalizable to longer deployments. Future research should therefore examine how extended periods (e.g., 5 to 14 days) of physical work and sleep restriction impact these stress responses among personnel. Understanding how these stress systems respond when exposed to occupational demands over longer durations will provide fire agencies with important knowledge for managing fatigue-related risks among personnel combating extended wildfires. In addition to firefighting, physically demanding occupations that have consecutive 7- to 14-day work periods and reported sleep disturbances (e.g., offshore oil and gas installations; Parkes 2012) may benefit from further examining concurrent stress responses to these demands.

Considering that cortisol and certain cytokines (i.e., IL-6, IL-8 and IL-4) were found to respond to a single simulated deployment, it is necessary further research examines how repeated periods of combined sleep restriction and wildland firefighting work affects these responses. Indeed, there is likely to be an increased demand for more firefighting deployments each year, given that summers are becoming increasingly longer (Bureau of Meteorology et al. 2014) and there is a decrease in the numbers of

personnel, especially volunteers, involved in Australian (McLennan et al. 2005) and United States of America (Karter et al. 2013) wildland firefighting activities. As a consequence, this may expose firefighting personnel to repeated periods of combined physical work and sleep restriction. Physically demanding occupations that involve shift rotations and have reported sleep disturbances (e.g., offshore oil and gas installations; Parkes 2012), may also encounter repeated exposure to these demands. Although physiological responses are capable of adapting to repeated stressors (McEwen 1998), if the stress exposure is severe (e.g., length of exposure and shortened time between exposures), frequent experience may exacerbate the inflammatory and cortisol response over a longer period of time (e.g., over a fire season). Prolonged inflammatory and cortisol levels may increase the risk of adverse long-term health outcomes such as cardiovascular disease (CVD; Lippi et al. 2008; Rosmond et al. 2003; Willerson et al. 2004) and depression (Dantzer et al. 2008; Mackin et al. 2004). In addition to cortisol, research should examine how exposure to sleep loss and physical work among firefighters' may alter other hormonal axes, such as the growth hormone/insulin-like growth factor axis and the luteinizing hormone/testosterone axis, linked to the pathophysiology of CVD (Colao 2008; Hyde et al. 2011).

7.2.3 Psychophysiological relationships and fire-ground monitoring of stress responses

At present, occupational-based research investigating psychophysiological relationships between stress responses is limited. Study 4 advances this area by showing that when exposed to sleep restriction while performing simulated firefighting work, both positive and negative mood dimensions relate to certain inflammatory and cortisol responses. The observed increases in positive mood dimensions related to a rise in IL-6, IL-8 and TNF-α provide the first evidence of this

relationship in an occupational setting. One application of these findings could be that psychological mood measures can be used to monitor physiological responses on the fire-ground. Compared to collecting and analysing blood and/or saliva samples to evaluate inflammatory and HPA-axis function, monitoring these responses using mood measures offers a less invasive and more practical option (e.g., more immediate results, no storage of samples etc.). However, further assessment of positive mood is necessary. It is possible that current research suggesting the relationship between positive mood and immune markers could be a function of affective arousal (i.e., level of activation to de-activation) rather than valance (i.e., level of pleasantness to unpleasantness) related to the expression of positive moods (Marsland et al. 2007). If this is the case, measures that examine the arousal and valance dimensions of mood in detail would allow for further insight regarding how positive mood and physiological responses are related and what relevance this has for the health of firefighters and personnel with similar occupational demands (e.g., military, search and rescue personnel). Examples of mood measures which assess valance and arousal dimensions include the bioinformational model (Lang 1978) and the self assessment manikin (Bradley et al. 1994). Additional follow-up testing that tracks how mood and cytokine responses relate post-deployment could enable a greater understanding of the complex relationships between positive mood and the immune system.

Positive associations between an increase in negative mood dimensions and elevated cytokine (i.e., IL-6, TNF-α, IL-10) and cortisol levels during physical work and restricted sleep provide information for fire agencies about subjective fire-ground indicators of physiological changes. These findings provide a basis from which to investigate whether the magnitude of inflammatory and cortisol alterations associated with a negative mood carry relevance to the pathogenesis of health outcomes such as

cardiovascular and metabolic diseases and depression, especially in light of their increased prevalence in some firefighting populations (An et al. 2015; Carey et al. 2011; Cook et al. 2013; Kales et al. 2007; Wolkow et al. 2014). Ultimately, this could help establish thresholds for mood measures that accurately reflect and help predict health-related cortisol and inflammatory changes.

The present controlled laboratory study allowed for the quantification of acute inflammatory, SAM system, HPA-axis and psychological stress responses to sleep restriction while performing multiple days of simulated physical work. An evidence-based approach was taken to replicate a campaign fire deployment by using highly representative simulated fire suppression tasks, sleeping conditions and types of food and drink provided. However, the artificial environment did not capture some elements of live firefighting. For instance, limited access to research space and equipment meant that it was only possible to simultaneously assess firefighters in groups of 3 to 5, which is different to crew sizes in some parts of the world (e.g., ~ 20 firefighters in rural USA; Cuddy et al. 2007). Although research is limited, interactions between people in small groups when compared to larger groups can impact psychological responses differently. For instance, smaller group size and density may evoke greater hostility and aggressiveness respectively (Doll et al. 1971; Schettino et al. 1976). Therefore, the psychophysiological relationships observed in Study 4 are most applicable to tanker-based teams that operate in groups of 3 to 5 (Phillips et al. 2012). Additional testing that verifies if the psychological measures examined reflect acute physiological changes among firefighting teams of different sizes will ensure their ecological validity for application to larger groups on the fire-ground. Furthermore, although females make up a smaller percentage (17%) of wildland firefighters in Australia (McLennan et al. 2005), research with larger samples will help determine how sex,

and other demographic factors (e.g., age, BMI), impact firefighters' psychophysiological stress responses to physical work and restricted sleep. Together, these next steps (i.e., establishing thresholds and validating with different firefighting conditions and larger samples) will help refine the ability of psychological measures to accurately monitor firefighters, and if necessary, intervene before their health is impaired.

The physical intensity, radiant heat and life or death consequences of live fire suppression activities are difficult to simulate away from the fire-ground. These real-world stressors encountered by personnel may elicit significant physiological and psychological stress responses. In the current study, the simulation of physical work and sleep restriction in isolation, may therefore remove other frequently encountered stressors from firefighters working conditions. Consequently, it is possible the current results slightly underestimate firefighters' neuroendocrine, inflammatory and psychological responses when exposed to these, and other demands faced in real world settings. Preparatory or post-fire work performed in mild temperatures, such as that simulated in the current study, does however represent a significant proportion of firefighting (Budd et al. 1997; Raines et al. 2013; Rodríguez-Marroyo et al. 2012). Moreover, on completion of the simulated deployment, firefighters provided feedback indicating that the duration, intensity and timing of the physical work and sleep restriction examined in the current study reflected exposure to these stressors involved in preparatory or post-fire work on the fire-ground. This makes the findings from this thesis highly applicable to a large portion of firefighting operations, in addition to related industries which expose personnel to sleep restriction while performing physical work in mild temperatures (e.g., maintenance workers and other emergency services). Nevertheless, confirming the findings from this thesis in the field represents

an essential next step for this area of research, which despite the methodological challenges, will afford insights with greater ecological validity to fire and other hazardous occupational settings.

7.2.4 Stress responses and chronic health outcomes

Acute increases in pro-inflammatory cytokines (IL-8 and IL-6; Study 1), cortisol (Study 2) and an imbalance between these systems (i.e., immune-endocrine relationship; Study 3) evident in this body of work highlight potential stress response pathways which, over time, may adversely impact physical and psychological health. For instance, chronically elevated cortisol and inflammatory responses as well as altered immune-endocrine functioning have been implicated in the pathophysiology of adverse long-term health outcomes, such as cardiovascular and metabolic diseases (Lippi et al. 2008; Nijm et al. 2007; Rosmond et al. 2003) and depression (Dantzer et al. 2008; Mackin et al. 2004). This may be of further concern to Australian wildland fire agencies who have an ageing volunteer firefighter workforce (McLennan et al. 2005), given the potentially harmful impact older age can have on increasing the circulation of both IL-6 and IL-8 (Bruunsgaard et al. 2001; Rink L et al. 1998) and prolonging the IL-8 response to occupational stressors (Dutheil et al. 2013). This may suggest that older firefighters need extended recovery times following a fire-ground deployment. Further research could also investigate potential fire-ground strategies such as a moderate ingestion of carbohydrates (e.g., up to 60 grams per hour of physical work) and certain supplements (e.g., quercetin; Walsh et al. 2011) shown to dampen inflammation, thereby also preventing potential increases in evening cortisol.

Expression of positive associations between negative mood and cortisol, as well as inflammatory changes (Study 4), if persistent, have been further linked to chronic

health outcomes such as depression (Lutgendorf et al. 2008; Musselman et al. 2001). Indeed mood disorders (An et al. 2015; Carey et al. 2011; Cook et al. 2013), along with CVD are particularly prevalent among some firefighting populations (Kales et al. 2007; Wolkow et al. 2014). However, there is no evidence of an increase in CVD-related mortality (Glass et al. 2014) or a higher prevalence of CVD risk factors for Australian wildland firefighters when compared to the general population (Wolkow et al. 2014). Therefore, research is needed to establish if the acute stress responses observed after a single deployment are independently related to long-term health outcomes among wildland firefighters worldwide. Additional studies that employ longitudinal designs are necessary to understand if and how multiple deployments to the fire-ground over a fire season, and over multiple fire seasons impact cortisol, inflammatory and psychophysiological responses over the long-term. Gathering further morbidity and mortality data from this population of Australian wildland firefighters will help determine the degree to which responses may be linked to adverse health outcomes (e.g., CVD, depression). Insights provided by such research may help fire agencies in determining whether additional precautions are needed to mitigate the potential risks that physical work and sleep restriction pose to firefighters' health over their life cycle.

7.3 References

Abedelmalek, S, Souissi, N, Chtourou, H, Denguezli, M, Aouichaoui, C, Ajina, M, Aloui, A, Dogui, M, Haddouk, S and Tabka, Z 2013, 'Effects of Partial Sleep Deprivation on Proinflammatory Cytokines, Growth Hormone, and Steroid Hormone Concentrations During Repeated Brief Sprint Interval Exercise', *Chronobiology International: The Journal of Biological and Medical Rhythm Research*, vol. 30, no. 4, pp. 502-509.

Åkerstedt, T 1990, 'Psychological and psychophysiological effects of shift work', *Scandinavian Journal Of Work, Environment and Health*, vol. 16, Supplement 1, pp. 67-73.

Åkerstedt, T, Kecklund, G and Selén, J 2010, "Early Morning Work—Prevalence And Relation To Sleep/Wake Problems: A National Representative Survey', *Chronobiology International: The Journal of Biological and Medical Rhythm Research*, vol. 27, no. 5, pp. 975-986.

An, SJ, Chung, YK, Kim, BH, Kwak, KM, Son, JS, Koo, JW, Ju, YS and Kwon, YJ 2015, 'The effect of organisational system on self-rated depression in a panel of male municipal firefighters', *Annals Of Occupational And Environmental Medicine*, vol. 27, pp. 1-7.

Barak, Y 2006, 'The immune system and happiness', *Autoimmunity Reviews*, vol. 5, no. 8, pp. 523-527.

Belenky, G, Wesensten, NJ, Thorne, DR, Thomas, ML, Sing, HC, Redmond, DP, Russo, MB and Balkin, TJ 2003, 'Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: a sleep dose-response study', *Journal of Sleep Research*, vol. 12, no. 1, pp. 1-12.

Berk, LS, Felten, DL, Tan, SA, Bittman, BB and Westengard, J 2001, 'Modulation of neuroimmune parameters during the eustress of humor-associated mirthful laughter', *Alternative Therapies in Health and Medicine*, vol. 7, no. 2, pp. 62-72.

Bierwolf, C, Struve, K, Marshall, L, Born, J and Fehm, HL 1997, 'Slow wave sleep drives inhibition of pituitary-adrenal secretion in humans', *Journal of Neuroendocrinology*, vol. 9, pp. 479-484.

Boudjeltia, KZ, Faraut, B, Esposito, MJ, Stenuit, P, Dyzma, M, Antwerpen, PV, Brohée, D, Vanhamme, L, Moguilevsky, N, Vanhaeverbeek, M and Kerkhofs, M 2011, 'Temporal dissociation between myeloperoxidase (MPO)-modified LDL and MPO elevations during chronic sleep restriction and recovery in healthy young men', *PLoS ONE*, vol. 6, no. 11, pp. e28230-e28230.

Bradley, M and Lang, P 1994, 'Measuring emotion: The self assessment manikin and the semantic differential', *Journal of Behavior Therapy and Experimental Psychiatry*, vol. 25, no. 1, pp. 49-59.

Brose, A, Schmiedek, F, Lövdén, M and Lindenberger, U 2012, 'Daily variability in working memory is coupled with negative affect: The role of attention and motivation', *Emotion*, vol. 12, no. 3, pp. 605-617.

Bruunsgaard, H 2005, 'Physical activity and modulation of systemic low-level inflammation', *Journal of Leukocyte Biology*, vol. 78, no. 4, pp. 819-835.

Bruunsgaard, H, Pedersen, M and Pedersen, BK 2001, 'Aging and proinflammatory cytokines', *Current Opinion in Hematology*, vol. 8, no. 3, pp. 131-136.

Budd, GM, Brotherhood, JR, Hendrie, AL, Jeffery, SE, Beasley, FA, Costin, BP, Wu, Z, Baker, MM, Cheney, NP and Dawson, MP 1997, 'Project Aquarius 4. Experimental bushfires, suppression procedures, and measurements', *International Journal of Wildland Fire*, vol. 7, no. 2, pp. 99-104.

Bureau of Meteorology and Commonwealth Scientific and Industrial Research Organisation (CSIRO) 2014, *State of the Climate 2014*, Australian Goverment.

Buysse, DJ, Reynolds, CF, 3rd, Monk, TH, Berman, SR, Kupfer, DJ 1989, 'The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research', *Psychiatry Res,* vol. 28, no 2, pp. 193-213.

Carey, MG, Al-Zaiti, SS, Dean, GE, Sessanna, L and Finnell, DS 2011, 'Sleep problems, depression, substance use, social bonding, and quality of life in professional firefighters', *Journal Of Occupational And Environmental Medicine/American College Of Occupational And Environmental Medicine*, vol. 53, no. 8, pp. 928-933.

Cater, H, Clancy, D, Duffy, K, Holgate, A, Wilison, B and Wood, J 2007, 'Fatigue on the fireground: the DPI experience', *Bushfire Cooperative Research Centre/Australasian Fire Authorities Council Conference Research Forum*, Hobart, Tasmania.

Chandola, T, Heraclides, A and Kumari, M 2010, 'Psychophysiological biomarkers of workplace stressors', *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 1, pp. 51-57.

Chao, CC, Janoff, EN, Hu, SX, Thomas, K, Gallagher, M, Tsang, M and Peterson, PK 1991, 'Altered cytokine release in peripheral blood mononuclear cell cultures from patients with the chronic fatigue syndrome', *Cytokine*, vol. 3, no. 4, pp. 292-298.

Chrousos, GP 1995, 'The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation', *The New England Journal Of Medicine*, vol. 332, no. 20, pp. 1351-1362.

Colao, A 2008, 'The GH-IGF-I axis and the cardiovascular system: clinical implications', *Clinical Endocrinology,* vol. 69, no. 3, pp. 347-358.

Cook, B and Mitchell, W 2013, *Occupational health effects for firefighters: The extent and implications of physical and psychological injuries*, Centre of Full Employment and Equity.

Cuddy, J, Gaskill, S, Sharkey, B, Harger, S and Ruby, B 2007, 'Supplemental feedings increase self-selected work output during wildfire suppression', *Medicine and Science in Sports and Exercise*, vol. 39, no. 6, pp. 1004-1012.

Dantzer, R, O'Connor, J, Freund, G, Johnson, R and Kelley, K 2008, 'From inflammation to sickness and depression: when the immune system subjugates the brain', *Nature Reviews Neuroscience*, vol. 9, no. 1, pp. 46-56.

Desantis, AS, Diezroux, AV, Hajat, A, Aiello, AE, Golden, SH, Jenny, NS, Seeman, TE and Shea, S 2012, 'Associations of salivary cortisol levels with inflammatory markers: The Multi-Ethnic Study of Atherosclerosis', *Psychoneuroendocrinology*, vol. 37, pp. 1009-1018.

Doll, RE and Gunderson, EKE 1971, 'Group size, occupational status and psychological symptomatology in an extreme environment', *Journal of Clinical Psychology*, vol. 27, no. 2, pp. 196-198.

Dutheil, F, Trousselard, M, Perrier, C, Lac, G, Chamoux, A, Duclos, M, Naughton, G, Mnatzaganian, G and Schmidt, J 2013, 'Urinary interleukin-8 is a biomarker of stress in emergency physicians, especially with advancing age-the JOBSTRESS* randomized trial', *PLoS ONE*, vol. 8, no. 8, pp. e71658-e71658.

Elenkov, IJ 2008, 'Neurohormonal-cytokine interactions: Implications for inflammation, common human diseases and well-being', *Neurochemistry International*, vol. 52, pp. 40-51.

Elenkov, IJ and Chrousos, GP 2002, 'Stress Hormones, Proinflammatory and Antiinflammatory Cytokines, and Autoimmunity', *Annals of the New York Academy of Sciences*, vol. 966, no. 1, pp. 290-303.

Faraut, B, Boudjeltia, KZ, Dyzma, M, Rousseau, A, David, E, Stenuit, P, Franck, T, Van Antwerpen, P, Vanhaeverbeek, M and Kerkhofs, M 2011, 'Benefits of napping and an extended duration of recovery sleep on alertness and immune cells after acute sleep restriction', *Brain, Behavior, and Immunity*, vol. 25, no. 1, pp. 16-24.

Ferguson, SA, Aisbett, B, Jay, SM, Onus, K, Lord, C, Sprajcer, M and Thomas, MJW 2011, 'Design of a valid simulation for researching physical, physiological and cognitive performance in volunteer firefighters during bushfire deployment', *in Proceedings of Bushfire Cooperative Research Centre/ Australasian Fire and Emergency Service Authorities Council Conference Research Forum*, ed. RP Thornton, Sydney, pp. 196-204.

Geiger-Brown, J, Rogers, VE, Trinkoff, AM, Kane, RL, Bausell, RB and Scharf, SM 2012, 'Sleep, Sleepiness, Fatigue, and Performance of 12-Hour-Shift Nurses', *Chronobiology International: The Journal of Biological and Medical Rhythm Research*, vol. 29, no. 2, pp. 211-219.

Geurts, SAE and Sonnentag, S 2006, 'Recovery as an explanatory mechanism in the relation between acute stress reactions and chronic health impairment', *Scandinavian Journal Of Work, Environment and Health*, vol. 32, no. 6, pp. 482-492.

Glass, D, Sim, M, Pircher, S, Del Monaco, A, Dimitriadis, C, Miosge, J, Vander Hoorn, S and Gordon, I 2014, *Australian Firefighters' Health Study*, Monash Centre for Occupational and Environmental Health, Melbourne, Victoria.

Gundersen, Y, Opstad, PK, Reistad, T, Thrane, I and Vaagenes, P 2006, 'Seven days' around the clock exhaustive physical exertion combined with energy depletion and sleep deprivation primes circulating leukocytes', *European Journal of Applied Physiology*, vol. 97, no. 2, pp. 151-157.

Hyde, Z, Norman, PE, Flicker, L, Hankey, GJ, McCaul, KA, Almeida, OP, Yeap, BB 2011. 'Elevated LH predicts ischaemic heart disease events in older men: the Health in Men Study', *European Journal of Endocrinology,* vol. 164, no. 4, pp. 569-577.

Irwin, MR, Olmstead, R, and Carroll, JE 2015. 'Sleep Disturbance, Sleep Duration, and Inflammation: A Systematic Review and Meta-Analysis of Cohort Studies and Experimental Sleep Deprivation', *Biological Psychiatry*, doi: 10.1016/j.biopsych.2015.05.014.

Jenkins, JL, Fredericksen, K, Stone, R and Tang, N 2007, 'Strategies to improve sleep during extended search and rescue operations', *Prehospital Emergency Care: Official Journal Of The National Association Of EMS Physicians And The National Association Of State EMS Directors*, vol. 11, no. 2, pp. 230-233.

Juster, R, McEwen, B and Lupien, S 2010, 'Allostatic load biomarkers of chronic stress and impact on health and cognition', *Neuroscience and Biobehavioral Reviews*, vol. 35, no. 1, pp. 2-16.

Kales, SN, Soteriades, ES, Christophi, CA and Christiani, DC, 2007, 'Emergency duties and deaths from heart disease among firefighters in the United States', *The New England Journal Of Medicine*, vol. 356, no. 12, pp. 1207-1215.

Karter, M and Stein, G 2013, *United States Fire Department Profile Through 2012*, National Fire Protection Association.

Kemeny, ME 2007, 'Emotions and the Immune System', in R, Ader, (ed.), *Psychoneuroimmunology*, Elsevier, pp. 619-629.

Koh, KB 1998, 'Emotion and immunity', *Journal of Psychosomatic Research*, vol. 45, no. 2, pp. 107-115.

Lang, P 1978, *Anxiety: Toward a psychophysiological definition*, Psychiatric diagnosis: Exploration of biological predictors, Spectrum, New York.

Lippi, G, Franchini, M, Salvagno, G, Montagnana, M and Guidi, G 2008, 'Higher morning serum cortisol level predicts increased fibrinogen but not shortened APTT', *Journal Of Thrombosis And Thrombolysis*, vol. 26, no. 2, pp. 103-105.

Lorton, D, Lubahn, CL, Estus, C, Millar, BA, Carter, JL, Wood, CA and Bellinger, DL 2006, 'Bidirectional communication between the brain and the immune system: implications for physiological sleep and disorders with disrupted sleep', *Neuroimmunomodulation*, vol. 13, no. 5-6, pp. 357-374.

Lundberg, U 1999, 'Coping with stress: neuroendocrine reactions and implications for health', *Noise and Health*, vol. 1, no. 4, pp. 67-74.

Lundberg, U 2010, 'Neuroendrocrine Measures', in R Contrada and A Baum (eds), *The Handbook of Stress Science: Biology, Psychology, and Health*, Springer.

Lundeland, B, Gundersen, Y, Opstad, PK, Thrane, I, Zhang, Y, Olaussen, RW and Vaagenes, P 2012, 'One week of multifactorial high-stress military ranger training affects Gram-negative signalling', *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 72, no. 7, pp. 547-554.

Lutgendorf, SK, Weinrib, AZ, Penedo, F, Russell, D, DeGeest, K, Costanzo, ES, Henderson, PJ, Sephton, SE, Rohleder, N, Lucci, JA, 3rd, Cole, S, Sood, AK and Lubaroff, DM 2008, 'Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients', *Journal Of Clinical Oncology: Official Journal Of The American Society Of Clinical Oncology*, vol. 26, no. 29, pp. 4820-4827.

Mackin, P and Young, AH 2004, 'The role of cortisol and depression: exploring new opportunities for treatments', *Psychiatric Times*, vol. 21, no. 5, pp. 92-95.

Maier, SF 2003, 'Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition', *Brain, Behavior, and Immunity*, vol. 17, pp. 69-85.

Marques, AH, Silverman, MN and Sternberg, EM 2010. 'Evaluation of Stress Systems by Applying Noninvasive Methodologies: Measurements of Neuroimmune Biomarkers in the Sweat, Heart Rate Variability and Salivary Cortisol'. *Neuroimmunomodulation,* vol. 17, no. 3, pp. 205-208.

Marsland, AL, Pressman, S and Cohen, S 2007, 'Positive Affect and Immune Function', in R Ader (ed.), *Psychoneuroimmunology*, Elsevier, pp. 761-779.

Matsunaga, M, Isowa, T, Kimura, K, Miyakoshi, M, Kanayama, N, Murakami, H, Sato, S, Konagaya, T, Nogimori, T, Fukuyama, S, Shinoda, J, Yamada, J and Ohira, H 2008, 'Associations among central nervous, endocrine, and immune activities when positive emotions are elicited by looking at a favorite person', *Brain, Behavior, and Immunity*, vol. 22, pp. 408-417.

McEwen, BS 1998, 'Stress, adaptation, and disease - Allostasis and allostatic load', *Annals of the New York Academy of Sciences*, vol. 840, pp. 33-44.

McEwen, BS 2006, 'Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load', *Metabolism: Clinical And Experimental*, vol. 55, Supplement 2, pp. S20-S23.

McEwen, BS, Biron, CA, Brunson, KW, Bulloch, K, Chambers, WH, Dhabhar, FS, Goldfarb, RH, Kitson, RP, Miller, AH, Spencer, RL and Weiss, JM 1997, 'The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions', *Brain Research Reviews*, vol. 23.

McEwen, BS and Seeman, T 1999, 'Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load', *Annals of the New York Academy of Sciences*, vol. 896, pp. 30-47.

McLennan, J and Birch, A 2005, 'A potential crisis in wildfire emergency response capability? Australia's volunteer firefighters', *Environmental Hazards*, vol. 6, pp. 101-107.

Miller, GE, Cohen, S and Ritchey, AK 2002, 'Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model', *Health Psychology*, vol. 21, no. 6, pp. 531-541.

Mittwoch-Jaffe, T, Shalit, F, Srendi, B and Yehuda, S 1995, 'Modification of cytokine secretion following mild emotional stimuli', *Neuroreport*, vol. 6, no. 5, pp. 789-792.

Musselman, DL, Miller, AH, Porter, MR, Manatunga, A, Gao, F, Penna, S, Pearce, BD, Landry, J, Glover, S, McDaniel, JS and Nemeroff, CB 2001, 'Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: Preliminary findings', *The American Journal of Psychiatry*, vol. 158, no. 8, pp. 1252-1257.

Nieman, DC, Davis, JM, Henson, DA, Walberg-Rankin, J, Shute, M, Dumke, CL, Utter, AC, Vinci, DM, Carson, JA, Brown, A, Lee, WJ, McAnulty, SR and McAnulty, LS 2003, 'Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run', *Journal of Applied Physiology*, vol. 94, no. 5, pp. 1917-1925.

Nieman, DC, Luo, BB, Dréau, D, Henson, DA, Shanely, RA, Dew, D and Meaney, MP 2014, 'Immune and inflammation responses to a 3-day period of intensified running versus cycling', *Brain, Behavior, and Immunity*, vol. 39, pp. 180-185.

Nijm, J, Kristenson, M, Olsson, AG and Jonasson, L 2007, 'Impaired cortisol response to acute stressors in patients with coronary disease. Implications for inflammatory activity', *Journal of Internal Medicine*, vol. 262, no. 3, pp. 375-384.

Opal, SM and DePalo, VA 2000, 'Anti-inflammatory cytokines', *Chest*, vol. 117, no. 4, pp. 1162-1172.

Opstad, PK 1994, 'Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men', *European Journal of Endocrinology*, vol. 131, no. 1, pp. 56-66.

Opstad, PK and Aakvaag, A 1981, 'The effect of a high calory diet on hormonal changes in young men during prolonged physical strain and sleep deprivation', *European Journal of Applied Physiology*, vol. 46, no. 1, pp. 31-39.

Pandey, AK and Kar, SK 2011, 'REM sleep deprivation of rats induces acute phase response in liver', *Biochemical and Biophysical Research Communications*, vol. 410, no. 2, pp. 242-246.

Parkes, KR 2012, 'Shift schedules on North Sea oil/gas installations: a systematic review of their impact on performance, safety and health', *Safety Science*, vol. 50, no. 7, pp. 1636-1651.

Pedersen, BK and Febbraio, MA 2008, 'Muscle as an endocrine organ: focus on muscle-derived interleukin-6', *Physiological Reviews*, vol. 88, no. 4, pp. 1379-1406.

Pedersen, BK and Fischer, CP 2007, 'Beneficial health effects of exercise--the role of IL-6 as a myokine', *Trends in Pharmacological Sciences*, vol. 28, no. 4, pp. 152-156.

Petrovsky, N 2001, 'Towards a unified model of neuroendocrine–immune interaction', *Immunology and Cell Biology*, vol. 79, no. 4, pp. 350-357.

Phillips, M, Payne, W, Lord, C, Netto, K, Nichols, D and Aisbett, B 2012, 'Identification of physically demanding tasks performed during bushfire suppression by Australian rural firefighters', *Applied Ergonomics*, vol. 43, no. 2, pp. 435-441.

Piazza, JR, Charles, ST, Stawski, RS and Almeida, DM 2013, 'Age and the association between negative affective states and diurnal cortisol', *Psychology and Aging*, vol. 28, no. 1, pp. 47-56.

Powell, R and Copping, A 2010, 'Sleep Deprivation and Its Consequences in Construction Workers', *Journal of Construction Engineering and Management*, vol. 136, no. 10, pp. 1086-1092.

Raines, J, Snow, R, Petersen, A, Harvey, J, Nichols, D and Aisbett, B 2013, 'The effect of prescribed fluid consumption on physiology and work behavior of wildfire fighters', *Applied Ergonomics*, vol. 44, no. 3, pp. 404-413.

Rink L, Cakman I and H, K 1998, 'Altered cytokine production in the elderly', *Mechanisms of Ageing and Development*, vol. 102, no. 199-209.

Rodríguez-Marroyo, JA, López-Satue, J, Pernía, R, Carballo, B, García-López, J, Foster, C and Villa, JG 2012, 'Physiological work demands of Spanish wildland firefighters during wildfire suppression', *International Archives Of Occupational And Environmental Health*, vol. 85, no. 2, pp. 221-228.

Rosmond, R, Wallerius, S, Wanger, P, Martin, L, Holm, C and Björntorp , P 2003, 'A 5-year follow-up study of disease incidence in men with an abnormal hormone pattern', *Journal of Internal Medicine*, vol. 254, pp. 386-390.

Ruby, BC, Schoeller, DA, Sharkey, BJ, Burks, C and Tysk, S, . 2003, 'Water turnover and changes in body composition during arduous wildfire suppression', *Medicine and Science in Sports and Exercise*, vol. 35, no. 10, pp. 1760-1765.

Ruby, BC, Shriver, TC, Zderic, TW, Sharkey, BJ, Burks, C and Tysk, S, 2002, 'Total energy expenditure during arduous wildfire suppression', *Medicine and Science in Sports and Exercise*, vol. 34, no. 6, pp. 1048-1054.

Schettino, AP and Borden, RJ 1976, 'Sex differences in responses to naturalistic crowding: Affective reactions to group size and group density', *Personality and Social Psychology Bulletin*, vol. 2, no. 1, pp. 67-70.

Sparrow, WA, Hughes, KM, Russell, AP and Le Rossignol, PF 1999, 'Effects of practice and preferred rate on perceived exertion, metabolic variables and movement control', *Human Movement Science*, vol. 18, no. 2-3, pp. 137-153.

Späth-Schwalbe, E, Hansen, K, Schmidt, F, Schrezenmeier, H, Marshall, L, Burger, K, Fehm, HL and Born, J 1998, 'Acute effects of recombinant human interleukin-6 on endocrine and central nervous sleep functions in healthy men', *The Journal Of Clinical Endocrinology And Metabolism*, vol. 83, no. 5, pp. 1573-1579.

Spies, K, Hesse, F and Hummitzsch, C 1996, 'Mood and capacity in Baddeley's model of human memory', *Zeitschrift für Psychologie mit Zeitschrift für angewandte Psychologie*, vol. 204, no. 4, pp. 367-381.

Starkie, R, Ostrowski, SR, Jauffred, S, Febbraio, M and Pedersen, BK 2003, 'Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans', *The Federation of American Societies for Experimental Biology (FASEB) Journal*, vol. 17, no. 8, pp. 884-886.

Steensberg, A, Fischer, CP, Keller, C, Moller, K and Pedersen, BK 2003, 'IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans', *American Journal of Physiology - Endocrinology and Metabolism*, vol. 285, no. 2, pp. 433-437.

Takahashi, K, Iwase, M, Yamashita, K, Tatsumoto, Y, Ue, H, Kuratsune, H, Shimizu, A and Takeda, M 2001, 'The elevation of natural killer cell activity induced by laughter in a crossover designed study', *International Journal of Molecular Medicine*, vol. 8, no. 6, pp. 645-650.

Thomas, KS, Motivala, S, Olmstead, R and Irwin, MR 2011, 'Sleep depth and fatigue: role of cellular inflammatory activation', *Brain, Behavior, and Immunity*, vol. 25, no. 1, pp. 53-58.

Turnbull, AV and Rivier, CL 1999, 'Regulation of the Hypothalamic-Pituitary-Adrenal Axis by Cytokines: Actions and Mechanisms of Action', *Physiological Reviews*, vol. 79, no. 1, pp. 1-71.

Van Dongen, HP, Maislin, G, Mullington, JM and Dinges, DF 2003, 'The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation', *Sleep*, vol. 26, no. 2, pp. 117-126.

van Zuiden, M, Kavelaars, A, Amarouchi, K, Maas, M, Vermetten, E, Geuze, E and Heijnen, CJ 2012, 'IL-1beta reactivity and the development of severe fatigue after military deployment: a longitudinal study', *J Neuroinflammation*, vol. 9, p. 205.

Vgontzas, AN, Bixler, EO, Chrousos, GP and Pejovic, S 2008, 'Obesity and sleep disturbances: Meaningful sub-typing of obesity', *Archives of Physiology and Biochemistry*, vol. 114, no. 4, pp. 224-236.

Vgontzas, AN, Mastorakos, G, Bixler, EO, Kales, A, Gold, PW and Chrousos, GP 1999, 'Sleep deprivation effects on the activity of the hypothalamic-pituitary-adrenal and growth axes: potential clinical implications', *Clinical Endocrinology*, vol. 51, no. 2, pp. 205-215.

Vincent, G, Ferguson, SA, Larsen, B, Wolkow, A, Tran, J and Aisbett, B 2015, 'Sleep restriction during simulated wildfire suppression: effect on physical task performance ', *PLoS ONE*, vol. 10, no. 1.

Walsh, NP, Gleeson, M, Pyne, DB, Nieman, DC, Dhabhar, FS, Shephard, RJ, Oliver, SJ, Bermon, S and Kajeniene, A 2011, 'Position statement. Part two: Maintaining immune health', *Exercise Immunology Review*, vol. 17, pp. 64-103.

Weinkove, C 1991, 'ACP Broadsheet No 127: April 1991. Measurement of catecholamines and their metabolites in urine', *Journal Of Clinical Pathology*, vol. 44, no. 4, pp. 269-275.

Willerson, JT and Ridker, PM 2004, 'Inflammation as a cardiovascular risk factor', *Circulation*, vol. 109, no. Supplement 1, pp. II2-II10.

Wolkow, A, Netto, K, Langridge, P, Green, J, Nichols, D, Sergeant, M and Aisbett, B 2014, 'Coronary Heart Disease Risk in Volunteer Firefighters in Victoria, Australia', *Archives of Environmental and Occupational Health*, vol. 69, no. 2, pp. 112-120.

Wu, H, Stone, WS, Hsi, X, Zhuang, J, Huang, L, Yin, Y, Zhang, L and Zhao, Z 2010, 'Effects of Different Sleep Restriction Protocols on Sleep Architecture and Daytime Vigilance in Healthy Men', *Physiological Research*, vol. 59, no. 5, pp. 821-829.

Yehuda, S, Sredni, B, Carasso, RL and Kenigsbuch-Sredni, D 2009, 'REM Sleep Deprivation in Rats Results in Inflammation and Interleukin-17 Elevation', *Journal of Interferon and Cytokine Research*, vol. 29, no. 7, pp. 393-398.

Appendices

Table of Appendices

Appendix A: Approval to conduct research

A.1 Deakin University Human Ethics Research approval letter

DEAKIN UNIVERSITY

Human Ethics Research

Office of Research Integrity
Research Services Division
70 Elgar Road Burwood Victoria
Postal: 221 Burwood Highway
Burwood Victoria 3125 Australia
Telephone 03 9251 7123 Facsimile 03 9244 6581
research-ethics@deakin.edu.au

Memorandum

To: Dr Brad Aisbett
School of Exercise and Nutrition Sciences
B

cc:

From: Deakin University Human Research Ethics Committee (DUHREC)

Date: 06 October, 2010

Subject: 2010-170
Awake, smoky and hot: Workplace stressors when fighting bushfires

Please quote this project number in all future communications

The application for this project was considered at the DU-HREC meeting held on 02/08/2010.

Approval has been given for Dr Brad Aisbett, School of Exercise and Nutrition Sciences, to undertake this project from 6/10/2010 to 6/10/2014.

The approval given by the Deakin University Human Research Ethics Committee is given only for the project and for the period as stated in the approval. It is your responsibility to contact the Human Research Ethics Unit immediately should any of the following occur:

- Serious or unexpected adverse effects on the participants
- Any proposed changes in the protocol, including extensions of time.
- Any events which might affect the continuing ethical acceptability of the project.
- The project is discontinued before the expected date of completion.
- Modifications are requested by other HRECs.

In addition you will be required to report on the progress of your project at least once every year and at the conclusion of the project. Failure to report as required will result in suspension of your approval to proceed with the project.

DUHREC may need to audit this project as part of the requirements for monitoring set out in the National Statement on Ethical Conduct in Human Research (2007).

Human Research Ethics Unit
research-ethics@deakin.edu.au
Telephone: 03 9251 7123

A.2 Medical questionnaire

Occupational and environmental factors impacting firefighter performance

Name_____

1 What is your age?_____ years

2 Gender: Male ☐ Female ☐

3 Domestic status:

Married / Living with a partner	☐
Separated / Divorced	☐
Widowed	☐
Single	☐

4 Do you have children living at home with you? Yes ☐ No ☐

5 If yes, how many? _____ and how old are they? 1)_____ 2)_____ 3)_____ 4)_____ 5) _____

6 Do you consume caffeinated products (eg coffee, tea, cola, energy drinks, chocolate bars etc)?
Yes ☐ No ☐
If YES, adding all of these together, how many items do you normally consume each day?
_____

7 Do you describe yourself as a:

☐ Regular smoker (I smoke one or more cigarettes per day)

☐ Occasional smoker (I do not smoke every day)

☐ Ex-smoker (I used to smoke but not anymore)

☐ Non-smoker (I have never smoked regularly)

8 How often do you drink alcohol?
☐ Never ☐ Less than once per week ☐ Once or twice per week ☐ Once every two days ☐ Daily

9 On a typical drinking occasion, how many drinks do you have? (One drink equals a glass of beer, a glass of wine or a shot of liquor).
☐ None ☐ Less than 2 drinks ☐ 2-4 drinks ☐ 5-6 drinks

10 Have you travelled overseas in the last four weeks? ☐ No ☐ Yes
If YES, when and where did you travel?
_____

WORK

11 Are you, or have you ever been, involved in shift work? ☐ Yes ☐ No
If YES, when were you involved in shift work, and for how long?
_____
_____

12 Are you currently employed? ☐ Yes ☐ No
If YES, what is your current occupation?
_____

13 How often do you work?
☐ Full-time ☐ Part-time ☐ Casual ☐ Seasonal ☐ Self-employed

FIREFIGHTING HISTORY

14 Years of fire fighting experience (volunteer and/or salaried?
_____

15 How long have you been a member of the CFS?

_____

16 What training have you completed?

_____

17 Approximate number of campaign deployments? _____

18 When were you last called for a job to which you responded/attended?

_____

HEALTH

19 *Do you have a history of:*	Yes	No	Don't know
Serious accident, head injury or concussion?	☐	☐	☐
Epilepsy or other neurological disorders?	☐	☐	☐
Unexplained loss of consciousness?	☐	☐	☐
Migraine headaches?	☐	☐	☐
Respiratory problems?	☐	☐	☐
Chronic depression or another psychiatric problem?	☐	☐	☐
Cardiovascular disease (e.g. heart attack, stroke)?	☐	☐	☐
Substance abuse?	☐	☐	☐
Recreational drug use?	☐	☐	☐

20 *Has anyone ever told you that you?*	Yes	No	Don't Know
Are overweight?	☐	☐	☐
Have high blood pressure?	☐	☐	☐
Have a heart murmur?	☐	☐	☐
Are asthmatic?	☐	☐	☐
Are diabetic?	☐	☐	☐

21 *Have you ever had?*	Yes	No	Don't Know
Chest pain, chest discomfort, chest tightness or chest heaviness?	☐	☐	☐
Shortness of breath out of proportion to exercise undertaken?	☐	☐	☐
Sensations of abnormally fast and/or irregular heart beat?	☐	☐	☐
Episodes of fainting, collapse or loss of consciousness?	☐	☐	☐
Abnormal bleeding (i.e. longer than normal bleeding time) or bruising?	☐	☐	☐

If you answered 'yes' to any parts of Questions 20, 21 and 22 above, please provide details regarding any restrictions or cautions that may need to be taken during the course of the study.

_____
_____
_____

22 Have you ever suffered any musculoskeletal injury or had a disorder that has impaired your movement or functioning?

☐ Yes ☐ No ☐ Don't Know

If YES, please elaborate:

_____
_____
_____

23 Do you have a cardiac pacemaker or other implanted electro-medical device?
☐ Yes ☐ No ☐ Don't Know
If YES, please elaborate:

_____
_____
_____

24 Are you currently taking any medications? ☐ Yes ☐ No
If YES, please list the medications

_____
_____
_____

25 Do you have any allergies (e.g. to any food, Finalgon or Deepheat creams, tapes or bandaids (adhesives), latex etc)? If YES, please list the allergies

_____
_____
_____

26 Will you be having a medical procedure or travelling by aeroplane in the next month?
☐ Yes ☐ No ☐ Don't Know

SLEEP
27 How many hours of sleep do you need to feel rested? _____ hours

28 How satisfied are you with the amount of sleep you get?
Very dissatisfied 1 2 3 4 5 6 7 8 9 10 Very Satisfied

29 Overall, how would you rate the quality of your sleep?
☐ very poor ☐ poor ☐ fair ☐ good ☐ very good ☐ excellent

30 Have you ever been diagnosed with a sleeping problem? ☐ No ☐ Yes
If YES, please describe

_____
_____
_____

31 How often do you take naps? (e.g. never, occasionally, once a day, twice a week)

_____

32 *How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?* (This refers to your usual way of life in recent times. If you have not performed a listed activity, make a guess at what you are likely to do during that activity). PLEASE TICK ONE BOX PER LINE

	Would never doze	Slight chance	Moderate chance	High chance
Sitting and reading	0☐	1☐	2☐	3☐
Watching TV	0☐	1☐	2☐	3☐
Sitting inactive in a public place (eg theatre, meeting)	0☐	1☐	2☐	3☐
As a passenger in a car for an hour without a break	0☐	1☐	2☐	3☐
Lying down in the afternoon when circumstances permit	0☐	1☐	2☐	3☐
Sitting and talking to someone	0☐	1☐	2☐	3☐
Sitting quietly after lunch without alcohol	0☐	1☐	2☐	3☐
In a car, while stopped for a few minutes in traffic	0☐	1☐	2☐	3☐

Please answer the following in relation to sleep timing

- Please read each question very carefully before answering.
- Please answer each question as honestly as possible.
- Answer ALL questions
- Each question should be answered independently of others. Do NOT go back and check your answers.

- What time would you get up if you were entirely free to plan your day?
  5:00 – 6:30 AM ☐
  6:30 – 7:45 AM ☐
  7:45 – 9:45 AM ☐
  9:45 – 11:00 AM ☐
  11:00 – 12 Noon ☐
  12 Noon – 5:00 AM ☐
- What time would you go to bed if you were entirely free to plan your evening?
  8:00 – 9:00 PM ☐
  9:00 – 10:15 PM ☐
  10:15 – 12:30 AM ☐
  12:30 – 1:45 AM ☐
  1:45 – 3:00 AM ☐
  3:00 AM – 8:00 PM ☐
- If there is a specific time at which you have to get up in the morning, to what extent do you depend on being woken up by an alarm clock?
  Not all dependent ☐
  Slightly dependent ☐
  Fairly dependent ☐
  Very dependent ☐
- How easy do you find it to get up in the morning (when you are not woken up unexpectedly)?
  Not all easy ☐
  Not very easy ☐
  Fairly easy ☐
  Very easy ☐
- How alert do you feel during the first half-hour after you wake up in the morning?
  Not all alert ☐
  Slightly alert ☐
  Fairly alert ☐
  Very alert ☐
- How hungry do you feel during the first half hour after you wake up in the morning?
  Not all hungry ☐
  Slightly hungry ☐
  Fairly hungry ☐
  Very hungry ☐
- During the first half-hour after you wake up in the morning, how tired do you feel?
  Very tired ☐
  Fairly tired ☐
  Fairly refreshed ☐
  Very refreshed ☐
- If you have no commitments the next day, what time would you go to bed compared to your usual bedtime?
  Seldom or never later ☐
  Less than one hour later ☐
  1 – 2 hours later ☐
  More than two hours later ☐

- You have decided to engage in some physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 7:00 – 8:00 am. Bearing in mind nothing but your own internal "clock", how do you think you would perform?
  - Would be in good form ☐
  - Would be in reasonable form ☐
  - Would find it difficult ☐
  - Would find it very difficult ☐
- At what time of day do you feel you become tired as a result of need for sleep?
  - 8:00 – 9:00 PM ☐
  - 9:00 – 10:15 PM ☐
  - 10:15 PM – 12:45 AM ☐
  - 12:45 – 2:00 AM ☐
  - 2:00 – 3:00 AM ☐
- You want to be at your peak performance for a test that you know is going to be mentally exhausting and will last for two hours. You are entirely free to plan your day. Considering only your own internal "clock", which ONE of the four testing times would you choose?
  - 8:00 AM – 10:00 AM ☐
  - 11:00 AM – 1:00 PM ☐
  - 3:00 PM – 5:00 PM ☐
  - 7:00 PM – 9:00 PM ☐
- If you got into bed at 11:00 PM, how tired would you be?
  - Not at all tired ☐
  - A little tired ☐
  - Fairly tired ☐
  - Very tired ☐
- For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which ONE of the following are you most likely to do?
  - Will wake up at usual time, but will NOT fall back asleep ☐
  - Will wake up at usual time and will doze thereafter ☐
  - Will wake up at usual time but will fall asleep again ☐
  - Will NOT wake up until later than usual ☐
- One night you have to remain awake between 4:00 – 6:00 AM in order to carry out a night watch. You have no commitments the next day. Which ONE of the alternatives will suite you best?
  - Would NOT go to bed until watch was over ☐
  - Would take a nap before and sleep after ☐
  - Would take a good sleep before and nap after ☐
  - Would sleep only before watch ☐
- You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own internal "clock" which ONE of the following time would you choose?
  - 8:00 AM – 10:00 AM ☐
  - 11:00 AM – 1:00 PM ☐
  - 3:00 PM – 5:00 PM ☐
  - 7:00 PM – 9:00 PM ☐
- You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 10:00 – 11:00 PM. Bearing in mind nothing else but your own internal "clock" how well do you think you would perform?
  - Would be in good form ☐
  - Would be in reasonable form ☐
  - Would find it difficult ☐
  - Would find it very difficult ☐

- Suppose that you can choose your own work hours. Assume that you worked a FIVE hour day (including breaks) and that your job was interesting and paid by results). Which FIVE CONSECUTIVE HOURS would you select?
  5 hours starting between 4:00 AM and 8:00 AM ☐
  5 hours starting between 8:00 AM and 9:00 AM ☐
  5 hours starting between 9:00 AM and 2:00 PM ☐
  5 hours starting between 2:00 PM and 5:00 PM ☐
  5 hours starting between 5:00 PM and 4:00 AM ☐
- At what time of the day do you think that you reach your "feeling best" peak?
  5:00 – 8:00 AM ☐
  8:00 – 10:00 AM ☐
  10:00 AM – 5:00 PM ☐
  5:00 – 10:00 PM ☐
  10:00 PM – 5:00 AM ☐
- One hears about "morning" and "evening" types of people. Which ONE of these types do you consider yourself to be?
  Definitely a "morning" type ☐
  Rather more a "morning" than an "evening" type ☐
  Rather more an "evening" than a "morning" type ☐
  Definitely an "evening" type ☐

33 Do you have any other condition or injury not previously mentioned that the researchers should be aware of (i.e. that would prevent you from undertaking your normal duties)?
☐ No ☐ Yes

If YES, please elaborate

_____

_____

Thank you for taking the time to fill in this questionnaire

I believe the information I have provided to be true and correct.
SIGNED:_____ DATE:_____

bushfire CRC **This project is funded by the Bushfire CRC**

A.3 Consent form

TO: ***Participants***

Consent Form

Date:

Full Project Title: Awake, smoky and hot: Workplace stressors when fighting bushfires

Reference Number: [2010-***170]*.**

I have read and I understand the attached Plain Language Statement.

I freely agree to participate in this project according to the conditions in the Plain Language Statement.

I have been given a copy of the Plain Language Statement and Consent Form to keep.

The researcher has agreed not to reveal my identity and personal details, including where information about this project is published, or presented in any public form.

I give my specific consent to be filmed and photographed throughout testing and understand that the researchers may contact me again for my data to be used for other research purposes.

Participant's Name (printed) ……

Signature …… Date ……

Should you wish to return your consent form (and have lost your reply-paid envelope), please send the forms to:

Dr Brad Aisbett
School of Exercise and Nutrition Sciences
Deakin University
Burwood VIC 3125
Phone: 03 9244 6474 Fax: 03 9244 6017
Email: brad.aisbett@deakin.edu.au

TO: Participants

Revocation of Consent Form

(To be used for participants who wish to withdraw from the project)

Date:

Full Project Title: Awake, smoky and hot: Workplace stressors when fighting bushfires

Reference Number: [2010-***170]*.**

I hereby wish to WITHDRAW my consent to participate in the above research project and understand that such withdrawal WILL NOT jeopardise my relationship with Deakin University or Country Fire Authority.

Participant's Name (printed) …….

Signature …….Date ……

Please mail or fax this form to:
Dr Brad Aisbett
School of Exercise and Nutrition Sciences
Deakin University
Burwood VIC 3125
Phone: 03 9244 6474 Fax: 03 9244 6017
Email: brad.aisbett@deakin.edu.au

Appendix B: Questionnaires

B.1 Pre- and post-testing injury and illness questionnaire

Pre-testing Questionnaire:

All participants:

Since you submitted your pre-participation medical questionnaire have you:

- Suffered an injury or illness? yes/no (please circle)
- Taken any form of medication in the last week? yes/no (please circle)

  If yes, what type of medication?_____

  What was the dosage you took?_____
- Experienced any recent muscle or joint pain (e.g. back pain, muscle cramps or stiffness etc)? yes/no (please circle)

  Remember: Please tell a researcher if you experience any twinges, pain or other discomfort whilst you're here.

Additional medical questions:

- Are you a smoker? yes/no (please circle)

If yes, approximately how many cigarettes do you smoke per day?_____

What strength of cigarette do you smoke?_____

Debrief or post-testing questionnaire:

Did you experience any twinges, pain of discomfort while you were here? yes/no (please circle)

If yes, on a scale of 1 to 10, how would you rate this

pain?_____

Do you think it affected your physical work during the testing period in

anyway?_____

Female participants only:

Since you submitted your pre-participation medical questionnaire have you:

- Taken any form of medication (including oral contraceptives)? yes/no (please circle)

If yes, what type of medication?_____

Do you know the name of your medication?_____

What dosage did you take?

take?_____

- When was your last

  period?_____
- Do you typically experience any of the following symptoms associated with menstruation:

  cramps

  bloating

  tenderness

  other (please explain)

B.2 Mood Scale II

Please indicate to what extent (Not At All, Somewhat/Sometimes, Mostly/Generally) you experienced each emotion by placing a circle around the appropriate response.

Energetic	Not At All	Somewhat/Sometimes Mostly/Generally
Good	Not At All	Somewhat/Sometimes Mostly/Generally
Miserable	Not At All	Somewhat/Sometimes Mostly/Generally
Grouchy	Not At All	Somewhat/Sometimes Mostly/Generally
Inactive	Not At All	Somewhat/Sometimes Mostly/Generally
Uneasy	Not At All	Somewhat/Sometimes Mostly/Generally
Lively	Not At All	Somewhat/Sometimes Mostly/Generally
Contented	Not At All	Somewhat/Sometimes Mostly/Generally
Blue	Not At All	Somewhat/Sometimes Mostly/Generally
Mean	Not At All	Somewhat/Sometimes Mostly/Generally
Weary	Not At All	Somewhat/Sometimes Mostly/Generally
Alarmed	Not At All	Somewhat/Sometimes Mostly/Generally
Alert	Not At All	Somewhat/Sometimes Mostly/Generally
Satisfied	Not At All	Somewhat/Sometimes Mostly/Generally
Depressed	Not At All	Somewhat/Sometimes Mostly/Generally
Annoyed	Not At All	Somewhat/Sometimes Mostly/Generally
Lazy	Not At All	Somewhat/Sometimes Mostly/Generally
Insecure	Not At All	Somewhat/Sometimes Mostly/Generally
Cheerful	Not At All	Somewhat/Sometimes Mostly/Generally
Calm	Not At All	Somewhat/Sometimes Mostly/Generally
Sad	Not At All	Somewhat/Sometimes Mostly/Generally
Angry	Not At All	Somewhat/Sometimes Mostly/Generally
Drowsy	Not At All	Somewhat/Sometimes Mostly/Generally
Afraid	Not At All	Somewhat/Sometimes Mostly/Generally
Vigorous	Not At All	Somewhat/Sometimes Mostly/Generally
Pleased	Not At All	Somewhat/Sometimes Mostly/Generally
Downcast	Not At All	Somewhat/Sometimes Mostly/Generally
Burned up	Not At All	Somewhat/Sometimes Mostly/Generally

Sluggish Mostly/Generally	Not At All	Somewhat/Sometimes
Jittery Mostly/Generally	Not At All	Somewhat/Sometimes
Active Mostly/Generally	Not At All	Somewhat/Sometimes
Happy Mostly/Generally	Not At All	Somewhat/Sometimes
Low Mostly/Generally	Not At All	Somewhat/Sometimes
Irritated Mostly/Generally	Not At All	Somewhat/Sometimes
Hopeless Mostly/Generally	Not At All	Somewhat/Sometimes
Steady Mostly/Generally	Not At All	Somewhat/Sometimes

B.3 Samn-Perelli Fatigue Scale

When you are asked to "Rate your fatigue (1-7)" fill in the number that is your best estimate of your average fatigue level at that moment in time. Use this scale:

1 = fully alert, wide awake

2 = very lively, responsive, but not at peak

3 = okay, somewhat fresh

4 = a little tired, less than fresh

5 = moderately tired, let down

6 = extremely tired, very difficult to concentrate

7 = completely exhausted, unable to function effectively

Appendix C: Copy of published paper

REVIEW PAPER

International Journal of Occupational Medicine and Environmental Health 2015;28(2):183–208
http://dx.doi.org/10.13075/ijomeh.1896.00227

EFFECTS OF WORK-RELATED SLEEP RESTRICTION ON ACUTE PHYSIOLOGICAL AND PSYCHOLOGICAL STRESS RESPONSES AND THEIR INTERACTIONS: A REVIEW AMONG EMERGENCY SERVICE PERSONNEL

ALEXANDER WOLKOW[1,2], SALLY FERGUSON[2,3], BRAD AISBETT[1,2], and LUANA MAIN[1]

[1] Deakin University, Burwood, Australia
Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences
[2] Bushfire Co-Operative Research Centre, East Melbourne, Australia
[3] Central Queensland University, Wayville, Australia
Appleton Institute

Abstract
Emergency work can expose personnel to sleep restriction. Inadequate amounts of sleep can negatively affect physiological and psychological stress responses. This review critiqued the emergency service literature (e.g., firefighting, police/law enforcement, defense forces, ambulance/paramedic personnel) that has investigated the effect of sleep restriction on hormonal, inflammatory and psychological responses. Furthermore, it investigated if a psycho-physiological approach can help contextualize the significance of such responses to assist emergency service agencies monitor the health of their personnel. The available literature suggests that sleep restriction across multiple work days can disrupt cytokine and cortisol levels, deteriorate mood and elicit simultaneous physiological and psychological responses. However, research concerning the interaction between such responses is limited and inconclusive. Therefore, it is unknown if a psycho-physiological relationship exists and as a result, it is currently not feasible for agencies to monitor sleep restriction related stress based on psycho-physiological interactions. Sleep restriction does however, appear to be a major stressor contributing to physiological and psychological responses and thus, warrants further investigation.

Key words:
Sleep, Cytokines, Stress, Cortisol, Mood, Psycho-physiological

INTRODUCTION AND REVIEW OBJECTIVES

Inadequate sleep quality and quantity is a common problem in modern society, which, in turn, can negatively affect psychological and physiological functioning [1]. Evidence suggests that periods of partial and total sleep deprivation/restriction can impair immune function (e.g., above and below normal pro- and anti-inflammatory cytokine levels) [2,3], hormone secretion (e.g., higher and flatter diurnal cortisol levels) [4,5] and instigate adverse psychological changes (e.g., symptoms of anxiety and

This review was completed as part of a lead author's (Alexander Wolkow) PhD.
Received: February 18, 2014. Accepted: August 13, 2014.
Corresponding author: A. Wolkow, Centre for Physical Activity and Nutrition Research School of Exercise and Nutrition Sciences, Deakin University, 221 Burwood Hwy, Burwood, VIC 3125, Australia (e-mail: awolkow@deakin.edu.au).

depression) [1,6–9]. Furthermore, an increasing body of evidence has demonstrated a link between sleep restriction and negative long-term physical and mental health outcomes [2,10–16]. For instance, inadequate or disrupted sleep has been associated with cardiovascular and metabolic diseases [10,12,14–16], and depression [6–8]. Worldwide, cardiovascular diseases (CVDs) are the leading cause of death [17], while depression remains the leading cause of disability [18]. The links that exist between widespread chronic, long-term negative health outcomes and sleep, underscore the need to examine and characterize acute psychological and physiological stress responses to sleep restriction and deprivation.

To fully evaluate and understand the relationship between acute sleep restriction and physical and mental health, an integrated approach that takes into account both acute psychological and physiological responses to this stressor should be considered. While the understanding of how stress affects psycho-physiological responses and their interaction is still limited, specific findings suggest that psychological health and well-being may influence physiological processes and vice versa [19–21]. Indeed, evidence suggests that an increase in stress exposure simultaneously induces both physiological (i.e., higher and flatter diurnal cortisol levels and/or abnormally high or low cytokine levels) and psychological changes (i.e., mood and behavioral disturbances), and that these responses can be positively or negatively correlated with one another [22–25].

Under normal circumstances, cytokines and glucocorticoids (e.g., cortisol) form a feedback loop, whereby stress elicits the release of pro-inflammatory cytokines which activate the hypothalamic pituitary adrenal (HPA)-axis and results in the release of cortisol and anti-inflammatory cytokines [26–29]. In turn, cortisol and anti-inflammatory cytokines negatively feedback to suppress and regulate the inflammatory response [26–29]. However, exposure to intense or prolonged stress can disrupt this feedback loop causing an enhanced/up-regulated inflammatory state and HPA-axis disturbances [28,30,31].

These maladaptations are typically associated with negative physiological (e.g., CVD and metabolic syndrome) and psychological health outcomes (e.g., depression) [28,32,33], and in chronic situations, may be underlying these stress-related diseases [30,31,34,35]. For instance, elevated levels of sleep regulating cytokines interleukin (IL)-6, IL-1β and TNF-α have been positively associated with CVD [36–38], metabolic syndrome [39] and depression [40,41]. Furthermore, higher, flatter diurnal cortisol patterns have been related to depression [42,43]. Other distinctive parts of the cortisol stress response such as elevated morning cortisol levels measured in plasma have also been positively associated with CVD and metabolic syndrome related features (e.g., glucose intolerance, insulin sensitivity, hypertension, atherosclerosis) [44–46]. Despite these associations, the exact direction and magnitude of a 'normal' acute change in cytokine, cortisol and mood responses to different periods of sleep restriction is largely unknown, as is the degree to which these acute stress responses are quantifiable risk factors to health [47]. Accordingly, an integrated approach that combines assessment of multiple responses and their interactions may help evaluate and assist in contextualizing the significance of these stress responses [24,25,28,48,49] to sleep restriction.

One of the most common causes of inadequate sleep are work-related factors [50]. For instance, early start times and shift work can cause a misalignment to the circadian rhythm of physiological functions [50]. Reduced sleep opportunities, as a result of extended work hours, long commutes, overtime and being on-call can also disrupt sleep [50]. In addition, exposure to environmental (e.g., light and noise), physical (e.g., intense physical work) and/or psychological (e.g., critical decisions, life threatening situations) work-related stressors can disrupt the circadian rhythm and prevent adequate

sleep [14,50,51]. Emergency (e.g., firefighting, law enforcement/police, some emergency medical services) and defense force services (e.g., military, navy, army) are a unique group of occupations in which personnel is exposed to non-standard scheduling of work hours whilst completing physical work demands on a daily basis [52–58]. For instance, emergency personnel can perform long hours of intermittent physically intense work (up to 15-h) with little rest between consecutive shifts, which can last up to a week [55,56,59–61].

Furthermore, some personnel have reported that the constant readiness (i.e., hyper-vigilance) to respond to an emergency alarm felt while on-duty can transfer to the off-duty environment [62]. This state of hyper-vigilance in combination with excessive work hours and exposure to other occupational stressors, could place personnel of these physically demanding occupations at an increased risk of suffering from inadequate sleep. Indeed, a higher prevalence of sleep disturbances has been reported among firefighting, police, paramedic and military personnel when compared to other occupations [60,61,63–66]. For the purpose of this review, further mention of 'emergency services' or 'emergency personnel' will refer to firefighting, police, paramedic/ambulance and defense force personnel, unless stated otherwise.

The high prevalence of adverse long-term health outcomes (e.g., CVD, metabolic syndrome and depression) associated with sleep restriction reported among these occupations [67–70] is of further concern for emergency services. For instance, Courtney et al. [68] have found paramedic personnel in Australia to have a higher prevalence of sleep-related mental health outcomes (i.e., depression and anxiety) when compared to community samples. Furthermore, the findings among police have revealed that officers reporting shorter sleep durations had a significantly greater number of metabolic syndrome related factors when compared to non-police workers [71] or officers who received more sleep [72].

Given the high prevalence of sleep restriction [61,63,64] and negative sleep-related health outcomes reported among emergency personnel [67–70], it is important to understand how work-related sleep restriction affects personnel's acute psychological and physiological stress responses. Therefore, the 1st part of this review will identify and critique any gaps in the available occupational-based literature that has investigated the effects of sleep restriction on acute hormonal, inflammatory and psychological (i.e., mood, anxiety levels, perceived stress) responses among personnel of emergency services. This review is focused on understanding how personnel respond to modest periods of chronic sleep restriction (i.e., 1–7 nights) that could reflect a single shift, a working week or deployment to a large emergency event (e.g., large bushfires/wildfires). Therefore, research investigating extended periods of reduced sleep (e.g., 8-week military training) will not be examined in this review. Furthermore, the independent influence that night-shifts in emergency work have on stress responses is beyond the scope of this review and therefore, will not be evaluated.

There is growing support in the literature to simultaneously measure multiple responses and their interactions to assess the relevance/importance of stress responses [28,73]. Accordingly, it would be valuable for services to know whether a psycho-physiological approach can help contextualize the significance of acute stress responses to sleep restriction and therefore, assist services to efficiently monitor the acute health of their personnel in the field. For example, if exposure to work-related stressors such as sleep restriction elicits similar or related psychological and physiological responses, then monitoring the health of personnel could be achieved by using self-report measures (e.g., psychological questionnaires). Physiological assessments (e.g., blood samples) could then compliment these measures to provide a more complete picture of the personnel's stress related health.

Therefore, the 2nd part of this review will interrogate the pertinent emergency service-based literature to determine if a psycho-physiological approach can help contextualize the significance of any acute stress responses to assist emergency services monitor the health of their personnel.

While this review intends to provide a comprehensive evidence-based inclusive of various emergency-based occupations, the sleep and stress response research to date has focused mainly on soldiers. Consequently, the balance of literature in this review from each uniform service reflects what is currently available. Furthermore, where emergency-specific research is not available, findings from the wider stress response literature that has investigated periods of sleep restriction similar in length to that demonstrated during emergency work (i.e., 1–7 nights) will be reviewed, and where possible, their transferability to personnel in emergency occupations will be examined.

MATERIAL AND METHODS

Study selection and literature search strategy

This narrative review searched for sleep and stress-related research conducted in emergency-based occupations, the duties of which could be described as physically demanding. Although narrative, the source articles were identified using a systematic search strategy of the global database Ebsco Host to search health-related databases (Academic Search Complete, The Allied and Complementary Medicine Database, CINAHL, Global Health, Health Source (Consumer and Nursing/Academic Editions), MasterFILE, MEDLINE/PubMed, PsycARTICLES, PsycBOOKS, PsycEXTRA, Psychology and Behavioral Sciences, PsycINFO, PsychTESTS and SPORTDiscus) to identify relevant English-language studies published between January 1985 and September 2013.

The occupation-based key words used for the search included: 'firefighters', 'fire fighters', 'fire-fighters', 'police', 'law enforcement', 'paramedics', 'ambulance personnel', 'soldiers', 'navy', 'military' and 'defense force' searched together with sleep and stress response related words of key interest that included: 'sleep deprivation', 'sleep restriction', 'cortisol', 'cytokines', 'mood' and 'psycho-physiological'. Each of the key words mentioned was searched for individually and in conjunction with each other. In addition, relevant articles were identified from the references provided in the original articles retrieved.

The search results were screened and obviously irrelevant or duplicate articles were omitted. Further articles from non-peer-reviewed sources were excluded from the search results. Abstracts and full-texts of the remaining results were then scanned and included in the final review if they met the inclusion criteria outlined in Table 1.

Table 1. Inclusion criteria for the literature review

Inclusion criteria	Explanation
Participants	active duty emergency (e.g., firefighting, police/law enforcement, paramedic/ambulance personnel, rescue workers) or defense force (e.g., army, navy) personnel in physically demanding occupations or healthy adults exposed to periods of sleep restriction similar to emergency personnel (see below)
Period of sleep restriction/deprivation	complete or partial sleep restriction (i.e., < 7 h sleep) from 1–8 consecutive nights
Shift type	single day or consecutive shifts with periods of restricted sleep no specific night shifts
Physiological stress responses	pro- and/or anti-inflammatory cytokines and/or cortisol
Psychological stress responses	a valid and reliable subjective mood, behavior and/or anxiety questionnaire (e.g., Profile of Mood States, Brunel Mood Scale, State-Trait Anxiety Inventory)

RESULTS AND DISCUSSION

The impact of work-related sleep restriction on physiological stress responses among emergency service personnel

Sleep restriction and changes to cortisol

Despite the high prevalence of sleep disturbances reported among police [61,64,65], firefighting [66], paramedic [60] and military personnel [74,75], only one emergency service-based study has investigated the specific effect of restricted sleep on physiological stress responses [76]. Goh et al. [76] have found no significant difference in the overall daily cortisol levels between control (i.e., 8 h sleep) and sleep deprived military personnel (i.e., 1 night of total sleep deprivation). Though there was a significant increase in cortisol levels at 1:30 p.m. on the day after sleep deprivation [76].

Similar results for daily cortisol levels have been found in non-emergency service-based investigations [77–79], indicating that in isolation, a single night of complete sleep restriction may not be a sufficient stressor to significantly affect the overall diurnal release of cortisol among emergency personnel. Determining the isolated (i.e., with no other significant external physical or psychological stressors present) effect that controlled periods of shortened sleep may have on emergency responders' physiological responses is difficult due to their multi-stressor environments (e.g., emergency incidents that can last hours or days and expose personnel to sleep deprivation and physical work) [52,60,61] and consequently, it has not been investigated to a great extent. However, the findings from multi-day military studies may provide some insight into the possible effect restricted sleep opportunities between periods of physical work and military-related demands (e.g., food and water restriction) have on stress responses.

To date, several studies have investigated soldiers' hormonal changes in response to receiving as little as 1–2-h of total sleep across the course of near continuous physical training spanning 3–7 days [80,81]. For instance, Opstad and Aakvaag [81] have reported that the normal circadian variation in the morning and evening cortisol levels on day 1 and 4 of the military training disappeared, indicating an abnormal circadian cortisol release (Table 2). In a more recent study, Opstad [80] employed a high frequency cortisol sampling method to further investigate the effect of a similar 5-day physical training course with minimal sleep (i.e., 1–3-h of total sleep over the course) on military cadets' circadian cortisol release (Table 2). Similar to control conditions (i.e., no physical training and an 8-h sleep opportunity), cortisol levels followed a normal diurnal rhythm on day 1 of the course. However, throughout the rest of the course, mean cortisol levels remained consistently elevated (+130–140%) and over the final 24-h period, the circadian rhythm had almost disappeared [80]. Moreover, 4–5 days after completing the course the circadian cortisol rhythm remained significantly different from the control period [80], adding further support to the possibility of a disrupted circadian cortisol rhythm following consecutive days of sleep restriction during military training. Neither study [80,81], however, controlled for sleep duration or frequency. Instead, the participants slept when possible between training activities and it was estimated by the authors that the participants obtained 1–3-h of total sleep over the course [80,81]. The lack of control over sleep variables (i.e., timing, duration and frequency) limits the ability to make definite conclusions regarding whether sleep duration, frequency and/or rhythm disruption influenced the diurnal dysregulation of cortisol or not.

Furthermore, the participants were also performing physical work and had a substantially restricted daily energy intake during the training course [80,81]. These factors potentially confound the interpretation of these findings, as exposure to physical work and energy restriction has also been found to disrupt normal diurnal cortisol

Table 2. Emergency service based sleep restriction and stress response studies

Reference	Sample	Intervention/ Design	Sleep restriction (SR)	Other stressors	Stress responses	Timing of samples	Main findings
Lieberman et al. [82]	male soldiers (N = 13)	baseline followed by an 84-h lab-based military training	baseline: 4× nights of *ab libitum* sleep training: 7×1 h sleep opportunities (6.2±0.4 h total sleep)	physical work, restricted energy intake, hot environment	POMS	POMS: ~ 8:00 a.m. day 1, 3 and 4 during baseline week and on day 1, 3 and 4 during training	POMS: increase from baseline to training in tension (day 3: 3.4, +74%; day 4: 2.7, +52%; p = 0.01), depression (day 3: 5.4, +216%; day 4: 5.6, +329%; p = 0.02), confusion (day 3: 5.3, +120%; day 4: 7.4, +176%; p < 0.001), fatigue (day 3: 9.4, +145%; day 4: 12.0, +194%; p < 0.001) & anger (day 3: 8.4, +420%; day 4: 9.4, +324%; p = 0.002), vigor (day 3: –3.5, –65%; day 4: –3.4, –57%; p < 0.001)
Lieberman et al. [83]	male army officers (N = 31)	pre-training assessment followed by 53-h training exercise and post-training assessment	pre-training: 1× *ab libitum* sleep (5.3±0.2 h) training: intermittent sleep (3±0.3 h total sleep)	physical work, hot environment and restricted energy intake	Salivary cortisol; POMS	cortisol: pre-training (day 1: 6:00 p.m., day 2: 6:00 a.m., 12:00 p.m., 6:00 p.m.), during training (day 3: 6:00 a.m., 12:00 p.m., 6:00 p.m.) post-training (day 4: 12:00 p.m., 6:00 p.m., day 5: 6:00 a.m.) POMS: pre-training (day 1: 6:00 p.m.), during training (day 4: 12:00 p.m.) post-training (day 5: 5:00 a.m.)	cortisol: decreased from pre-training at 6:00 a.m. (day 2) to day 3 at 6:00 a.m. (–7.7 nmol×l^{-1}, -39%; p < 0.001), day 5 at 6:00 a.m. (–3.6 nmol×l^{-1}, –18%; p < 0.05) increased from pre-training at 6:00 p.m. to post-training at 6:00 p.m. (day 1 vs. 3: 6.1 nmol×l^{-1}, +105%; day 1 vs. 4: 4.4 nmol×l^{-1}, +76%; day 2 vs. 3: 8 nmol×l^{-1}, +207%; day 2 vs. 4: 6.3 nmol×l^{-1}, +164%, p < 0.001) POMS: change from pre- to post-training in tension (2.3, +53%; p < 0.002), depression (3.2, +168%; p < 0.001), confusion (7.8, +252%; p < 0.001), fatigue (17.7, +466%; p < 0.001), anger (2.5, +86%; p ≤ 0.01), vigor (–15.2, –75%; p < 0.001)

Opstad [80]	male military cadets (N = 10)	baseline (1 week prior) followed by 5-day training course and recovery (4–5 days post-training)	training: intermittent sleep totalling 1–3 h baseline and recovery: 9–10 h sleep opportunities	physical work and restricted energy intake	blood serum cortisol	~ 4 h intervals for 24 h a week prior to the course (baseline), on days 1–2, days 4–5 of training and 4–5 days post-training (recovery)	24-h mean cortisol increased from baseline (283±24 nmol×l^{-1}) to days 1–2 (519±30 nmol×l^{-1}; +83%), days 4–5 (556±30 nmol×l^{-1}; 96%), but normal after recovery (287±25 nmol×l^{-1}; significance not reported) circadian cortisol rhythm different from baseline ($p = 0.0016$) and disappeared days 4–5 morning cortisol on days 1–2 and 4–5 increased above baseline (+130–140%; $p < 0.00005$)
Lundeland et al. [97]	male military cadets (N = 8)	7-day ranger-training course	intermittent sleep totalling ~ 7 h (1 h/24 h)	physical work and restricted energy intake	blood plasma cortisol and cytokines (LPS-simulated TNF-α, IL-1β, IL-6)	7:00 a.m. baseline and during training on day 3, 5, 7	cortisol: increased from baseline (413 nmol×l^{-1}) to day 3 (505 nmol×l^{-1}) and day 5 (874 nmol×l^{-1}) then decreased on day 7 (631 nmol×l^{-1}; $p < 0.05$) cytokines: LPS whole-blood: TNF-α increased from baseline (40.2 ng×10^6) to day 3 (125.5 ng×10^6; $p < 0.05$) then decreased to day 5 (56.1 ng×10^6; $p < 0.05$) IL-1β increased from baseline (68.8 ng×10^6) to day 3 (192.8 ng×10^6) then decreased to day 5 (62.6 ng×10^6; $p < 0.05$) IL-6 increased from baseline (240.9 ng×10^6) to day 3 (419.8 ng×10^6) then decreased to day 5 (212.1 ng×10^6; $p < 0.05$)

Table 2. Emergency service based sleep restriction and stress response studies – cont.

Reference	Sample	Intervention/ Design	Sleep restriction (SR)	Other stressors	Stress responses	Timing of samples	Main findings
Gundersen et al. [94]	male military cadets (N = 8)	7-day ranger-training course	intermittent sleep totalling ~ 7 h (1 h/24 h)	physical work and restricted energy intake	blood plasma cortisol and cytokines (TNF-α, IL-1β, IL-10, IL-1ra, IL-6); LPS-simulated (TNF-α, IL-1β)	7:00 a.m. baseline and during training on day 2, 4, 7	cortisol: increased from baseline (599±34 nmol×l^{-1}) to day 2 (1 072±82 nmol×l^{-1}, +79%) cytokines: IL-1ra increased from baseline (207±15 pg×ml^{-1}, +306%; $p < 0.05$) (841±437 pg×ml^{-1}, +306%; $p < 0.05$) IL-6 increased from below detection at baseline to day 2 (10.6±1.3 pg×ml^{-1}) and 4 (6.8±1.6 pg×ml^{-1}; $p < 0.05$) LPS whole-blood: TNF-α increased from below detection at baseline to day 7 (150.1±25.3 pg×ml^{-1}; $p < 0.05$) IL-1β increased from baseline (8.9±2.8 pg×ml^{-1}) to day 7 (45.2±6.3 pg×ml^{-1}, +408%; $p < 0.05$)
Slaven et al. [106]	male and female police officers (N = 391)	reported on sleep quality and depression symptoms	n.a.	n.a.	CES-D; PSQI	CES-D: once to assess depressive symptoms in 1 week PSQI: once to assess sleep quality and behavior in 1 month	correlation between CES-D and PSQI global score, and PSQI components (data not reported; $p < 0.001$) CES-D increased across increasing quintiles of PSQI for males (4.72, 5.60, 7.88, 12.67, 12.65; $p < 0.001$) and females (5.53, 6.21, 13.08, 10.88, 12.63, $p = 0.001$)
Bøyum al. [93]	male military cadets (N = 87)	5–7-day training course	intermittent sleep totalling 2–3 h	physical work and restricted energy intake	blood plasma cytokines (IL-1α, IL-6, IL-1β, IL-2, IL-3, IL-4)	between 6:00–7:00 a.m. each day	12–20% decrease in IL-6 on days 4–7 of training ($p < 0.05$) no significant change in other cytokines

Goh et al. [76]	male military personnel (N = 14)	control night followed by single night of SR	control: 1×8 h sleep SR: 1×40 h	no	saliva cortisol	control and SR day 1: 8:00 a.m., 1:30 p.m., 6:00 p.m., 9:00 p.m., 12:00 a.m. control day 2: 8:00 a.m., 1:30 p.m., 6:00 p.m. SR day 2: 3:00 a.m., 6:00 a.m., 8:00 a.m., 1:30 p.m., 6:00 p.m.	cortisol increased at 13:30 h after SR (16.56 nmol×l^{-1}; +200%; p < 0.01) normal circadian cortisol rhythm
Opstad and Aakvagg [81]	male military cadets (N = 11)	5-day training course while receiving high or low calorie intake; follow-up 11 days post-training	training: intermittent sleep totalling 1–2 h follow-up: not reported	physical work and restricted energy intake	blood serum cortisol	Training: 6:30–7:30 a.m. (except day 4: 9:00–10:00 a.m.) and 6:00–7:00 p.m. follow-up: 6:30–7:30 a.m. and 6:00–7:00 p.m.	cortisol decreased from day 2 to 4 in both groups (low calorie: –220 µmol×l^{-1}, –31%; high calorie: –200 µmol×l^{-1}, –33%; sig and change not reported, estimated from graphs provided), and normal diurnal pattern had disappeared on days 1 and 4

n.a. – non-applicable; CES-D – Center for Epidemiologic Studies Depression Scale; IL – interleukin; OR – odds ratio; PSQI – Pittsburgh Sleep Quality Index; POMS – Profile of Mood States; LPS – lipopolysaccharide; SR – sleep restriction.

levels [84,85]. Therefore, which stressor or combination of stressors has the greatest effect on the participants' cortisol response remains to be determined.

Although both Opstad [80] and Opstad and Aakvaag [81] have observed a dysregulated cortisol response, different discrete parts of the cortisol circadian cycle were investigated in each study. For instance, in the more recent study, Opstad [80] has reported that daily cortisol secretion increased significantly over the 5-day training course, while the earlier study [81] reported a decline in the morning (i.e., 8:00 a.m.) cortisol production. Both increases and decreases in cortisol level have been demonstrated following stress exposure and could indicate allostatic load (i.e., wear and tear) on the endocrine system expressed as either an intensified or suppressed cortisol production [86,87]. The increased acute daily levels of cortisol have been associated with insulin resistance, which could accelerate the progression of type II diabetes, atherosclerosis and hypertension [44,45,88].

Conversely, persons exposed to chronic stress have demonstrated inadequate morning (salivary) cortisol levels one hour after awakening [89]. McEwen and Seeman [90] define an inadequate cortisol response as a form of allostatic load that occurs when the HPA-axis produces too little cortisol in response to a stressor, which as a result, causes immune mediators (e.g., inflammatory cytokines) and other systems that are normally contained by cortisol, to become overactive. Consequently, hyperactivity of these systems can increase the risk of auto-immune and inflammatory disorders (e.g., rheumatoid arthritis and multiple sclerosis) [27,90].

Methodological differences between the studies cited above could have contributed to the conflicting results for cortisol. For instance, single day cortisol sampling implemented by Opstad and Aakvaag [81] provides less stable measures of cortisol when compared to a multi-day sampling assessment [91], such as that adopted by Opstad [80]. Furthermore, the morning rise in cortisol known as the cortisol awakening response depends closely on awakening time [92]. Therefore, variation in diurnal cortisol demonstrated between these studies could be also due to the differences in the time of cortisol sample collection after awakening. For instance, Opstad [80] and Opstad and Aakvaag [81] collected morning cortisol at 8:00 a.m. and between 6:30 and 7:30 a.m. respectively, yet neither study reported when the participants slept, limiting the ability to take into account what effect awakening time had on the morning cortisol levels in these studies. In addition, cortisol was examined by both Opstad [80] and Opstad and Aakvaag [81] using blood, while Goh et al. [76] used saliva samples. Evidence suggests that a high cortisol response can occur among individuals as a result of drawing blood (i.e., venepuncture) [79]. Therefore, the sampling methods could further explain different findings for cortisol between these studies [76,80,81].

The mixed findings for diurnal cortisol could also be due to differences in the duration and frequency of sleep deprivation and restriction examined. For instance, Goh et al. [76] have reported that a single night of total sleep deprivation had no effect on overall diurnal cortisol levels. Meanwhile, significant changes were reported following extreme periods of sleep restriction endured over consecutive days examined in the military training studies [80,81].

Sleep restriction and changes to cytokines

To date, research has investigated what impact sleep restriction during military operations has on pro- and anti-inflammatory cytokines [93,94]. For instance, Bøyum et al. [93] have investigated IL-6, IL-1α, IL-1β, IL-2 and IL-4 levels among military cadets before and during 5–7 days of a continuous military training combined with sleep (i.e., 2–3-h of total sleep) and calories restriction. Bøyum et al. [93] have found a ~12–20% reduction in IL-6 on days 4–7 ($p < 0.05$) (Table 2) [93], but no

change in any of the other investigated markers. The decline in IL-6 is in contrast to the findings from the modest (3–6-h sleep per night) sleep restriction studies of similar duration, which have demonstrated an increase in daily cytokine levels among healthy adults following sleep deprivation [3,95,96].

Furthermore, Gundersen et al. [94] have found IL-6 levels to significantly increase from baseline to days 2 (10.6±1.3 pg×ml^{-1}) and 4 (+6.8±1.6 pg×ml^{-1}) (Table 2) during a 7-day training course that comprised almost identical sleep restriction periods and physical work intensities to those investigated by Opstad et al. [80,81]. However, by completion of the course, IL-6 had returned to baseline levels [94]. In contrast, IL-1ra levels have been reported by Gundersen et al. [94] to increase throughout the training course (+306%; $p < 0.05$) (Table 2). Furthermore, the pro-inflammatory cytokines TNF-α (from below detection to 150.1±25.3 pg×ml^{-1}; $p < 0.05$) and IL-1β (+408%; $p < 0.05$) were found to increase from baseline to completion of the training course (Table 2) [94].

Using an almost identical training protocol, Lundeland et al. [97] have investigated cadets' IL-6, TNF-α and IL-1β levels in LPS-simulated whole blood (Table 2). Findings for IL-6 were similar to those of Gundersen et al. [94], whereas TNF-α and IL-1β levels were also found to increase from baseline to day 3 (+212% and +180%, respectively), then decreased to day 5 (–55% and –68%, respectively; $p < 0.05$) [97]. The increase and then the decrease in cytokine levels could indicate either an adaptation to the training course or possibly, failure to continue the same workload, however, this is difficult to determine with no performance data presented [97].

Interestingly, Bøyum et al. [93] have observed contrasting findings for IL-6 when compared to the studies by Gundersen et al. [94] and Lundeland et al. [97]. The decline in IL-6 observed by Bøyum et al. [93] over a short duration (i.e., 4–7 days) of activity was unexpected and suggested by the authors [93] to be a result of plasma expansion due to excessive water intake. This particular cytokine does however, have both anti- and pro-inflammatory actions in the immune response [98]. Therefore, the significant reduction in IL-6 could indicate possible dysregulation of the immune system.

While no emergency service research has examined the effect of sleep restriction on inflammatory cytokines in a controlled setting, the findings from the wider stress response literature indicate that pro-inflammatory cytokines IL-6, IL-1β, IL-1ra and TNF-α significantly increase or decrease from baseline following single [77,99] as well as multiple nights [100] of complete and partial sleep restriction among healthy subjects. For instance, a single 40-h period of total sleep deprivation caused an +89% increase from baseline in TNF-α levels at 5:00 p.m. ($p < 0.01$) and +95% increase at 8:00 p.m. among healthy men ($p < 0.05$) (Table 3) [77]. A similar period (i.e., 40 h) of sleep deprivation investigated by Frey et al. [99] has also been found to induce a significant increase from baseline in the morning and afternoon levels of IL-1β ($p < 0.05$) (Table 2). Similar to Bøyum et al. [93], Frey et al. [99] have also found that severe sleep deprivation resulted in a decrease in IL-6 levels throughout most of the day ($p < 0.05$) (Table 3).

Conversely, Vgontzas et al. [100] have reported that the 24-h secretion of IL-6 increased when sleep was restricted to 6-h per night for one week ($p < 0.05$) (Table 3). Additionally, Vgontzas et al. [100] have found that a multi-day sleep restriction period was associated with an increased 24-h secretion of TNF-α in men only ($p < 0.01$) (Table 3). As previously mentioned, the inconsistent findings for IL-6 levels, could in part be due to the pro- and anti-inflammatory roles of this cytokine [98]. Furthermore, the variable findings for IL-6 indicate that this marker responds differently to a short period of total sleep deprivation (i.e., 40-h) compared to a week of modest sleep restriction [99,100].

Table 3. Healthy general population based sleep deprivation and stress responses studies

Reference	Sample	Intervention/ Design	Sleep restriction (SR)	Other stressors	Stress responses	Timing of samples	Main findings
Frey et al. [99]	healthy men and women (N = 19)	3 baseline days and nights, followed by single SR period and recovery sleep	baseline: 3×8 h sleep opportunities SR: 1×40 h SR recovery: 1×8 h sleep opportunity	no	salivary cortisol and blood plasma cytokines (IL-1β, IL-1ra and IL-6) subjective stress (visual analog scale)	cortisol: every 1 h beginning 2 h into SR period and ending after 38 h cytokines: every 30 min throughout SR period subjective stress: every 2 h	cortisol: no significant change subjective stress: increase at 10:00 a.m. (12 mm, +41%), 2:00 p.m. (10 mm, +33%), 4:00 p.m. (10 mm, +38%), 8:00 p.m. (11 mm, +35%), 10:00 p.m. (20 mm, +80%; all $p < 0.05$) all magnitude changes estimated from graphs provided cytokines: IL-1β increased at 9:00 a.m. (0.25 pg×ml^{-1}, +125%), 2:00 p.m. (0.20 pg×ml^{-1}, +114%), 3:00 p.m. (0.43 pg×ml^{-1}, +213%) and 4:00 p.m. (0.33 pg×ml^{-1}, +245%; $p < 0.05$) IL-1ra increased at 9:00 a.m. (35 pg×ml^{-1}, +23%), 10:00 a.m. (30 pg×ml^{-1}, +21%), 11:00 a.m. (20 pg×ml^{-1}, +13%), 7:00 p.m. (40 pg×ml^{-1}, +24%) and 12:00 a.m. (30 pg×ml^{-1}, +16%; $p < 0.05$) IL-6 decreased at 9:00 a.m. (−0.2 pg×ml^{-1}, −12%), 10:00 a.m. (−0.6 pg×ml^{-1}, −40%), 11:00 a.m. (−0.7 pg×ml^{-1}, −44%) 12:00 p.m. (−0.8 pg×ml^{-1}, −50%), 1:00 p.m. (−0.4 pg×ml^{-1}, −29%), 2:00 p.m. (−0.3 pg×ml^{-1}, −21%), 3:00 p.m. (−0.2 pg×ml^{-1}, −13%), 4:00 p.m. (−0.1 pg×ml^{-1}, −6%), 5:00 p.m. (−0.4 pg×ml^{-1}, − 31%) and 6:00 p.m. (−0.2 pg×ml^{-1}, −13%; $p < 0.05$)

Vgontzas et al. [100]	healthy adults (N = 25)	4 nights of normal sleep followed by 8 nights of partial SR	normal sleep: 4×8 h sleep opportunities SR: 8×6 h sleep opportunities PSG: each night	no	blood plasma cortisol and cytokines (TNF-α and IL-6)	cortisol and cytokines: 24 h sampling (8:00 a.m. to 7:30 a.m. next day) every 30 min on day 4 (i.e., baseline) and day 12 after SR	cortisol: peak morning cortisol decreased after SR (44.14 ± 16.55 nmol×l^{-1}, $p < 0.05$) cytokines: 24-h secretion of IL-6 (0.80 ± 0.3 pg×ml^{-1}; $p < 0.05$) and TNF-α (in men only; 0.26 ± 0.1 pg×ml^{-1}, $p < 0.01$) increased during SR
Thomas et al. [103]	healthy men and women (N = 31)	3 days and nights in a sleep laboratory	adaptation night: 1×7 h sleep opportunity sleep testing: 2×7 h sleep opportunities	no	fatigue (Vitality subscale from Health Survey); LPS cytokines (IL-6 and TNF-α)	cytokines: 11:00 p.m. pre-testing and 8:00 a.m. post-testing fatigue: evening following 1st night of testing	cytokines: evening IL-6 associated with fatigue ($r = 0.17$, $p = 0.05$) evening IL-6 negatively associated with SWS ($r^2 = 0.17$, $p = 0.29$) and positively associated with REM sleep ($r^2 = 0.26$, $p < 0.01$) fatigue: less SWS associated with fatigue the next day ($\beta = 0.55$, $p = 0.02$)
Chennaoui et al. [77]	healthy men (N = 12)	4 night SR experiment (2× baseline nights, 1×SR night and 1× recovery night)	baseline: 2×8 h sleep SR: 1×40 h SR period recovery: 1×8 h sleep PSG: continuous	no	plasma cortisol and cytokines (TNF-α, IL-6)	cytokines and cortisol: every 3 h during day 2 and 4 at 8:00 a.m., 11:00 a.m., 2:00 p.m., 5:00 p.m., 8:00 p.m. and 11:00 p.m.	cytokines: increase from baseline in TNF-α at 5:00 p.m. (+89%, $p < 0.01$) and 8:00 p.m. (+95%, $p < 0.05$) no significant change in IL-6 cortisol: no significant change

Table 3. Healthy general population based sleep deprivation and stress responses studies – cont.

Reference	Sample	Intervention/ Design	Sleep restriction (SR)	Other stressors	Stress responses	Timing of samples	Main findings
Wu et al. [104]	healthy men (N = 10)	completed earlier-night SR followed by later-night SR, separated by 10 nights of unrestricted sleep	earlier-night SR: 1×7.4 h sleep, 4×3 h sleep (12:00–3:00 a.m.), 1×7.4 h sleep (recovery later-night SR: 1×7.4 h sleep, 4×3 h sleep (3:00–6:00 a.m.), 1× ~ 7.4 h (recovery) PSG: each night during SR	no	serum cortisol, STAI	STAI and cortisol: 7:00 a.m. each morning	STAI: positive correlations between anxiety and SR (earlier-night SR: r = 0.990, p = 0.001; later-night SR: r = 0.946, p = 0.015) anxiety increased after night 1 of SR ($Z1 = 7.501$, p = 0.006), in both conditions and remained high ($Z2 = 12.643$, p = 0.000; $Z3 = 11.556$, p = 0.001; $Z4 = 9.682$, p = 0.002) cortisol: both conditions caused a decreased morning cortisol from baseline to the 3rd (earlier-night: –102.6 nmol×l^{-1}; later-night: –17.6 nmol×l^{-1}; p = 0.046), 4th (earlier-night: –110.6 nmol×l^{-1}; later-night: –3.03 nmol×l^{-1}; p = 0.010) then returned to baseline on the recovery night negative correlation between cortisol and SR duration (r = –0.955, p = 0.012)
Kajtna et al. [105]	healthy men (N = 22)	completed either SR protocol (N = 9) or normal sleep conditions (N = 13)	SR group: 1×8 h sleep (adaptation), 1×40 h SR period and 1×8 h sleep (recovery) control: not reported PSG: recorded during adaptation and recovery nights	no	BRUMS, serum cortisol	BRUMS: administered in the evenings after adaptation night and SR period cortisol: every 4 h	BRUMS: change in fatigue (4.67, +140%) and vigor (–2.56, –74%; both p < 0.05) following SR cortisol: no significant change no correlation between BRUMS and cortisol

STAI – State-Trait Anxiety Inventory for adults; BRUMS – Brunel Mood Scale; PSG – polysomnography; SWS – slow wave sleep; REM – rapid-eye movement sleep.
Other abbreviations as in Table 2.

Sleep restriction and simultaneous cortisol and cytokine changes

In addition to cytokines, Gundersen et al. [94] have found soldiers' cortisol levels increased from baseline on day 2 (+79%) and day 4 (+74%) of training, while Lundeland et al. [97] have reported increased cortisol levels on day 3 (+22%) and day 5 (+73%). But similarly to IL-6, cortisol had returned towards baseline levels by day 7 (Table 2) [94,97]. The simultaneous increase of both cytokine and cortisol levels is similar to what has been observed among individuals with stress-related illness (e.g., depression, metabolic syndrome, CVD) [28,30,31,101] and therefore, could indicate dysregulation of the bi-directional feedback loop [28,30,31]. However, given that some of these markers returned towards baseline levels by completion of the military training, it is likely that the soldiers' endocrine and inflammatory processes were able to adapt to the stressors of sleep restriction and physical work to prevent adverse outcomes [94].

Similar to other military-based studies in this area [80,81,93], Gundersen et al. [94] have not controlled for sleep duration or frequency and the participants also performed continuous physical work and had a reduced energy intake. Both physical work and energy restriction are stressors capable of causing a change in cytokine [102] and/or cortisol levels [84,85]. Therefore, while these [94,97] and other multi-day military-based studies [80,81,93] provide an insight into the effect sleep restriction may have on personnel's acute cytokine and/or cortisol response, the lack of scientific control demonstrated in available military-based literature [80,81,93,94] clouds the true relationship between the stress of sleep restriction and immune and hormonal responses among emergency service personnel.

Furthermore, the demands investigated in these studies are military-specific. For instance, the extreme sleep restriction endured by military personnel differs somewhat to the moderate, partial sleep restriction civilian emergency service personnel, such as police, firefighters and paramedics, are typically exposed to [55,60,61]. Yet, given the possible dysregultion of the cytokine and cortisol bi-directional feedback loop, further research should be focused on determining how varying amounts of controlled sleep restriction may affect emergency personnel's hormonal and immunological responses.

While Gundersen et al. [94] and Lundeland et al. [97] have reported that cortisol and cytokine levels were able to recover towards baseline, there is currently insufficient emergency service literature from which one could draw conclusions regarding the optimal recovery time for personnel exposed to sleep restriction. Therefore, further investigation is needed to determine, more specifically, the amount and/or number of recovery sleep(s) required for hormonal and inflammatory markers to recover following various types of emergency work (e.g., firefighting and police work).

Such investigations may assist emergency services in optimizing work/shift structures to minimize negative stress-related health outcomes while still meeting the unique staffing demands (e.g., on-call, response capabilities, and mobilizing for multi-day emergencies) of their organizations.

The impact of sleep restriction on mood state among emergency service personnel

In addition to physiological responses, restricted and/or poor quality sleep may also adversely affect emergency workers psychological functioning [31,82,83]. Slaven et al. [106] have investigated self-reported depressive symptoms and sleep quality among police officers who completed the Centre for Epidemiological Studies Depression (CES-D) and Pittsburgh Sleep Quality Index (PSQI) questionnaires. The findings revealed strong correlations between both measures.

For instance, mean CES-D depressive symptom scores increased across increasing quintiles of the PSQI global sleep quality score for males and females ($p < 0.001$),

indicating that depressive symptoms in male and female officers significantly increased as subjective sleep quality deteriorated [106]. However, the use of self-report measures when investigating sleep may be negatively affected by reporting/recall bias, demonstrated by a propensity to subjectively overestimate sleep length [107]. While it should be noted that the CES-D is highly sensitive to sleep, which is reflected in this study by the strong correlation between depression and sleep among officers, future research would benefit from more objective sleep measures (e.g., activity monitors, polysomnography). Furthermore, the cross-sectional design of this study [106] limits the ability to make causal inferences.

Prospective study designs, multi-day military-based studies have examined soldiers' psychological responses to periods of objectively measured (i.e., activity monitors) sleep restriction and near constant physical work [82,83]. Lieberman et al. [83] have assessed the mood state of soldiers before and after a 53 h continuous physical combat training exercise in which they had 3±0.3 h of total sleep. Using the Profile of Moods States (POMS), Lieberman et al. [83] have reported a significant change from pre- to post-training in each of the mood subscales (i.e., tension, depression, confusion, fatigue, anger and vigor) (Table 2). In a more recent study, Lieberman et al. [82] have assessed soldiers' mood also using POMS during an 84-h laboratory-based military training simulation, which controlled for the length and frequency of sleep opportunities (i.e., 7×1-h sleep breaks) and the type and duration of military activities per day. Over the course, the soldiers had a total of 6.2±0.4 h of sleep, and like their earlier study, all mood subscales significantly worsened over the duration of the training (Table 2). These findings indicate that decrements in mood can persist with 6 h of total sleep while performing an extended (i.e., 84-h) period of physical military work under more controlled laboratory settings.

Both the above mentioned studies [82,83] failed to include a control group. Consequently, it is difficult to determine how much of the reported change in psychological responses were due to the lack of sleep, or a combination of other stressors present (e.g., physical work and energy restriction). Therefore, it is not possible to know which of the stressors, or which combination of stressors is the most damaging. Accordingly, caution should be taken when generalizing findings from multi-day military-based studies to other emergency services (e.g., firefighting, police, emergency medical) routinely exposed to different occupational demands (e.g., only partial sleep deprivation or more intermittent physical work) [52].

Furthermore, these studies [82,83] used wrist-worn activity monitors to determine the participants' sleep duration and frequency. Although suited to field research, caution should be taken when using activity monitors to measure more complex sleep parameters (e.g., sleep architecture) [108]. As a result, it is difficult to determine for certain whether the psychological stress responses observed in these studies are attributed to reduced sleep duration alone or changes to sleep architecture.

Impact of sleep restriction on psycho-physiological stress responses among emergency service personnel

To date only a limited number of studies have investigated psycho-physiological responses to sleep restriction [103,105,109,110] and none has been an emergency service-based study. Thomas et al. [103] have reported that evening stimulated production of IL-6 in healthy adults was weakly associated with subjective feelings of fatigue the next day ($r = 0.17$; $p = 0.05$) and have concluded that this relationship was mediated by a reduced amount of slow wave sleep (SWS). Indeed, earlier studies have also reported that increases in circulating levels of IL-6 correlate with decreases in SWS [111,112].

A number of studies have also examined interaction between cortisol and psychological responses to various stressors [25,48], yet only a small number of studies have investigated how sleep restriction, specifically, may affect

the interaction between these responses [105,109,110]. Furthermore, such studies have generally focused on participants with either a sleep disorder or mental illness [109,110] and therefore, were not included in this review. Indeed, to the best of the authors' knowledge, only one study has examined interaction between subjective mood (assessed using the Brunel mood scale) and cortisol levels pre and post a 40-h period of sleep deprivation [105]. However, no correlation has been reported between responses in this study [105]. As such, future research needs to determine whether psycho-physiological relationships exist among healthy emergency responders (free of clinical mental and/or sleep disorders) exposed to acute sleep restriction on the job.

While emergency service research is yet to examine statistical relationships (e.g., correlation) between psycho-physiological changes to sleep restriction, Lieberman et al. [83] have simultaneously investigated soldiers' psychological and physiological responses to sleep loss during simulated combat. As previously described, the soldiers received minimal sleep and had a restricted energy intake while completing a field-exercise that involved almost continuous physical work in an intermittently hot environment (Table 2) [83]. The soldiers completed the POMS questionnaire pre-, during and post-field and cortisol was measured at 6:00 a.m., 12:00 p.m. and 6:00 p.m.

The results demonstrated that the soldiers' mood, including vigor, fatigue, confusion, depression and tension, significantly deteriorated from pre- to post-field (Table 2) [83]. Furthermore, the *post hoc* analysis of cortisol revealed lower levels in the morning and higher levels in the evening during the field-exercise compared to the pre-field levels [83]. In addition, evening cortisol measurements on day 3 and 4 during the combat training were higher than pre-training samples [83]. The low morning and high evening cortisol levels reported by Lieberman et al. [83] could indicate a disrupted cortisol circadian rhythm. Indeed, previous research has demonstrated that a low awakening cortisol response and high evening cortisol levels have both been associated with negative health outcomes (e.g., depression) [113].

On the other hand, the increase in the evening cortisol levels reported by Lieberman et al. [83] could also indicate that the participants were more active than usual at these times due to the around-the-clock physical work involved in the military training. Consequently, an inadequate (i.e., low) cortisol response in this instance could be problematic and indicate another component of allostatic load [90]. Given how the cortisol response to different stressors can vary (e.g., prolonged and inadequate responses), yet still indicate possible dysregulation of the HPA-axis highlights the need to investigate all aspects of this physiological stress response in future studies.

Moreover, investigating how the cortisol response interacts with other psychological stress responses could help to further contextualize the significance of the response [28]. Indeed the acute psychological and physiological findings by Lieberman et al. [83] may demonstrate that the deterioration in mood during the military training could be associated with a disrupted cortisol response (i.e., low morning and high evening levels). Yet without statistical analysis of the interactions between these markers, it is difficult to determine what the psycho-physiological relationship was (under these conditions).

The lack of a control group is a further limitation of this study [83], making it challenging to determine if the findings were due to sleep restriction, other stressors (i.e., heat stress, energy restriction and physical work) or a combination of these demands. However, this study [83] represents the only available emergency service-based research that examines concurrent changes in the acute psychological and physiological responses to sleep restriction.

Laboratory-based sleep studies [99,104] have also demonstrated concurrent physiological and psychological changes similar to those observed in the field by Lieberman et al. [83]. Using a crossover study design, Wu et al. [104]

have investigated the effect of an earlier-night (i.e., sleep from 12:00 to 3:00 a.m.) and later-night (i.e., sleep from 3:00 to 6:00 a.m.) sleep restriction protocol on subjective anxiety (measured using the State-Trait Anxiety Inventory; STAI) and cortisol levels. For each condition, the participants completed an unrestricted baseline sleep, 4 nights of sleep restriction followed by a recovery night and blood samples (for cortisol analysis) were taken each morning (at 7:00 a.m.) [104].

In both conditions, STAI increased from baseline after the 1st night of reduced sleep and then continued to increase each day for the duration of the conditions [104]. Furthermore, positive correlations between STAI and sleep restriction (earlier-night sleep restriction: $r = 0.990$, $p = 0.00$; later-night sleep restriction: $r = 0.946$, $p = 0.015$) were reported and both sleep periods resulted in the reduced morning cortisol levels [104]. Following the recovery night, cortisol and STAI in both conditions returned towards normal, but only cortisol reached baseline. While this study explored more controlled sleep periods, additional daily samples were needed to provide a more detailed measure of circadian changes in hormonal and mood responses.

Furthermore, the lack of statistical analyses between the psychological and physiological responses limits the ability to investigate any possible psycho-physiological interactions in response to the periods of sleep restriction examined.

In more extreme cases (e.g., natural or man-made disasters such as large bushfires/wildfires), emergency personnel have reported continuous periods of extended wakefulness lasting for more than 24-h [55]. When examined in controlled laboratory conditions, total sleep deprivation was found by Frey et al. [99] to elicit simultaneous physiological and psychological changes among healthy adults. For instance, Frey et al. [99] have reported a simultaneous increase in inflammatory cytokines (IL-1ra, IL-1b) in the morning and evening and higher subjective stress levels at most time points across the 40-h sleep deprivation period compared with baseline ($p < 0.05$). However, no significant effects for salivary cortisol levels were detected in this study.

CONCLUSIONS

A single night of sleep deprivation (either partial or full) may not be a sufficient stressor to significantly affect the overall daily release of cortisol among military emergency responders [76]. However, extreme sleep restriction over multiple days of emergency work (e.g., 1–7 h of sleep over 2–7 days) can:

- disrupt the circadian cortisol rhythm [80,81,83],
- disrupt (i.e., above and below baseline or control levels) pro- (i.e., IL-6, TNF-α and IL-1β) and anti-inflammatory cytokine levels (i.e., IL-1ra) [93,94,97],
- elicit adverse psychological responses (i.e., deterioration in mood) [82,83],
- cause a simultaneous increase in both cortisol and cytokine levels (i.e., IL-6) [94,97].

Taken together, this literature informs emergency services that exposure to more than one night of severe work-related sleep restriction experienced between shifts may influence their personnel's acute physiological (i.e., cortisol and cytokine levels) and psychological (i.e., mood) functioning.

The significance (i.e., abnormal or normal) of acute changes in physiological and psychological responses to restricted sleep experienced at work is of further importance to emergency services. Extreme sleep restriction over consecutive days of military training can result in the concurrent deterioration of both mood and disrupted diurnal cortisol levels [83,104]. However, interactions between these responses have not yet been statistically analyzed and therefore, it is difficult to determine, for certain, if a psycho-physiological relationship exists between these markers (under these conditions) among personnel.

Healthy individuals have also demonstrated adverse simultaneous, but not statistically evaluated, changes in multiple physiological (i.e., cortisol and cytokines) and psychological (i.e., STAI) responses following consecutive nights (lasting up to a week) of sleep restriction [100,104]. Conversely, the evidence of psycho-physiological interactions between stress responses following a single night of sleep deprivation remains equivocal [77,99,105]. However, it appears that the release of pro-inflammatory cytokines (i.e., IL-6) positively correlates with feelings of fatigue [103], indicating the negative effect immune dysregulation may have on general well-being.

Moreover, this finding offers some limited empirical support for the use of non-invasive measures of fatigue to evaluate immune function. However, on the whole, evidence investigating psycho-physiological responses to sleep restriction is still very limited and inconclusive. Therefore, it is not currently feasible for uniform services to develop practical means to efficiently monitor the acute stress of their personnel in the field, based on psycho-physiological interactions.

To date, only a limited number of studies have investigated the effect of work-related sleep restriction on acute stress responses among different forms of emergency personnel. In fact, this review of the literature uncovered only one police-based study [31] and no firefighting or emergency medical-related investigations. Meanwhile, the overwhelming majority of the research was focused on multi-day military studies [80–83,93,94,97]. As such, the periods of extreme sleep restriction (i.e., 1–2-h per 24-h) investigated are, in most cases, military specific. Furthermore, military-based studies expose personnel to stressors (such as continuous physical military activities and food and fluid restriction) not commonly experienced during more civilian emergency service work (e.g., firefighting and police work) [55,114].

Consequently, extrapolating findings from the defense occupations with different workloads to more civilian emergency services may under or overestimate the potential stress-related implications and lead to inappropriate advice/recommendations regarding sleep opportunities. Additionally, most military field-based studies have not controlled for sleep duration or frequency and failed to include a control group matched for age, sex or work experience.

As a result, it is difficult to determine how much of the reported change in physiological or psychological responses were due to sleep restriction, other occupational stressors or a combination of stressors and therefore, if sleep restriction (or a combination of stressors) was the most damaging stressor. Regardless, it appears that sleep restriction may be a major occupational stressor contributing to the reported physiological and psychological responses and therefore, warrants further investigation.

FUTURE RESEARCH DIRECTIONS

To further understand the impact of work-related sleep restriction on acute stress responses and to provide emergency services with the knowledge they need to protect the health of their personnel, future research should focus on:

- a wider range of emergency services,
- practical methods to monitor physiological and psychological health of the personnel exposed to work related sleep restriction,
- controlled periods of sleep restriction similar in duration to what is reported/experienced among civilian emergency services,
- concurrent measurement of multiple stress responses and statistical analyses of the psycho-physiological interactions (if any) between these responses,
- all aspects of the cortisol and cytokine response (e.g., normal, inadequate and prolonged responses) that characterize the nature and possible dysregulation of these systems,
- the amount and/or number of recovery sleep(s) required for cortisol and cytokine levels to return to baseline following work-related sleep restriction.

While the acute effect that work-related sleep restriction has on stress remains an important focus, future research could also benefit from longitudinal/follow-up studies to further understand the possible link between this stressor and negative long-term health outcomes. For instance, examining how stress responses recover following exposure to sleep restriction (via post/follow-up testing) during emergency work may provide insights into how acute responses translate into chronic physiological and psychological changes and ultimately, result in adverse long-term health-outcomes.

REFERENCES

1. Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med. 2007;3(5):19–28.
2. Mullington JM, Simpson NS, Meier-Ewert HK, Haack M. Sleep loss and inflammation. Best Pract Res Clin Endocrinol Metab. 2010;24:775–84, http://dx.doi.org/10.1016/j.beem.2010.08.014.
3. Vgontzas AN, Papanicolaou DA, Bixler EO, Lotsikas A, Zachman K, Kales A, et al. Circadian interleukin-6 secretion and quantity and depth of sleep. J Clin Endocrinol Metab. 1999;84(8):2603–7, http://dx.doi.org/10.1210/jcem.84.8.5894.
4. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet. 1999;354(9188):1435–9, http://dx.doi.org/10.1016/S0140-6736(99)01376-8.
5. Peter JH, Mark TOC, Nancy JR, Stephen JH, Jürgens N, Keri LHC, et al. Inflammation in human brain injury intracerebral concentrations of IL-1α, IL-1β, and their endogenous inhibitor IL-1ra. J Neurotrauma. 2007;24(10):1545–57, http://dx.doi.org/10.1089/neu.2007.0295.
6. Chung S, Son GH, Kim K. Circadian rhythm of adrenal glucocorticoid: Its regulation and clinical implications. Biochim Biophys Acta. 2011;1812(5):581–91.
7. Zimberg IZ, Dâmaso A, Del Re M, Carneiro AM, de Sá Souza H, de Lira FS, et al. Short sleep duration and obesity: Mechanisms and future perspectives. Cell Biochem Funct. 2012;30(6):524–9, http://dx.doi.org/10.1002/cbf.2832.
8. Reynolds AC, Dorrian J, Liu PY, Van Dongen HPA, Wittert GA, Harmer LJ, et al. Impact of five nights of sleep restriction on glucose metabolism, leptin and testosterone in young adult men. PLoS One. 2012;7(7):1–10, http://dx.doi.org/10.1371/journal.pone.0041218.
9. Babson KA, Trainor CD, Feldner MT, Blumenthal H. A test of the effects of acute sleep deprivation on general and specific self-reported anxiety and depressive symptoms: An experimental extension. J Behav Ther Exp Psychiatry. 2010;41(3):297–303, http://dx.doi.org/10.1016/j.jbtep.2010.02.008.
10. Gangwisch JE, Heymsfield SB, Boden-Albala B, Buijs RM, Kreier F, Pickering TG, et al. Short sleep duration as a risk factor for hypertension: Analyses of the first National Health and Nutrition Examination Survey. Hypertension. 2006;47(5):833–9, http://dx.doi.org/10.1161/01.HYP.0000217362.34748.e0.
11. Ganz FD. Sleep and immune function. Crit Care Nurse. 2012;32(2):e19–25, http://dx.doi.org/10.4037/ccn2012689.
12. Hall MH, Muldoon MF, Jennings JR, Buysse DJ, Flory JD, Manuck SB. Self-reported sleep duration is associated with the metabolic syndrome in midlife adults. Sleep. 2008;31(5):635–43.
13. Finnerty CC, Jeschke MG, Herndon DN, Gamelli R, Gibran N, Klein M, et al. Temporal cytokine profiles in severely burned patients: A comparison of adults and children. Mol Med. 2008;14(9–10):553–60.
14. Myles WS. Self-paced work in sleep deprived subjects. Ergonomics. 1987;30(8):1175–84.
15. Reynolds RM, Labad J, Strachan MWJ, Braun A, Fowkes FGR, Lee AJ, et al. Elevated fasting plasma cortisol is associated with ischemic heart disease and its risk factors in people with type 2 diabetes: The edinburgh type 2 diabetes study. J Clinic Endocrinol Metab. 2010;95(4), http://dx.doi.org/10.1210/jc.2009-2112.

16. McCanlies EC, Slaven JE, Smith LM, Andrew ME, Charles LE, Burchfiel CM, et al. Metabolic syndrome and sleep duration in police officers. Work. 2012;43(2):133–9.
17. World Health Organization. Global status report on noncommunicable disaeses 2010. Geneva: WHO; 2011.
18. World Health Organization. Depression. Fact Sheet Number 369. October 2012 [cited 2013 Jun 19]. Available from: http://www.who.int/mediacentre/factsheets/fs369/en/.
19. Aardal-Eriksson E, Eriksson TE, Holm AC, Lundin T. Salivary cortisol and serum prolactin in relation to stress rating scales in a group of rescue workers. Biol Psychiatry. 1999;46(6): 850–5, http://dx.doi.org/10.1016/S0006-3223(98)00381-3.
20. Ryff CD, Singer B. The contours of positive human health. Psychol Inquiry. 1998;9(1):1, http://dx.doi.org/10.1207/s15327965pli0901_1.
21. Ryff CD, Singer BH. Biopsychosocial challenges of the new millennium. Psychother Psychosom. 2000;69(4):170–7.
22. Brydon L, Walker C, Wawrzyniak A, Whitehead D, Okamura H, Yajima J, et al. Synergistic effects of psychological and immune stressors on inflammatory cytokine and sickness responses in humans. Brain Behav Immun. 2009;23(Suppl 1): 217–24, http://dx.doi.org/10.1016/j.bbi.2008.09.007.
23. Maas CJM, Snijders TAB. The multilevel approach to repeated measures for complete and incomplete data. Qual Quant. 2003;37(1):71, http://dx.doi.org/10.1023/A:1022545930672.
24. Chiodo S, Tessitore A, Cortis C, Cibelli G, Lupo C, Ammendolia A, et al. Stress-related hormonal and psychological changes to official youth Taekwondo competitions. Scand J Med Sci Sports. 2011;21(1):111–9, http://dx.doi.org/10.1111/j.1600-0838.2009.01046.x.
25. Jürimäe J, Mäestu J, Purge P, Jürimäe T, Sööt T. Relations among heavy training stress, mood state and performance for male junior rowers. Percept Mot Skills. 2002;95(2): 520–6, http://dx.doi.org/10.2466/pms.2002.95.2.520.
26. Besedovsky HO, Rey A. The cytokine-HPA axis feed-back circuit. Zeitschrift Rheumatologie. 2000;59 (Suppl 2):II26–30, http://dx.doi.org/10.1007/s003930070014.
27. Eskandari F, Sternberg EM. Neural-immune interactions in health and disease. Ann Ny Acad Sci. 2002;966:20–7, http://dx.doi.org/10.1111/j.1749-6632.2002.tb04198.x.
28. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: From HPA axis to glucocorticoid receptor dysfunction. Ann Ny Acad Sci. 2012;1261(1):55–63, http://dx.doi.org/10.1111/j.1749-6632.2012.06633.x.
29. Sternberg EM, Licinio J. Overview of neuroimmune stress interactions. Implications for susceptibility to inflammatory disease. Ann Ny Acad Sci. 1995;771:364–71, http://dx.doi.org/10.1111/j.1749-6632.1995.tb44695.x.
30. Makhija K, Karunakaran S. The role of inflammatory cytokines on the aetiopathogenesis of depression. Aust N Z J Psychiatry. 2013, http://dx.doi.org/10.1177/0004867413488220.
31. Zunszain PA, Anacker C, Cattaneo A, Carvalho LA, Pariante CM. Glucocorticoids, cytokines and brain abnormalities in depression. Prog Neuropsychopharmacol. 2011; 35(3):722–9, http://dx.doi.org/10.1016/j.pnpbp.2010.04.011.
32. Kunz-Ebrecht SR, Kirschbaum C, Marmot M, Steptoe A. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. Psychoneuroendocrinology. 2004;29(4):516–28, http://dx.doi.org/10.1016/S0306-4530(03)00072-6.
33. Mackin P, Young AH. The role of cortisol and depression: Exploring new opportunities for treatments. Psychiatr Times. 2004;21(5):92–5.
34. Pella D, Otsuka K, Singh RB. Metabolic syndrome: A disease of the brain. Open Nutraceutic J. 2011;4(1):107–18, http://dx.doi.org/10.2174/1876396001104010107.
35. Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia. 1998;41(10): 1241–8, http://dx.doi.org/10.1007/s001250051058.
36. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000;101(15):1767–72, http://dx.doi.org/10.1161/01.CIR.101.15.1767.

37. Cesari M, Penninx BWJ, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation. 2003;108(19):2317–22, http://dx.doi.org/10.1161/01.CIR.0000097109.90783.FC.
38. Bennet AM, van Maarle MC, Hallqvist J, Morgenstern R, Frostegård J, Wiman B, et al. Association of TNF-alpha serum levels and TNFA promoter polymorphisms with risk of myocardial infarction. Atherosclerosis. 2006;187(2):408–14, http://dx.doi.org/10.1016/j.atherosclerosis.2005.09.022.
39. You TJ, Nicklas BJ, Ding JZ, Penninx BWJH, Goodpaster BH, Bauer DC, et al. The metabolic syndrome is associated with circulating adipokines in older adults across a wide range of adiposity. J Gerontol A Biol. 2008;63(4):414–9, http://dx.doi.org/10.1093/gerona/63.4.414.
40. Hiles SA, Baker AL, de Malmanche T, Attia J. A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: Exploring the causes of heterogeneity. Brain Behav Immun. 2012;26(7):1180–8, http://dx.doi.org/10.1016/j.bbi.2012.06.001.
41. Liu Y, Ho RC-M, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression. J Affect Disord. 2012;139(3):230–9, http://dx.doi.org/10.1016/j.jad.2011.08.003.
42. Doane LD, Franz CE, Prom-Wormley E, Eaves LJ, Mendoza SP, Hellhammer DH, et al. Negative emotionality, depressive symptoms and cortisol diurnal rhythms: Analysis of a community sample of middle-aged males. Horm Behav. 2011;60(2):202–9, http://dx.doi.org/10.1016/j.yhbeh.2011.05.003.
43. Heaney JLJ, Phillips AC, Carroll D. Ageing, depression, anxiety, social support and the diurnal rhythm and awakening response of salivary cortisol. Int J Psychophysiol. 2010;78(3):201–8, http://dx.doi.org/10.1016/j.ijpsycho.2010.07.009.
44. Reynolds RM, Walker BR. Human insulin resistance: The role of glucocorticoids. Diabetes Obes Metab. 2003;5(1):5–12, http://dx.doi.org/10.1046/j.1463-1326.2003.00221.x.
45. Walker BR, Soderberg S, Lindahl B, Olsson T. Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women. J Intern Med. 2000;247(2):198–204, http://dx.doi.org/10.1046/j.1365-2796.2000.00609.x.
46. Filipovský J, Ducimetiére P, Eschwége E, Richard JL, Rosselin G, Claude JR. The relationship of blood pressure with glucose, insulin, heart rate, free fatty acids and plasma cortisol levels according to degree of obesity in middle-aged men. J Hypertens. 1996;14(2):229–35, http://dx.doi.org/10.1097/00004872-199602000-00012.
47. Fortmann SP, Ford E, Criqui MH, Folsom AR, Harris TB, Hong Y, et al. CDC/AHA workshop on markers of inflammation and cardiovascular disease: Application to clinical and public health practice: Report from the population science discussion group. Circulation. 2004;110(25):e554–9, http://dx.doi.org/10.1161/01.CIR.0000148982.95775.BF.
48. Bouget M, Rouveix M, Michaux O, Pequignot J, Filaire E. Realationships among training stress mood and dehydroepiandrosterone sulphate/cortisol ratio in female cyclists. J Sport Sci. 2006;24(12):1297–302, http://dx.doi.org/10.1080/02640410500497790.
49. Filaire E, Legrand B, Lac G, Pequignot J. Training of elite cyclists: Effects on mood state and selected hormonal responses. J Sport Sci. 2004;22:1025–33, http://dx.doi.org/10.1080/02640410410001716751.
50. Burl S, Townend J, Njie-Jobe J, Cox M, Adetifa UJ, Touray E, et al. Age-dependent maturation of Toll-like receptor-mediated cytokine responses in Gambian infants. Plos One. 2011;6(4):e18185, http://dx.doi.org/10.1371/journal.pone.0018185.
51. Vgontzas AN, Zoumakis M, Bixler EO, Lin H-M, Prolo P, Vela-Bueno A, et al. Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: Physiologic and therapeutic implications. J Clinical Endocrinol Metab. 2003;88(5):2087–95, http://dx.doi.org/10.1210/jc.2002-021176.

52. Aisbett B, Wolkow A, Sprajcer M, Ferguson S. "Awake, smoky, and hot": Providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters. Appl Ergon. 2012;43(5):916–25, http://dx.doi.org/10.1016/j.apergo.2011.12.013.
53. Aisbett B, Phillips M, Nichols D. Work patterns of tanker-based bushfire suppression by Australian volunteer firefighters in south-east Australia. Paper presented at the Tassie Fire Conference: The Joint Australasian Fire Authorities Council/Bushfire Co-Operative Research Centre Conference; 2007 Sept 21; Hobart, Tasmania, Australia [cited 2013 Oct 16]. Available from: http://www.proceedings.com.au/tassiefire/powerpoint_pdf/Fri%201400%20Ballroom%202%20Aisbett.pdf.
54. Black JL. Heat stress in bushfire fighters: A practitioner's perspective. In: Hales JRS, editor. Heat stress: physical exertion and environment. East Melbourne: The Menzies Foundation; 1987. p. 29–36.
55. Cater H, Clancy D, Duffy K, Holgate A, Wilson B, Wood J. Fatigue on the fireground: The DPI experience. Paper presented at the Tassie Fire Conference: The Joint Australasian Fire Authorities Council/Bushfire Co-Operative Research Centre Conference; 2007 Sep 21; Hobart, Tasmania, Australia [cited 2013 Dec 10]. Available from: http://proceedings.com.au/tassiefire/papers_pdf/fri_wilson.pdf.
56. Cuddy JS, Gaskill SE, Sharkey BJ, Harger SG, Ruby BC. Supplemental feedings increase self-selected work output during wildfire suppression. Med Sci Sports Exerc. 2007;39(6):1004–12, http://dx.doi.org/10.1249/mss.0b013e318040b2fb.
57. Elliott G, Omodei M, Johnson C. How human factors drive decision making at fire ground level. Bushfire Co-Operative Research Centre FireNote 2009 [cited 2013 Oct 10]. Available from: http://www.bushfirecrc.com/managed/resource/0909_hf_firenote44_lowres.pdf.
58. Reisen F, Hansen D, Meyer CP. Exposure to bushfire smoke during prescribed burns and wildfires: Firefighters' exposure risks and options. Environ Int. 2011;37(2):314–21, http://dx.doi.org/10.1016/j.envint.2010.09.005.
59. Hunter L. The Campaign Fires: North-East/East Gippsland Fires 2003. Mt. Waverley, Vic.: Country Fire Authority; 2003.
60. Guyon A, Balbo M, Morselli LL, Tasali E, Leproult R, L'Hermite-Balériaux M, et al. Adverse effects of 2 nights of sleep restriction on the hypothalamic-pituitary-adrenal axis in healthy men. J Clin Endocrinol Metab. 2014;99(8):2861–8, http://dx.doi.org/10.1210/jc.2013-4254.
61. Neylan TC, Metzler TJ, Best SR, Weiss DS, Fagan JA, Liberman A, et al. Critical incident exposure and sleep quality in police officers. Psychosom Med. 2002;64(2):345–52, http://dx.doi.org/10.1097/00006842-200203000-00019.
62. Cook B, Mitchell W. Occupational health effects for firefighters: The extent and implications of physical and psychological injuries. Centre of Full Employment and Equity, Equity CoFEa; 2013 [cited 2013 Oct 10]. Available from: https://www.firecrisis.com.au/wp-content/uploads/2013/02/CofFEE-report-Final.pdf.
63. Van Mark A, Weiler SW, Schröder M, Otto A, Jauch-Chara K, Groneberg DA, et al. The impact of shift work induced chronic circadian disruption on IL-6 and TNF-α immune responses. J Occup Med Toxicol. 2010;5:18–22, http://dx.doi.org/10.1186/1745-6673-5-18.
64. Hartley TA, Burchfiel CM, Fekedulegn D, Andrew ME, Violanti JM. Health disparities in police officers: Comparisons to the U.S. general population. Int J Emerg Mental Health. 2011;13(4):211–20.
65. Rajaratnam SM, Barger LK, Lockley SW, Shea SA, Wang W, Landrigan CP, et al. Sleep disorders, health, and safety in police officers. JAMA. 2011;306(23):2567–78, http://dx.doi.org/10.1001/jama.2011.1851.
66. Abedelmalek S, Souissi N, Chtourou H, Denguezli M, Aouichaoui C, Ajina M, et al. Effects of partial sleep deprivation on proinflammatory cytokines, growth hormone, and steroid hormone concentrations during repeated brief sprint interval exercise. Chronobiol Int. 2013;30(4):502–9, http://dx.doi.org/10.3109/07420528.2012.742102.
67. Pejovic S, Basta M, Vgontzas AN, Kritikou I, Shaffer ML, Tsaoussoglou M, et al. Effects of recovery sleep after one

work week of mild sleep restriction on interleukin-6 and cortisol secretion and daytime sleepiness and performance. Am J Physiol Endocrinol Metab. 2013;305(7):E890–6, http://dx.doi.org/10.1152/ajpendo.00301.2013.

68. Courtney J, Francis A, Paxton S. Caring for the country: Fatigue, sleep and mental health in Australian rural paramedic shiftworkers. J Community Health. 2013;38(1):178–86, http://dx.doi.org/10.1007/s10900-012-9599-z.
69. Gleeson M, Blannin AK, Walsh NP, Bishop NC, Clark AM. Effect of low- and high-carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. Int J Sport Nutr. 1998;8(1):49–59.
70. Faraut B, Boudjeltia KZ, Dyzma M, Rousseau A, David E, Stenuit P, et al. Benefits of napping and an extended duration of recovery sleep on alertness and immune cells after acute sleep restriction. Brain Behav Immun. 2011;25(1):16–24, http://dx.doi.org/10.1016/j.bbi.2010.08.001.
71. Shiozaki M, Miyai N, Morioka I, Utsumi M, Koike H, Arita M, et al. [Assessment of the risk of ischemic heart disease and its relevant factors among Japanese police officers]. Sangyo Eiseigaku Zasshi. 2013;55(4):115–24. Japanese.
72. Charles LE, Ja KG, Andrew ME, Violanti JM, Fekedulegn D, Burchfiel CM. Sleep duration and biomarkers of metabolic function among police officers. J Occup Environ Med. 2011;53(8):831–7, http://dx.doi.org/10.1097/JOM.0b013e31821f5ece.
73. Silverman MN, Heim CM, Nater UM, Marques AH, Sternberg EM. Neuroendocrine and immune contributors to fatigue. PM R. 2010;2(5):338–46, http://dx.doi.org/10.1016/j.pmrj.2010.04.008.
74. Peterson AL, Goodie JL, Satterfield WA, Brim WL. Sleep disturbance during military deployment. Mil Med. 2008;173(3):230–5.
75. Moore J, Harper Smith A, Felice U, Walsh N. Three nights of sleep deprivation does not alter thermal strain during exercise in the heat. Eur J Appl Physiol. 2013;113(9):2353–60, http://dx.doi.org/10.1007/s00421-013-2671-2.
76. Goh VH, Tong TY, Lim C, Low EC, Lee LK. Effects of one night of sleep deprivation on hormone profiles and performance efficiency. Mil Med. 2001;166(5):427–31.
77. Chennaoui M, Sauvet F, Drogou C, Van Beers P, Langrume C, Guillard M, et al. Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-α) levels in healthy men. Cytokine. 2011;56(2):318–24, http://dx.doi.org/10.1016/j.cyto.2011.06.002.
78. Pejovic S, Vgontzas AN, Basta M, Tsaoussoglou M, Zoumakis E, Vgontzas A, et al. Leptin and hunger levels in young healthy adults after one night of sleep loss. J Sleep Res. 2010;19(4):552–8, http://dx.doi.org/10.1111/j.1365-2869.2010.00844.x.
79. Weckesser LJ, Plessow F, Pilhatsch M, Muehlhan M, Kirschbaum C, Miller R. Do venepuncture procedures induce cortisol responses? A review, study, and synthesis for stress research. Psychoneuroendocrinology. 2014;46:88–99, http://dx.doi.org/10.1016/j.psyneuen.2014.04.012.
80. Opstad PK. Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. Eur J Endocrinol. 1994;131(1):56–66, http://dx.doi.org/10.1530/eje.0.1310056.
81. Opstad PK, Aakvaag A. The effect of a high calory diet on hormonal changes in young men during prolonged physical strain and sleep deprivation. Eur J Appl Physiol. 1981;46(1):31–9, http://dx.doi.org/10.1007/BF00422172.
82. Lieberman HR, Niro P, Tharion WJ, Nindl BC, Castellani JW, Montain SJ. Cognition during sustained operations: Comparison of a laboratory simulation to field studies. Aviat Space Environ Med. 2006;77(9):929–35.
83. Lieberman HR, Bathalon GP, Falco CM, Kramer FM, Morgan CA, Niro P. Severe decrements in cognition function and mood induced by sleep loss, heat, dehydration, and undernutrition during simulated combat. Biol Psychiatry. 2005;57:422–9, http://dx.doi.org/10.1016/j.biopsych.2004.11.014.
84. Tomiyama AJ, Mann T, Vinas D, Hunger JM, Dejager J, Taylor SE. Low calorie dieting increases cortisol. Psychosom

Med. 2010;72(4):357–64, http://dx.doi.org/10.1097/PSY.0b013e3181d9523c.

85. Georgopoulos NA, Rottstein L, Tsekouras A, Theodoropoulou A, Koukkou E, Mylonas P, et al. Abolished circadian rhythm of salivary cortisol in elite artistic gymnasts. Steroids. 2011;76(4):353–7, http://dx.doi.org/10.1016/j.steroids.2010.10.013.
86. Viru AM, Hackney AC, Välja E, Karelson K, Janson T, Viru M. Influence of prolonged continuous exercise on hormone responses to subsequent exercise in humans. Eur J Appl Physiol. 2001;85(6):578–85, http://dx.doi.org/10.1007/s004210100498.
87. Juster R-P, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev. 2010;35(1):2–16, http://dx.doi.org/10.1016/j.neubiorev.2009.10.002.
88. Rosmond R, Dallman MF, Björntorp P. Stress-related cortisol secretion in men: Relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab. 1998;83(6):1853–9, http://dx.doi.org/10.1210/jc.83.6.1853.
89. Miller GE, Cohen S, Ritchey AK. Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. Health Psychol. 2002;21(6):531–41, http://dx.doi.org/10.1037/0278-6133.21.6.531.
90. McEwen BS, Seeman T. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. Ann NY Acad Sci. 1999;896:30–47, http://dx.doi.org/10.1111/j.1749-6632.1999.tb08103.x.
91. Golden S, Wand G, Malhotra S, Kamel I, Horton K. Reliability of hypothalamic–pituitary–adrenal axis assessment methods for use in population-based studies. Eur J Epidemiol. 2011;26:511–25, http://dx.doi.org/10.1007/s10654-011-9585-2.
92. Smyth N, Clow A, Thorn L, Hucklebridge F, Evans P. Delays of 5–15 min between awakening and the start of saliva sampling matter in assessment of the cortisol awakening response. Psychoneuroendocrinology. 2013;38(9):1476–83, http://dx.doi.org/10.1016/j.psyneuen.2012.12.013.
93. Bøyum A, Wiik P, Gustavsson E, Veiby OP, Reseland J, Haugen AH, et al. The effect of strenuous exercise, calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines. Scand J Immunol. 1996;43(2):228–35, http://dx.doi.org/10.1046/j.1365-3083.1996.d01-32.x.
94. Gundersen Y, Opstad P, Reistad T, Thrane I, Vaagenes P. Seven days' around the clock exhaustive physical exertion combined with energy depletion and sleep deprivation primes circulating leukocytes. Eur J Appl Physiol. 2006;97(2):151–7, http://dx.doi.org/10.1007/s00421-006-0150-8.
95. Vgontzas A, Bixler E, Lin H, Prolo P, Trakada G. IL-6 and its circadian secretion in humans. Neuroimmunomodulation. 2005;12:131–40, http://dx.doi.org/10.1159/000084844.
96. Shearer WT, Reuben JM, Mullington JM, Price NJ, Lee BN, Smith EO, et al. Soluble TNF-alpha receptor 1 and IL-6 plasma levels in humans subjected to the sleep deprivation model of spaceflight. J Allergy Clin Immun. 2001;107(1):165–70, http://dx.doi.org/10.1067/mai.2001.112270.
97. Lundeland B, Gundersen Y, Opstad P-K, Thrane I, Zhang Y, Olaussen RW, et al. One week of multifactorial high-stress military ranger training affects Gram-negative signalling. Scand J Clin Lab Invest. 2012;72(7):547–54, http://dx.doi.org/10.3109/00365513.2012.705017.
98. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, et al. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest. 1998;101(2):311–20, http://dx.doi.org/10.1172/JCI1368.
99. Frey DJ, Fleshner M, Wright KP. The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults. Brain Behav Immun. 2007;21(8):1050–7, http://dx.doi.org/10.1016/j.bbi.2007.04.003.
100. Vgontzas A, Zoumakis E, Bixler E, Lin H, Follett H, Kales A, et al. Adverse effects of modest sleep restriction on sleepiness, performance and inflammatory cytokines. J Clin Endocrinol Metab. 2004;89(5):2119–26, http://dx.doi.org/10.1210/jc.2003-031562.

101. Chrousos GP, Kino T. Glucocorticoid action networks and complex psychiatric and/or somatic disorders. Stress. 2007;10(2):213–9, http://dx.doi.org/10.1080/10253890701292119.
102. Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B, et al. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J Physiol. 2001;537(Pt 2):633–9, http://dx.doi.org/10.1111/j.1469-7793.2001.00633.x.
103. Thomas KS, Motivala S, Olmstead R, Irwin MR. Sleep depth and fatigue: Role of cellular inflammatory activation. Brain Behav Immun. 2011;25(1):53–8, http://dx.doi.org/10.1016/j.bbi.2010.07.245.
104. Wu H, Zhao Z, Stone WS, Huang L, Zhuang J, He B, et al. Effects of sleep restriction periods on serum cortisol levels in healthy men. Brain Res Bull. 2008;77(5):241–5, http://dx.doi.org/10.1016/j.brainresbull.2008.07.013.
105. Kajtna T, Štukovnik V, Dolenc Grošelj L. [Effect of acute sleep deprivation on concentration and mood states with a controlled effect of experienced stress]. Zdrav Vestn. 2011;80(5):354–61. Slovenian.
106. Slaven JE, Mnatsakanova A, Burchfield CM, Smith LM, Charles LE, Andrew ME, et al. Association of sleep quality with depression in police officers. Int J Emerg Ment Health. 2011;13(4):267–77.
107. Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. Self-reported and measured sleep duration: How similar are they? Epidemiology. 2008;19(6):838–45, http://dx.doi.org/10.1097/EDE.0b013e318187a7b0.
108. Reid K, Dawson D. Correlation between wrist activity monitor and electrophysiological measures of sleep in a simulated shiftwork environment for younger and older subjects. Sleep. 1999;22(3):378–85.
109. Backhaus J, Junghanns K, Hohagen F. Sleep disturbances are correlated with decreased morning awakening salivary cortisol. Psychoneuroendocrinology. 2004;29(9):1184–91, http://dx.doi.org/10.1016/j.psyneuen.2004.01.010.
110. Bouhuys A, Flentge F, van den Hoofdakker RH. Effects of total sleep deprivation on urinary cortisol, self-rated arousal, and mood in depressed patients. Psychiatry Res. 1990;34:149–62, http://dx.doi.org/10.1016/0165-1781(90)90016-X.
111. Burgos I, Richter L, Klein T, Fiebich B, Feige B, Lieb K, et al. Increased nocturnal interleukin-6 excretion in patients with primary insomnia: A pilot study. Brain Behav Immun. 2006;20(3):246–53, http://dx.doi.org/10.1016/j.bbi.2005.06.007.
112. Hong S, Mills PJ, Loredo JS, Adler KA, Dimsdale JE. The association between interleukin-6, sleep, and demographic characteristics. Brain Behav Immun. 2005;19(2):165–72, http://dx.doi.org/10.1016/j.bbi.2004.07.008.
113. Vreeburg SA, Hoogendijk WJG, DeRijk RH, van Dyck R, Smit JH, Zitman FG, et al. Salivary cortisol levels and the 2-year course of depressive and anxiety disorders. Psychoneuroendocrinology. 2013;38(9):1494–502, http://dx.doi.org/10.1016/j.psyneuen.2012.12.017.
114. Ruby BC, Shriver T, Zderic T, Sharkey B, Burks C, Tysk S. Total energy expenditure during arduous wildfire suppression. Med Sci Sports Exerc. 2002;34(6):1048–54, http://dx.doi.org/10.1097/00005768-200206000-00023.

Appendix D: Additional model details and data

D.1 Study 1

D.1.1 Summary of fit for REML model for cytokine profiles

Cytokine	Model	AIC	ΔAIC	Rank
Log IL-6	Independence – Unbanded	737.18	3.30	3
	Independence – Banded	737.57	3.69	4
	Unstructured	734.99	1.11	2
	Power Model	733.88	0.00	1
	Linear Trends, No Spline	797.79	63.91	10
	Common Spline for All Profiles	784.25	48.37	7
	Separate Spline for Each Sequence Day	774.99	41.11	5
	Separate Spline for Each Condition	787.25	53.37	9
	Common Spline for All Profiles – Combo Factor	784.25	50.37	8
	Common Spline for Each Condition and Sequence Day	780.60	46.72	6
Log IL-8	Independence – Unbanded	801.21	5.08	4
	Independence – Banded	801.16	5.03	3
	Unstructured	796.13	0.00	1
	Power Model	800.48	4.35	2
	Linear Trends, No Spline	842.03	45.90	9
	Common Spline for All Profiles	826.67	30.54	5
	Separate Spline for Each Sequence Day	831.62	35.49	7
	Separate Spline for Each Condition	829.88	33.75	6
	Common Spline for All Profiles – Combo Factor	826.67	30.54	5a
	Common Spline for Each Condition and Sequence Day	834.92	38.79	8
Log IL-1β	Independence – Unbanded	1284.03	2.83	4
	Independence – Banded	1283.20	2.00	2
	Unstructured	1281.20	0.00	1
	Power Model	1283.75	2.55	3
	Linear Trends, No Spline	1323.62	42.42	6
	Common Spline for All Profiles	1323.61	42.41	5
	Separate Spline for Each Sequence Day	1325.04	43.84	8
	Separate Spline for Each Condition	1324.60	43.40	7
	Common Spline for All Profiles – Combo Factor	1323.61	42.41	5a
	Common Spline for Each Condition and Sequence Day	1325.44	44.24	9

D.1.1 Continued

Cytokine	Model	AIC	ΔAIC	Rank
Raw TNF-α	Independence – Unbanded	1807.88	7.02	3
	Independence – Banded	1808.71	7.85	4
	Unstructured	1800.86	0.00	1
	Power Model	1807.14	6.28	2
	Linear Trends, No Spline	1874.33	73.47	9
	Common Spline for All Profiles	1863.88	63.02	5
	Separate Spline for Each Sequence Day	1866.62	65.76	7
	Separate Spline for Each Condition	1865.62	64.76	6
	Common Spline for All Profiles – Combo Factor	1863.88	63.02	5a
	Common Spline for Each Condition and Sequence Day	1867.44	66.58	8
Log IL-4	Independence – Unbanded	1326.18	0.48	2
	Independence – Banded	1325.70	0.00	1
	Unstructured	1334.43	8.73	4
	Power Model	1327.65	1.95	3
	Linear Trends, No Spline	1370.74	45.04	7
	Common Spline for All Profiles	1369.33	43.63	5
	Separate Spline for Each Sequence Day	1372.74	47.04	8
	Separate Spline for Each Condition	1369.86	44.16	6
	Common Spline for All Profiles – Combo Factor	1369.33	43.63	5a
	Common Spline for Each Condition and Sequence Day	1372.74	47.04	8a
Log IL-10	Independence – Unbanded	813.26	14.38	3
	Independence – Banded	814.94	16.06	4
	Unstructured	798.88	0.00	1
	Power Model	805.65	6.77	2
	Linear Trends, No Spline	825.89	27.01	5
	Common Spline for All Profiles	826.08	27.20	6
	Separate Spline for Each Sequence Day	827.60	28.72	8
	Separate Spline for Each Condition	826.66	27.78	7
	Common Spline for All Profiles – Combo Factor	826.08	27.20	6a
	Common Spline for Each Condition and Sequence Day	827.84	28.96	9

AIC = Akaike information criteria

D.1.2 Results of unstructured LMM for log-transformed IL-8 profile

Fixed Effects		**n.d.f**	***F* statistic**	***P***
condition		1	9.39	0.021
seqday		2	0.04	0.964
time		3	16.57	<0.001
condition.day		2	0.11	0.894
condition.time		3	0.54	0.656
day.time		6	0.52	0.789
condition.day.time		6	1.73	0.121
Random Effects	**Parameter**	**Estimate**		**SE**
profile.sample	V_11	0.4852		0.0718
	V_21	0.2750		0.0709
	V_22	0.7607		0.1129
	V_31	0.2865		0.0620
	V_32	0.2905		0.0734
	V_33	0.5205		0.0792
	V_41	0.2249		0.0573
	V_42	0.3209		0.0722
	V_43	0.3192		0.0643
	V_44	0.4751		0.0725

n.d.f = numerator degrees of freedom; SE = standard error

D.1.3 Results of unstructured LMM for log-transformed IL-6 profile

Fixed Effects		**n.d.f**	***F* statistic**	***P***
condition		1	1.87	0.222
seqday		2	3.10	0.050
time		3	24.65	<0.001
condition.day		2	0.19	0.829
condition.time		3	3.72	0.014
day.time		6	7.45	<0.001
condition.day.time		6	1.08	0.377
Random Effects	**Parameter**	**Estimate**		**SE**
profile.sample	V_11	0.5183		0.0787
	V_21	0.3172		0.0706
	V_22	0.6360		0.0963
	V_31	0.2533		0.0564
	V_32	0.3368		0.0657
	V_33	0.4179		0.0621
	V_41	0.3116		0.0658
	V_42	0.3735		0.0748
	V_43	0.3575		0.0628
	V_44	0.5593		0.0823

n.d.f = numerator degrees of freedom; SE = standard error

D.1.4 Pairwise comparison of predicted REML means for logged IL-6 profile between days and within the same condition and time of day

Condition	Time (h)	Comparison	Difference	LSD	*P*	Sig
SR	6.15	Day 1 v Day 2	0.5016	0.6158	0.0927	NS
SR	11.30	Day 1 v Day 2	0.4864	0.6927	0.1354	NS
SR	18.15	Day 1 v Day 2	0.1573	0.5586	0.5136	NS
SR	21.30	Day 1 v Day 2	0.0250	0.6415	0.9266	NS
SR	6.15	Day 1 v Day 3	0.7423	0.6158	0.0259	*
SR	11.30	Day 1 v Day 3	0.2983	0.6921	0.3294	NS
SR	18.15	Day 1 v Day 3	0.0151	0.5545	0.9486	NS
SR	21.30	Day 1 v Day 3	0.0071	0.6415	0.9791	NS
SR	6.15	Day 2 v Day 3	0.2407	0.6214	0.3768	NS
SR	11.30	Day 2 v Day 3	0.1880	0.6869	0.5250	NS
SR	18.15	Day 2 v Day 3	0.1422	0.5586	0.5534	NS
SR	21.30	Day 2 v Day 3	0.0321	0.6415	0.9058	NS
CON	6.15	Day 1 v Day 2	0.2461	0.6037	0.3543	NS
CON	11.30	Day 1 v Day 2	0.9543	0.6666	0.0131	*
CON	18.15	Day 1 v Day 2	0.0093	0.5386	0.9673	NS
CON	21.30	Day 1 v Day 2	0.1051	0.6230	0.6919	NS
CON	6.15	Day 1 v Day 3	0.6677	0.6039	0.0355	*
CON	11.30	Day 1 v Day 3	0.5432	0.6722	0.0948	NS
CON	18.15	Day 1 v Day 3	0.3199	0.5385	0.1944	NS
CON	21.30	Day 1 v Day 3	0.0035	0.6230	0.9893	NS
CON	6.15	Day 2 v Day 3	0.4216	0.6037	0.1371	NS
CON	11.30	Day 2 v Day 3	0.4111	0.6719	0.1833	NS
CON	18.15	Day 2 v Day 3	0.3293	0.5386	0.1836	NS
CON	21.30	Day 2 v Day 3	0.1086	0.6230	0.6822	NS

LSD = least significant differences; NS = not significant * = $P < 0.05$

D.2 Study 2

D.2.1 Summary of fit for REML model for logged cortisol profile

Model	AIC	ΔAIC	Rank
Independence model	1486.52	132.69	6
Power model without spline	1366.91	13.08	2
Power model with common spline for all profiles	1353.83	0.00	1
Power model with separate spline for each sequence day	1401.50	47.67	4
Power model with separate splines for each condition	1370.46	16.63	3
Power model with spline for each combination of condition and sequence day	1442.47	88.64	5

AIC = Akaike information criteria

D.2.2 Summary of fit for REML model for logged cortisol AUC

Model	**AIC**	**ΔAIC**	**Rank**
Identity model	580.86	14.48	3
Banded model	579.70	13.32	2
Unstructured model	566.38	0.00	1

AIC = Akaike information criteria

D.2.3 Summary of fit for REML model for daily HR

Model	**AIC**	**ΔAIC**	**Rank**
Independence model	76565.01	3125.14	2
AR model without spline	73439.87	0.00	1
AR model with common spline for all profiles	77982.30	4542.13	4
AR model with separate spline for each sequence day	77290.17	3850.30	3
AR model with separate splines for each condition	78398.92	4959.05	5
AR model with spline for each combination of condition and sequence day	78445.35	5005.48	6

AIC = Akaike information criteria; AR = autoregressive

D.2.4 Results of LMM for logged cortisol profile

Fixed Effects	**n.d.f**	***F* statistic**	***P***
condition	1	9.35	0.023
seqday	2	4.54	0.013
time	1	984.72	<0.001
condition.day	2	3.68	0.029
condition.time	1	8.2	0.005
day.time	2	5.59	0.004
condition.day.time	2	0.73	0.484
Random Effects	**Estimate**		**SE**
spline(time)	1.0566		0.5926
group	0.0022		0.0065
profile	0.0814		0.0175
profile.sample	0.4291		0.0461

n.d.f = numerator degrees of freedom; SE = standard error

D.2.5 Results of REML model for logged cortisol AUC

Fixed Effects	**n.d.f**	***F* statistic**	***P***
seqday	2	7.35	0.002
condition	1	5.54	0.025
Seqday.condition	2	8.22	0.001
Random Effects	**Parameter**	**Estimate**	**SE**
subject.seqday	V_11	30.53	7.52
	V_21	24.33	6.98
	V_22	33.21	8.18
	V_31	16.72	5.77
	V_32	26.22	6.91
	V_33	26.78	6.59

n.d.f = numerator degrees of freedom; SE = standard error

D.2.6 Results of REML for daily HR

Fixed Effects	**n.d.f**	***F* statistic**	***P***
condition	1	0.29	0.591
seqday	2	0.18	0.834
time	1	19.96	<0.001
condition.day	2	2.83	0.066
condition.time	1	1.24	0.266
day.time	2	5.86	0.003
condition.day.time	2	4.38	0.013
Random Effects	**Estimate**		**SE**
subject.day.time	95.43		1.76

n.d.f = numerator degrees of freedom; SE = standard error

D.3 Study 4

D.3.1 Interactions and slope (b) coefficients for Mood Scale II and cytokine response relationships

Interaction	Condition	Day	Time	Slope (b)
Mood Scale II and Daily Cytokine Profiles				
Condition × Activation for IL-6	SR			0.493
	CON			0.115
Day × Fear for IL-6		1		0.385
		2		0.047
		3		-0.993
Time × Happiness for IL-6			6:15	-0.294
			11:30	-0.033
			18:15	0.438
			21:30	0.084
Condition × Happiness for IL-8	SR			0.841
	CON			0.237
Day × Happiness for IL-8		1		0.841
		2		0.232
		3		-0.125
Day × Activation for IL-1β		1		0.552
		2		0.903
		3		1.803
Day × Fatigue for IL-1β		1		0.077
		2		1.081
		3		0.903
Condition × Happiness for TNF-α	SR			2.688
	CON			-0.257
Day × Happiness for TNF-α		1		2.688
		2		1.428
		3		-0.049
Mood Scale II and Morning Fasting Cytokine Measures				
Condition × Fear for TNF-α	SR		N/A	3.953
	CON		N/A	-0.447

D.3.2 Interactions and slope (b) coefficients for Samn-Perelli Fatigue Scale (both mean and profile) and cytokine and cortisol response relationships

Interaction	Condition	Day	Time	Slope (b)
Samn-Perelli Fatigue Scale Daily Mean and Cytokine Profiles				
Condition × Time × Samn-Perelli Fatigue Scale Daily Mean for IL-6	SR		6:15	0.159
	SR		12:00	0.532
	SR		18:15	0.281
	SR		21:30	0.350
	CON		6:15	0.045
	CON		12:00	0.156
	CON		18:15	0.237
	CON		21:30	0.097
Condition × Samn-Perelli Fatigue Scale Daily Mean for TNF-α	SR			1.710
	CON			0.529
Time × Samn-Perelli Fatigue Scale Daily Mean for TNF-α			6:15	1.710
			12:00	1.675
			18:15	1.092
			21:30	1.547
Condition × Samn-Perelli Fatigue Scale Daily Mean for IL-10	SR			0.037
	CON			-0.292
Samn-Perelli Fatigue Scale Daily Profile and Cortisol AUC				
Condition × Samn-Perelli Fatigue Scale Daily Profile for cortisol AUC	SR		N/A	0.131
	CON		N/A	-0.214
Samn-Perelli Fatigue Scale Daily Mean and Cortisol AUC				
Condition × Samn-Perelli Fatigue Scale Daily Mean for cortisol AUC	SR		N/A	1.275
	CON		N/A	-1.227

D.3.3 Total sleep time (mean ± SD) for each night in both conditions (h)

Night	CON	SR
Pre-study 1	7.3 ± 1.4	6.7 ± 0.9
Pre-study 2	6.7 ± 1.3	6.2 ± 1.4
1 (adaptation)	6.3 ± 0.9	6.4 ± 0.7
2	6.9 ± 0.4	3.6 ± 0.2*
3	6.9 ± 0.5	3.7 ± 0.2*

* = $P < 0.001$ between conditions