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RESEARCH ARTICLE

The Impact of Sleep Restriction and Simulated Physical Firefighting Work on Acute Inflammatory Stress Responses

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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 when 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 immunomodulatory 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 15 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 resulting 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 as 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 atherosclerosis and inflammatory changes that may result in CVD [20]. In a field setting, Main et al. [4] reported increased IL-6 across a shift of physical wildfire work without sleep disruption. However, firefighters’ IL-6 levels, along with IL-1β, IL-8 and IL-4 all exhibited an attenuated response across the second shift, possibly indicative of an adaptation [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, unchanged or fluctuations in 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 immunomodulatory 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 wildfire 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 effects 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 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 firefighter’s 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 fire agencies across Australia were recruited for this study. Participants were screened and excluded from the study if they had been diagnosed with any form of heart disease, diabetes, respiratory or sleep disorders. Participants were then randomly assigned to either a control (CON) or sleep restriction (SR) condition. For purposes of analysis, participants in each condition were then matched for age, sex and body mass index (BMI). There were no differences with regards to age, firefighting experience and BMI between conditions (Table 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 1). 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.

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 data collection 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; Fig 1). Conversely, participants in the SR condition had their bed time delayed, resulting in a 4-h

Table 1. Characteristics of participants in each condition (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CON (n = 18)</th>
<th>SR (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 ± 16</td>
<td>39 ± 15</td>
</tr>
<tr>
<td>Male:Female (n)</td>
<td>15:3</td>
<td>15:2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.1 ± 17.7</td>
<td>93.8 ± 20.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.1 ± 7.7</td>
<td>177.8 ± 7.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8 ± 5.0</td>
<td>29.6 ± 5.5</td>
</tr>
<tr>
<td>Firefighting experience (years)</td>
<td>8.7 ± 9.3</td>
<td>10.2 ± 6.4</td>
</tr>
</tbody>
</table>

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Fig 1. Firefighting work protocol for CON and SR conditions.

sleep opportunity (i.e., 02:00–06:00) on each of the 2 nights (Fig 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 (Fig 1). The testing environment was maintained at moderate temperatures (18–20°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 fluid consumption was recorded, the measurement of coffee and tea (i.e., caffeine) intake has only been reported in the results section. Energy and macronutrient intakes have also been described in the results section below.

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 3 (Fig 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. Participants’ cytokine levels were measured in both conditions at 4 identical time points across each of the 3 testing days (Fig 1).

Simulated physical firefighting work circuit. The physical work circuit comprised 6 simulated wildland firefighting tasks. Each of these tasks was designed to mimic the different physical demands involved in wildfire suppression [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, 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 [39, 40]. However, recent evidence indicates a close correlation between venous and capillary plasma IL-6 responses during or post-exercise [39] and at rest [41]. Conversely, other reports have found that venous and capillary concentrations of TNF-α [40] and IL-6 [39] differed at rest. However, these studies [39, 40] 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 [42]. Control over these factors in the current study limits their confounding influence on cytokine levels at rest.

Participants sampled 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; Fig 1). 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/min 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 micro bead 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.

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 was 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 (min) 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) was also recorded through the use of activity monitors worn on the wrist. Physical activity data (captured in 1-min 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 [43]. 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 homogeneity of variance and normality of the residuals from the resulting mixed model analysis. 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 fluid 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 [44]. 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 [45]. Model fit was assessed by Akaike Information Criterion (AIC) and small differences (AAIC) in this criterion compared to the minimum observed value in a set of candidate models were used to identify parsimonious models [46]. Predicted REML means constructed from the linear models fitted by the REML analysis were calculated and pairwise