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**Title:** Extreme occupational heat exposure is associated with elevated haematological and inflammatory markers in Fire Service Instructors

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**Author Conflict:** No competing interests declared

**Running Title:** Inflammation in Fire Instructors

**Abstract:** Fire Service Instructors (FSI) experience ~10 times more fire exposures than firefighters (FF), the increased physiological stress from this potentially puts them at risk of ill health and future cardiac events. This study aimed to establish whether FSI exhibit elevated biomarkers associated with cardiac event risk, identify if FSI experience systemic inflammation linked to fire exposure frequencies and evaluate a proposed exposure limit of 9 per month. Blood samples were collected from 110 Fire Service personnel (age: 44 {plus minus} 7 yrs; height: 178.1 {plus minus} 7.1 cm; body mass: 84.3 {plus minus} 12.0 kg, FSI n = 53, FF n = 57) for biomarker analysis. Work history details were collected from all participants. Participants with biomarker concentrations above healthy reference ranges were classified as "at risk". Neutrophil/lymphocyte ratio, platelet count, cardiac troponin T, interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ),

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immunoglobulin G (IgG) and C reactive protein (CRP) were greater in FSI than FF ( $p < 0.05$ ). Multiple regression analysis revealed 18.8% of IL-6, 24.9% of IL-1 $\beta$ , 29.2% of CRP, and 10.9% of IgG variance could be explained by number of heat exposures per month. Odds ratios revealed that those above the 9 per month exposure limit were 6-12 times more likely to be classified as "at risk" and were 16 times more likely to experience symptoms of ill health. Increased cytokine levels suggest FSI experience systemic inflammation, which is related to symptoms of ill health. We propose that an exposure limit could reduce the prevalence of these biomarker risk factors and ill health.

**New Findings:** Fire service instructors are frequently exposed to live fire scenarios, representing the most extreme chronic occupational heat exposure. These individuals report a series of unique health issues. This study seeks to identify if the number of exposures completed are associated with inflammatory and immunological markers and symptoms of ill health. Fire service instructors exhibit greater levels of inflammatory markers in comparison to firefighters. Fire exposure numbers are positively related with the prevalence of ill health and inflammation. Implementation of a proposed 9 exposure limit per month may be appropriate to minimise health issues.

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31 **NEW FINDINGS**

32

33 **What is the central question of this study?**

34 Fire service instructors are frequently exposed to live fire scenarios, representing the most extreme chronic  
35 occupational heat exposure. These individuals report a series of unique health issues. This study seeks to  
36 identify if the number of exposures completed are associated with inflammatory and immunological markers  
37 and symptoms of ill health.

38

39 **What is the main finding and its importance?**

40 Fire service instructors exhibit greater levels of inflammatory markers in comparison to firefighters. Fire  
41 exposure numbers are positively related with the prevalence of ill health and inflammation. Implementation of a  
42 proposed 9 exposure limit per month may be appropriate to minimise health issues.

43

44 **ABSTRACT**

45

46 Fire Service Instructors (FSI) experience ~10 times more fire exposures than firefighters (FF), the increased  
47 physiological stress from this potentially puts them at risk of ill health and future cardiac events. This study  
48 aimed to establish whether FSI exhibit elevated biomarkers associated with cardiac event risk, identify if FSI  
49 experience systemic inflammation linked to fire exposure frequencies and evaluate a proposed exposure limit of  
50 9 per month. Blood samples were collected from 110 Fire Service personnel (age:  $44 \pm 7$  yrs; height:  $178.1 \pm 7.1$   
51 cm; body mass:  $84.3 \pm 12.0$  kg, FSI n = 53, FF n = 57) for biomarker analysis. Work history details were  
52 collected from all participants. Participants with biomarker concentrations above healthy reference ranges were  
53 classified as “at risk”. Neutrophil/lymphocyte ratio, platelet count, cardiac troponin T, interleukin-6 (IL-6),  
54 interleukin-1 $\beta$  (IL-1 $\beta$ ), immunoglobulin G (IgG) and C reactive protein (CRP) were greater in FSI than FF  
55 ( $p < 0.05$ ). Multiple regression analysis revealed 18.8% of IL-6, 24.9% of IL-1 $\beta$ , 29.2% of CRP, and 10.9% of  
56 IgG variance could be explained by number of heat exposures per month. Odds ratios revealed that those above  
57 the 9 per month exposure limit were 6-12 times more likely to be classified as “at risk” and were 16 times more  
58 likely to experience symptoms of ill health. Increased cytokine levels suggest FSI experience systemic  
59 inflammation, which is related to symptoms of ill health. We propose that an exposure limit could reduce the  
60 prevalence of these biomarker risk factors and ill health.

61

## 62 INTRODUCTION

63

64 Within the UK Fire and Rescue Service, Fire Service Instructors (FSI) are responsible for the training of newly  
65 recruited and operational firefighters (FF). FSI regularly experience fire scenarios (commonly referred to as  
66 wears) to enable them to deliver training on breathing apparatus use, search and rescue techniques and fire  
67 behaviour. On average FSI report completing ~13 exposures a month, compared to ~1 fire incident attended by  
68 FF in the same period (Watkins, Hayes, Watt, & Richardson, 2018a). During exposures FSI typically experience  
69 maximum core temperatures of 37.9 – 38.5°C, with maximum heart rates of 134 – 147 b.min<sup>-1</sup> (Eglin, Coles, &  
70 Tipton, 2004; Watkins, Hayes, Watt, & Richardson, 2019b; Watt et al., 2016); indicating that these individuals  
71 undergo a moderate level of physiological strain on a regular basis.

72

73 A recent survey of FSI highlighted that 41% reported experiencing new symptoms of ill health since starting  
74 their career as a FSI (Watkins et al., 2018a). Symptoms included: severe fatigue, broken sleep, mood swings and  
75 headaches. Smith et al. (2000) hypothesised that symptoms such as these may be associated with chronic  
76 systemic inflammation when occurring in relation to repeated high volume physical strain without sufficient  
77 rest. However, it is difficult to ascertain whether inflammation is the cause of these symptoms, as there are  
78 limited possibilities to study individuals who experience high levels of strain over long periods of time  
79 (Carfagno & Hendrix, 2014). Previous assessment of military recruits during training courses have identified  
80 symptoms, including sleep disturbances, fatigue and confusion, that coincide with elevated interleukin-6 (IL-6),  
81 tumour necrosis factor alpha (TNF $\alpha$ ) and C reactive protein (CRP) (Booth, Probert, Forbes-Ewan, & Coad,  
82 2006; Gomez-Merino, Chennaoui, Burnat, Drogou, & Guezennec, 2003). The presence of elevated cytokine IL-  
83 6 has also been reported in FSI in comparison to non-fire exposed controls (Watt et al., 2016; Watkins, Hayes,  
84 Watt, & Richardson, 2019a;), although only a small sample of the FSI population have been assessed.

85

86 Inflammation is linked to the presence of atherosclerosis and cardiovascular event risk (Moriya, 2019).  
87 Numerous haematological factors such as IL-6, CRP, and platelet counts (PLT) are involved in the  
88 inflammatory process and are also predictors of cardiovascular event risk (Pearson et al., 2003; Ridker, Rifai,  
89 Stampfer, & Hennekens, 2000; Sharma & Berger, 2011). FSI, along with FF, have an increased risk of a  
90 cardiovascular event following a live fire (Kales et al. 2007), it is also the leading cause of death within FF  
91 (Fahy, Leblanc, & Molis, 2015). Currently, research has focused on the immediate impact that live fire has to  
92 the risk of cardiac events (Fahs et al., 2011; Fernhall, Fahs, Horn, Rowland, & Smith, 2012; Hunter et al., 2017).  
93 Findings by Watt et al (2016) and Watkins et al., (2019b) highlight that FSI exhibit increases in PLT, IL-6, and  
94 cardiac troponin T (cTnT), a marker of cardiac muscle damage, after a training-based fire exposure, however the  
95 long term implications of this acute response are not yet fully understood. It can be theorised that if a live fire  
96 causes an increased risk, with minimal recovery time between fires, this risk may be elevated further by  
97 subsequent exposures if nothing is done to reduce the risk between events. It is therefore of importance to  
98 establish whether the number of fire exposures experienced is linked to an elevation in haematological markers  
99 associated with cardiovascular risk on a daily basis.

100

101 The frequency of wear completion by FSI may be instrumental in the scale and time course of inflammation  
102 development. A survey of FSI highlighted that there is currently no evidenced based guidance on the number of

103 wears a FSI should remain below in any given period (Watkins et al., 2018a). Although 55% of FSI did have a  
104 limit set by management, they varied across services from 2 - 10 per week. In the USA instructors have been  
105 reported to complete 3 -5 live fires per day over a period of several weeks or months (Fent et al., 2019). The  
106 most commonly reported limit to exposure numbers in the UK was 9 per month, which is not a formal policy,  
107 rather one proposed by the UK Chief Fire Officers' Association (2015). Concerns regarding the number of  
108 wears performed was also a key theme when FSI offered their opinions about their working practices, with 35%  
109 of FSI indicating they felt that they completed too many wears. Being able to establish a suitable wear limit that  
110 might minimise the risk of fatigue symptoms occurring and reduce cardiac event risk factors, would enable  
111 evidence based guidelines for FSI across the world. Such information could have the potential to reduce FSI  
112 cardiac events risks and symptoms of ill health and prolong time spent in the career.

113  
114 This study aimed to establish whether FSI exhibit an altered baseline immunological level compared to FF. In  
115 addition, it aimed to identify if altered immunological levels were related to the number of wears completed by  
116 FSI. The final aim of the study was to evaluate a proposed limit of 9 wears a month, to establish if completing  
117 wear numbers above or below this number was associated with immunological markers above upper reference  
118 ranges and reports of ill health. There are therefore four hypotheses within this study: (1) FSI will exhibit greater  
119 IL-6, CRP, cTnT and platelet volume and counts than FF, (2) increased fire exposure numbers will be correlated  
120 with elevated levels of cytokines, (3) those above the 9 wear limit will exhibit cytokine levels above the  
121 reference limits, and (4) there will be an association between immunological markers and reports of ill health.

122

## 123 **METHODS**

124

### 125 *Ethical Approval*

126

127 One hundred and ten individuals (age:  $44 \pm 7$  yrs; height:  $178.1 \pm 7.1$  cm; body mass:  $84.3 \pm 12.0$  kg) were  
128 recruited from Fire and Rescue Services across the UK to participate in the study. Of the 110 recruits, 53 were  
129 FSI (age:  $45 \pm 8$  yrs; height:  $176.6 \pm 8.1$  cm; body mass:  $84.1 \pm 8.1$  kg) and 57 were FF (age:  $44 \pm 7$  yrs; height:  
130  $179.5 \pm 5.8$  cm; body mass:  $84.4 \pm 10.1$  kg). Of the FSI, 47 (89%) were male and 6 (11%) were female. Of the  
131 FF, 55 (96%) were male and 2 (4%) were female. Independent fitness testing was not conducted as part of this  
132 research study, however all participants were operational personnel and therefore were required to meet national  
133 fitness standards. Fitness standards in the UK Fire and Rescue Service state that operational personnel should  
134 maintain a minimum maximal volume of oxygen uptake of  $42 \text{ mL.kg}^{-1}.\text{min}^{-1}$  (Stevenson, Wilsher, & Sykes,  
135 2009), which is assessed in Service by trained staff. All participants took part in physical activity 2 – 3 times a  
136 week, with 32% reporting activity 4 – 5 times a week and 5% performing physical activity >5 times a week.  
137 Participants gave informed written consent and completed a medical questionnaire prior to taking part.  
138 Participants were required to abstain from caffeine and exercise for 12 hours prior to the study commencing,  
139 they were also asked to avoid alcohol for 24 hours prior to taking part. Adherence to these parameters was  
140 checked via a questionnaire. The study was approved by the University of Brighton Research Ethics Committee  
141 (reference number: ESREGC/09/15) and conformed to the Declaration of Helsinki (2013), except for  
142 registration in a database.

143

### ***Experimental Design***

Participants provided a single venous blood sample and working history details on one occasion. This was collected at the Welkin Laboratories, University of Brighton or on location at Fire Stations and Fire Training Centres. Samples were collected from all FSI between 8:00 - 10:00am. Samples were collected from FF between 8:00 - 10:00am or from 5:00 - 7:00pm, depending on their availability due to work requirements and rotas. All samples were collected prior to any heat exposure that day and a minimum of 12 hours post the last fire exposure.

### ***Venous Blood Collection***

A 10mL venous blood sample was taken at rest from the anti-cubital fossa by a trained phlebotomist. All samples were analysed within 2hr of collection for complete blood counts using an automated haematology analyser (XT2000i, Sysmex, UK) and then centrifuged at 4,500 rpm for 10 min at 4°C for plasma separation. Plasma was stored in a -86°C freezer for subsequent ELISA analyses of: IL-6, TNF $\alpha$ , IL1- $\beta$ , CRP (R & D Systems, Minneapolis, USA) and IgG (eBioscience, Thermo Fischer Scientific, Massachusetts, USA). The inter-assay coefficient of variation (CV%) for each ELISA was: 5.5% IL-6, 8.7% TNF $\alpha$ , 11.5% IL-1 $\beta$ , CRP 11.7% and IgG 6.0%. Plasma samples were also analysed for high sensitivity cTnT using an electrochemiluminescence assay [Roche Modular E170 (fifth generation), Basel, Switzerland], which had a blank of 3 ng.L<sup>-1</sup> and CV at the upper reference limit (14 ng.L<sup>-1</sup>) of < 8% (Westermann, Neumann, Sørensen, & Blankenberg, 2017). Upper reference limits were identified for all haematological variables, with limits for variables measured via ELISA analysis increased by the corresponding inter-assay CV. IL-6 and CRP limits represent increased risk of a cardiovascular event. The upper quartile for IL-6 of 2.28pg.mL<sup>-1</sup> was selected as Ridker et al., (2000) identified that those above this value have a 2.3 times higher relative risk of a future myocardial infarction than those in the lowest quartile. For CRP a value of >3 mg.L<sup>-1</sup> was chosen as it represents the high risk category, with a 2 fold increase in relative risk of cardiovascular disease compared to those with < 1.0 mg.L<sup>-1</sup> (Pearson et al., 2003). All other variable limits represent healthy upper reference ranges. Limits and their sources are detailed in Table 2.

### ***Participant Details and Working History***

Participants were asked if in the previous month they had suffered from symptoms of ill health, identified by Watkins et al., (2018a) (fatigue, broken sleep, heavy sweating, heart palpitations, mood swings, coughing, and breathing problems) or any other illnesses. Participants were also asked to provide a brief work history. Details of their time in the Fire and Rescue Service, if they were currently a FSI, and the time they had spent in their current role were collected. Participants also reported how many fire exposures (in either a training or operational capacity) they had completed in the previous week and in the last month.

### ***Statistical Analysis***

Data were analysed using IBM SPSS Statistics 22 and presented as mean  $\pm$  SD, unless otherwise stated. Data were tested for normality and sphericity. Differences in haematological variables between FSI and FF were assessed via independent samples t-tests, or Mann-Whitney U tests when data were not normally distributed.

187 Cohens  $d_s$  effect sizes are presented for t-test comparisons (Lakens, 2013) whilst  $r$  effect sizes are given for  
188 Mann-Whitney U tests (Field et al., 2009), effect sizes are interpreted as recommended by Cohen (1988).  
189 Pearson's chi-squared analysis was conducted to establish if there was an association between occupational  
190 group (FSI vs FF) and occurrence of symptoms of ill health (YES vs NO), with subsequent odds ratios  
191 conducted where significant associations occurred.

192  
193 Multiple regression analysis was conducted to identify the relationship between work history and demographic  
194 variables (BMI, age, time in service, number of wears completed per week, number of wears completed per  
195 month) with haematological dependent variables. Where dependent variables did not meet normality  
196 assumptions bootstrap re-sampling was conducted. Where significant regression models were identified,  
197 regression coefficient statistical significance was interrogated for each predictor variable. Regression analysis  
198 was then rerun with only significant predictor variables to define the model.

199  
200 Haematological markers identified as predicted from exposure numbers had data classified as either above ("at  
201 risk") or below ("healthy") upper reference range values (see Table 2). To evaluate the effectiveness of the  
202 current FSI proposed limit of 9 wears a month, Pearson's chi squared analysis was conducted to identify  
203 associations between those performing  $\leq 9$  or  $> 9$  exposures a month and those in the "at risk" or "healthy"  
204 groups. Pearson's chi squared analysis was also conducted to determine if reference range groups ("at risk" vs  
205 "healthy") were associated with ill health symptoms, with follow up odds ratios conducted for significant  
206 associations. An a priori power analysis was performed using previously reported differences in IL-6 between  
207 FSI and a control group (Watkins et al., 2019a), on the basis of the effect size (Cohen's  $d = 1.11$ ) associated  
208 with those differences and a statistical power of 80% a minimum of 28 participants were required. Due to the  
209 limited previous data available for the association of the specific symptoms of ill health with elevated cytokine  
210 levels, an a priori test based on a chi squared analysis with a medium effect ( $w = 0.3$ ) and 80% power was  
211 conducted, identifying that a minimum of 88 participants were required. Significance level was set at  $p < 0.05$ .

212

## 213 **RESULTS**

214

### 215 *Differences between FSI and FF*

216

217 FSI and FF had similar demographic details (Table 1). FSI had completed a greater number of fire exposures in  
218 the previous week and month ( $p < 0.001$ ) in comparison to FF (Table 1). The maximum number of exposures  
219 completed in a week and month by FSI were 8 and 20, respectively. In total 37 (69%) FSI were within the  
220 suggested limit of  $\leq 9$  wears per month, whilst 16 were not. No FF completed  $> 3$  exposures per month.

221

222 Reports of ill health were made by 3 (5%) FF and 16 (30%) FSI. FF who reported illness all reported colds. Of  
223 the FSI who reported illness, 10 (19%) had severe fatigue, 8 (15%) had a cold, 8 (15%) had broken sleep, 4  
224 (8%) suffered from a cough, 4 (8%) had heavy sweating, 2 (4%) had mood swings, 1 (2%) suffered from heart  
225 palpitations and 1 (2%) had sinusitis. Pearson's chi squared analysis revealed a significant association between  
226 group and presence of illness ( $\chi^2 (1) = 11.941, p = 0.001$ ), with FSI 7.78 (95% CI 2.12 - 28.62) times more likely  
227 to have a symptom of ill health than FF.

228

229 Analysis of CBC between FSI and FF revealed increased levels of NEUT ( $p = 0.029$ ), PLT ( $p < 0.001$ ), BASO  
230 ( $p = 0.003$ ), and neutrophil lymphocyte ratio (NLR) ( $p = 0.044$ ) in FSI compared to FF. FF had increased levels  
231 of EO ( $p = 0.032$ ) than FSI. Analysis of other haematological variables revealed increases in cTnT ( $p < 0.001$ ),  
232 IL-6 ( $p = 0.002$ ), IL-1 $\beta$  ( $p = 0.006$ ), CRP ( $p = 0.005$ ), and IgG ( $p < 0.001$ ) in comparison to FF. See Table 2 for  
233 details of haematological levels.

234  
235  
236

#### *Relationship between Deterministic Variables and Haematological Markers*

237 Multiple regression analysis of haematological variables revealed significant models for IL-6, IL-1 $\beta$ , CRP and  
238 IgG only ( $p < 0.05$ ). For these four haematological markers, age, time in service, and week exposure number  
239 were not predictor variables ( $p > 0.05$ ). Following remodeling with only significant predictors, month exposure  
240 number explained 18.8% of IL-6 variance, 24.9% of IL-1 $\beta$  variance, 29.2% of CRP variance, and 10.9% of IgG  
241 variance (see Figure 1). BMI also explained an additional 3% of CRP variance.

242

#### *“At Risk” Reference Values Associated with Wear Limits and Ill Health*

243  
244  
245 Haematological variables related to monthly wear numbers (IL-6, IL-1 $\beta$ , CRP and IgG) were grouped according  
246 to the upper reference range criteria. Those below the reference limit were classified as the “healthy” group,  
247 whilst those above were classified as the “at risk” group. The current suggested monthly wear limit of 9 wears  
248 was associated with groupings for IL-6, IL-1 $\beta$ , CRP and IgG, see Table 3 for the association statistics and odds  
249 ratios. The 9 wear monthly limit was also associated with the presence of symptoms of ill health ( $\chi^2 (1) =$   
250  $26.803, p < 0.001$ ). Those above the wear limit were 15.74 times (95% CI 2.26 - 32.80) more likely to suffer  
251 symptoms of ill health than those below the limit. The reference limit groupings for IL-6, IL-1 $\beta$ , CRP and IgG  
252 were also associated with the presence of symptoms of ill health, see Table 4 for the association statistics and  
253 odds ratios.

254

## **DISCUSSION**

255

256  
257 This study aimed to evaluate the concentrations of immunological markers related to health problems in FSI and  
258 FF in association with exposure history and the proposed 9 wears per month limit. Differences in numerous  
259 immunological markers were noted between FSI and FF, with FSI having greater levels of biomarkers  
260 associated with future cardiac events, such as NLR, IL-6 and CRP. In addition, monthly wear numbers were  
261 positively related to IL-6, IL-1 $\beta$ , CRP and IgG concentrations. The current proposal of a 9 wear limit may be  
262 reasonable, with those above the limit more likely to be in “at risk” groups for raised IL-6, IL-1 $\beta$ , CRP and IgG.  
263 Moreover, those in “at risk” groups were more likely to experience symptoms of ill health.

264

265 Of the FSI involved in this study, 30% reported symptoms of ill health, compared to 5% of FF. The occurrences  
266 reported are lower than the 41% of FSI and 21% of FF who reported ill health in a previous survey of fire  
267 service personnel (Watkins et al., 2018a). However, that survey referred to frequent symptoms since the  
268 beginning of their job role, whereas this study gathered information of symptoms experienced within the  
269 previous month. The type of symptoms reported in this study are however similar to that previously referred to,  
270 reaffirming the prevalence of a set of symptoms in the FSI population different from their FF counterparts.

271 Comparison of FSI and FF resting haematological variables revealed that NEUT, PLT, BASO, NLR, cTnT, IL-  
272 6, IL-1 $\beta$ , CRP, and IgG were all greater in FSI.

273

#### 274 *Acute Inflammatory and Haematological Changes with Heat Exposure*

275 Numerous previous studies offer an assessment of haematological and inflammatory responses to heat exposure.  
276 Following live fire exposures an increase of 19 – 85% in WBC and 32 – 54% in NEUT have typically been  
277 reported, with extended durations and greater temperatures resulting in larger increases when comparing studies  
278 (Smith, Petruzzello, Chludzinski, Reed, & Woods, 2005; Smith et al., 2011; Watkins et al., 2019b; Watt et al.,  
279 2016). Inflammatory markers such as IL-6 have also been noted to increase by 25 – 27% with live fire exposure  
280 (Walker et al., 2015; Watkins et al., 2019b; Watt et al., 2016).

281

282 The elevation of these markers has been postulated to be stimulated through numerous mechanisms.  
283 Sympathoadrenal activation, as a result of physiological strain, increases circulating catecholamines, which can  
284 reduce the interaction between leukocytes and endothelial cells and consequently cause the demargination of  
285 leukocytes from the vasculature (Shephard, 2003; Smith et al., 2005; Walsh & Whitham, 2006). Tissue damage  
286 can stimulate an inflammatory response, with endothelial cells releasing IL-6 and subsequently stimulating the  
287 release other inflammatory markers, such as C-reactive protein, and leukocyte transmigration as part of the acute  
288 phase response reaction (Bruunsgaard et al., 1997). However, it is now well documented that muscles release  
289 IL-6 during exercise and periods of elevated temperature without the presence of tissue damage (Fischer, 2006;  
290 Petersen & Pedersen, 2005; Welc et al., 2012). Endotoxin release from the gut during exercise with heat stress is  
291 also proposed as a stimulant for inflammation (Snipe, Khoo, Kitic, Gibson, & Costa, 2018; Starkie, Hargreaves,  
292 Rolland, & Febbraio, 2005). Research suggests that whilst these haematological markers may remain altered  
293 compared to baseline in the hours immediately following exposure, by 24 hours they typically have returned to  
294 resting levels (Walker et al., 2015). Consequently additional exposures with minimal recovery could result in  
295 the baseline elevations reported in this study.

296

#### 297 *Cardiovascular Event Risk*

298

299 An increased NLR represents neutrophilia, also indicated by the noted increase in NEUT, in combination with  
300 lymphocytopenia, and is an indicator of systemic inflammation (Guthrie et al., 2013). NLR is known to  
301 increase following acute exercise, remaining elevated but returning towards normal values 6 hrs post exercise  
302 (Nieman, 1998; Nieman, 2000). Neutrophils are involved in all stages of atherosclerosis, they increase the  
303 expression of adhesion molecules, limit vasodilation, and can lead to atherosclerotic plaque instability, making  
304 the plaque prone to rupture (Soehnlein, 2012). In contrast, regulatory T cell lymphocytes are involved in the  
305 inhibition of atherosclerosis by regulating the inflammatory response and therefore low lymphocyte counts  
306 represent a poorly regulated immune response (Shah et al., 2014). Consequently, the combined ratio of these  
307 two white blood cells acts as an independent predictor of cardiac mortality in patients with coronary artery  
308 disease (Papa et al., 2008), those with acute decompensated heart failure (Uthamalingam et al., 2011), patients  
309 with myocardial infarctions (Bhat et al., 2013), and in a healthy general population (Shah et al., 2014). Within a  
310 healthy population, higher NLR is associated with a greater incidence of coronary heart disease related deaths,

311 with an NLR >4.5 associated with 11% of deaths compared to 3.2% of deaths with an NLR of 1.5 – 3.0 and  
312 2.4% of deaths in those with an NLR of <1.5 (Shah et al., 2014). The upper range of NLR in a healthy  
313 population is 3.53, however an NLR >4.5 is associated with a hazard ratio for future cardiac events of 2.68  
314 (Forget et al., 2017; Shah et al., 2014). Fest et al., (2019) also states that being in the 3<sup>rd</sup> (1.60 – 1.91), 4<sup>th</sup> (1.92 –  
315 2.41) and 5<sup>th</sup> (>2.41) quartiles for NLR increased all cause mortality in comparison to the 1<sup>st</sup> quartile (<1.30).  
316 Whilst the exact values attributed to quartiles and high NLR are study dependent, FSI in this study exhibit a  
317 greater NLR (2.12) than FF (1.83), suggesting that FSI may be at a greater risk of a cardiovascular event than  
318 FF.

319

320 Elevated cTnT in FSI indicates possible low level myocardial damage. Baseline resting measurements of cTnT  
321 from healthy populations indicate typical levels of < 10 ng.L<sup>-1</sup> in marathon runners (Neilan et al., 2006;  
322 Richardson et al., 2018) and 6.2 ± 2.2 ng.L<sup>-1</sup> in elite floorball players (Wedin & Henriksson, 2015). This  
323 suggests that cTnT exhibited by FF and FSI are both below the healthy upper limit (< 14 ng.L<sup>-1</sup>) and within the  
324 range of other healthy active population groups. Increased resting levels of cTnT in healthy individuals are  
325 related to left ventricular wall thickening and left ventricular systolic dysfunction, in addition to all  
326 cardiovascular mortality (de Lemos et al., 2010). With each tertile increase of cTnT from non-detectable to > 14  
327 ng.L<sup>-1</sup>, the hazard ratio of cardiovascular mortality increases. Those with cTnT between 3 - 4.4 ng.L<sup>-1</sup> have a  
328 hazard ratio of 1.6 (95% CI 0.5 - 4.9), a cTnT of 4.4 - 6.6 ng.L<sup>-1</sup> suggests a ratio of 2.4 (95% CI 0.9 - 6.1), and  
329 individuals with cTnT 6.6 - 14 ng.L<sup>-1</sup> the hazard ratio of cardiovascular mortality is 4.6 (95% CI 2.1 - 10.0) (de  
330 Lemos et al., 2010). FF consequently have a lower hazard ratio of cardiovascular mortality than FSI, with FF  
331 exhibiting 3.00 ± 1.32 ng.L<sup>-1</sup> and therefore either falling in undetectable levels or within the first detectable  
332 tertile of 3 - 4.4 ng.L<sup>-1</sup>, compared to FSI who exhibited 4.41 ± 2.68 ng.L<sup>-1</sup> giving the average FSI a hazard ratio  
333 of 2.4.

334

335 MPV is also associated with incidence of myocardial infarction and coronary artery disease (Klovaite, Benn,  
336 Yazdanyar, & Nordestgaard, 2011; Sansanayudh et al., 2014). However, MPV displayed no differences between  
337 FSI and FF and although FSI PLT (234 ± 79 x10<sup>9</sup>.L<sup>-1</sup>) was greater than FF PLT (190 ± 35 x10<sup>9</sup>.L<sup>-1</sup>), it remained  
338 below the upper reference range. This is similar to the PLT noted in previous FF studies of 264 ± 53 x10<sup>9</sup>.L<sup>-1</sup>  
339 (Smith et al., 2011), 257 ± 62 x10<sup>9</sup>.L<sup>-1</sup> (Smith et al., 2014) and 241 ± 11 x10<sup>9</sup>.L<sup>-1</sup> (Hunter et al., 2017), and  
340 resting PLT previously reported in FSI (209 ± 43 x10<sup>9</sup>.L<sup>-1</sup>) (Watkins et al., 2019b). Consequently  
341 demonstrating that whilst elevated PLT has been noted post fire exposure (Smith et al., 2011; Hunter et al 2017;  
342 Watkins et al., 2019b), this increase is transient with minimal effect on daily baseline levels.

343

344 A key finding of this study is the significantly elevated levels of inflammatory cytokines IL-6, IL-1β and acute  
345 phase protein CRP, in FSI. IL-6 is involved in the increase of cell adhesion molecules, platelet reactivity and  
346 CRP release, and therefore may be involved in atherosclerosis formation (Lindmark, Diderholm, Wallentin, &  
347 Siegbahn, 2001; Schuett, Luchtefeld, Grothusen, Grote, & Schieffer, 2009). FSI exhibited a mean IL-6 of 1.66 ±  
348 2.29 pg.mL<sup>-1</sup>, in the third quartile of values reported in apparently healthy men (1.47 – 2.28 pg.mL<sup>-1</sup>), such  
349 concentrations have been linked to a 2.8 times higher risk of myocardial infarction (Ridker et al., 2000). IL-6  
350 levels in FSI reported in this study are not as great as those reported at rest by Watt et al (2016) (7.4 – 17.0

351 pg.mL<sup>-1</sup>). However, this present study included FSI with an average of  $6 \pm 5$  exposures a month, whereas FSI  
352 who participated in the study conducted by Watt et al (2016) had completed 15 wears in a 4 week period. The  
353 participants therefore experienced a greater number of exposures than the average experienced by the FSI  
354 involved in this study, further supporting the positive correlation identified between exposure number and IL-6  
355 values.

356

357 IL-1 $\beta$  is also involved in increasing the expression of adhesion molecules, inducing procoagulant activity, and  
358 the stimulation of CRP synthesis and release (Jialal, Devaraj, & Venugopal, 2004; Ridker, Thuren, Zalewski, &  
359 Libby, 2011). Although IL-1 $\beta$  has not yet been established as a predictor of cardiovascular events with set  
360 classifications of risk, perhaps due to the variability in its measurement in plasma (Ridker et al., 2011), gene  
361 polymorphisms causing increased IL-1 $\beta$  are associated with risk of coronary artery disease and cardiovascular  
362 events (Tsimikas et al., 2014). There is some effort to target IL-1 $\beta$  for atheroprotective interventions and there  
363 is some underpinning evidence for its involvement in raised risk (Ridker, 2016). This current study is the first  
364 study to identify elevated IL-1 $\beta$  levels in FSI.

365

366 CRP has also been detected in atherosclerotic lesions (Yasojima, Schwab, McGeer, & McGeer, 2001) and  
367 elevated levels have been reported to correlate with increased adhesion molecules and lower endothelium-  
368 dependent vasodilatory responses (Fichtlscherer et al., 2000; Jialal et al., 2004), although it is unlikely CRP is  
369 causal in these endothelial changes (Danesh & Pepys, 2009; Taylor, Giddings, & Van Den Berg, 2005). Whilst  
370 the mean CRP exhibited by FSI was  $1.62 \pm 2.40$  mg.L<sup>-1</sup>, the variation in levels was high (as demonstrated by the  
371 large SD). Of the FSI, 26.4% of individuals displayed CRP > 3.0 mg.L<sup>-1</sup> in comparison to just 3.5% FF. CRP >  
372 3.0 mg.L<sup>-1</sup> indicates a 2 fold increase in relative risk of cardiovascular disease compared to those with CRP <  
373 1.0 mg.L<sup>-1</sup> (Pearson et al., 2003). The elevated presence of IL-6, IL-1 $\beta$ , and CRP therefore demonstrates FSI  
374 experience chronic systemic inflammation and are at an increased risk of cardiovascular events.

375

### 376 *Wear Limits*

377

378 The measured concentration of IL-6, IL-1 $\beta$ , CRP and IgG were related to the number of wears that had been  
379 completed in the previous month, with 11 - 29% of variance in these markers explained by wear numbers. These  
380 findings support the theory that performing a greater number of fire exposures is associated with increased  
381 cytokine levels. The wear limit informally proposed and followed by some training centres is 9 wears per month  
382 (Watkins et al., 2018a). The evaluation of this limit indicates that those above the limit are more likely to be in  
383 the “at risk” group for IL-6 (OR = 6.27), IL-1 $\beta$  (OR = 7.00), CRP (OR = 12.43) and IgG (OR = 7.55). This  
384 suggests that those FSI conducting a greater number of wears may be at an increased risk of a future  
385 cardiovascular event. Moreover, our data suggests that individuals completing > 9 wears a month are 15.74  
386 times more likely to suffer symptoms of ill health than those below the limit.

387

### 388 *Health Symptoms*

389

390 The “at risk” groups for IL-6, IL-1 $\beta$ , CRP, and IgG were also associated with an increased likelihood of  
391 experiencing symptoms of ill health, such as fatigue, sleep disturbances, headaches, colds and flu like illnesses

392 (IL-6 OR = 5.63, IL-1 $\beta$  OR = 3.67, CRP OR = 6.05, IgG OR = 6.45). These symptoms are comparable to  
393 “sickness behaviours”, such as depression, fatigue and sleep disturbances, which are a consequence of the  
394 ability of inflammatory cytokines to effect central nervous system function. This may occur through numerous  
395 pathways, including: the transportation of cytokines across the blood brain barrier via cytokine receptors,  
396 stimulation of afferent nerves such as the vagus nerve and entering the brain via volume diffuse at  
397 circumventricular organs (Dantzer, O’Connor, Freund, Johnson, & Kelley, 2008; Johnson, 2002). Within both a  
398 military and sporting environment similar findings have been reported, with sleep disturbances, depressed mood  
399 and fatigue occurring with elevated levels of IL-1 $\beta$ , TNF $\alpha$ , CRP and IL-6 following repeated high levels of  
400 physiological strain (Booth et al., 2006; Gomez-Merino et al., 2003). Consequently, this study indicates that the  
401 symptoms reported by FSI may be related to systemic inflammation.

402

403 Cold and cough symptoms reported by FSI also suggest the possibility that immune function may be altered; it  
404 has previously been proposed that following periods of high physical strain humoral immunity is up-regulated  
405 and consequently cellular immunity is suppressed, increasing the risk of viral or bacterial infection (Buyukyazi  
406 et al., 2004; Smith, 2004). An increase in humoral immunity may also be related to an increase in allergies and  
407 immunoglobulins (Smith, 2004). IgG was elevated in FSI ( $1649 \pm 865 \text{ mg.dL}^{-1}$ ) in comparison to FF ( $835 \pm 695$   
408  $\text{mg.dL}^{-1}$ ), possibly indicating increased humoral immunity and inflammation. However, the balance between  
409 cellular and humoral immunity is also carefully controlled by the regulation of specific cytokines. IL-2, IL-12  
410 and interferon gamma (IFN $\gamma$ ) are crucial in the development of Th1 cells that are instrumental in cellular  
411 immunity, and IL-4, IL-5, IL-10, IL-6, and TNF $\alpha$  being involved in the development of Th2 cells that are  
412 involved in humoral immunity (Kidd, 2003; Lucille Lakier Smith, 2003). Whilst the elevated IL-6 in FSI could  
413 support the possible increase in humoral immunity, a larger array of cytokines and other biomarkers should be  
414 investigated in future studies to further understand the balance between humoral and cellular immunity in FSI.

415

#### 416 *Limitations*

417

418 Unlike IL-6, IL-1 $\beta$ , CRP and IgG some haematological variables that were greater in FSI than FF were not  
419 statistically associated with number of wears completed, or with any other demographic or work history details.  
420 In addition, not all variation in IL-6, IL-1 $\beta$ , CRP, and IgG was explained by monthly exposure numbers. It is  
421 reasonable to suggest that there are many other factors that were outside of the scope of this specific study that  
422 may have influenced these variables, such as the overall thermal load experienced from the wears completed,  
423 the type of wears completed (Watkins et al., 2019b), level of smoke/particulate exposure, stress levels of  
424 participants, and FSI use of hydration guidance, clothing (Watkins & Richardson, 2017) and pre/post cooling  
425 interventions (Watkins, Hayes, Watt, & Richardson, 2018b) for each wear performed. Stress, anxiety and  
426 depression have also previously been associated with systemic inflammation (Majd, Saunders, & Engeland,  
427 2019; Renna, O’Toole, Spaeth, Lekander, & Mennin, 2018), but were not measured in this study. It is unknown  
428 if levels of these psychological conditions differ between FF and FSI. Future research should further explore the  
429 influence that other factors may have on systemic inflammation in FSI.

430

431 Participants who reported themselves as FSI were classified as such, regardless of wear exposure number, to  
432 ensure the data represented the FSI population. This resulted in two FSI with no wear completions in the

433 previous month being included in the FSI group, despite them having a lower monthly wear number than the FF  
434 group mean. Due to the logistical difficulty of accessing this population and cost of blood sample analysis this  
435 study offers only a singular snapshot of FSI and FF immunological status. Presence of symptoms of ill health  
436 were also subjective and not verified by a medical professional. Furthermore, there are many risk factors of  
437 cardiovascular events, of which not all were collected in this study. The duration between wears was also not  
438 detailed as part of this study and should be included in future research. Overall, it is suggested that long term  
439 monitoring should be conducted to establish if any additional extraneous variables are associated with  
440 haematological values.

441

## 442 CONCLUSION

443

444 In conclusion, FSI specifically need to be targeted for interventions to help reduce the occurrence of elevated  
445 haematological measures, as findings from this study suggest they are at an increased risk of a future  
446 cardiovascular event and symptoms of ill health compared to FF. Moreover, increased levels of IL-6, IL-1 $\beta$ ,  
447 CRP, and IgG are associated with the number of wears FSI complete in a month. The current suggestion of a 9  
448 wear per month limit seems a reasonable guidance, as those above the limit are 15.74 times more likely to  
449 experience symptoms of ill health. Future research should investigate the impact that interventions designed to  
450 reduce the thermal load from frequent fire exposures have on the chronic systemic inflammation prevalent in  
451 FSI.

452

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676 **COMPETING INTERESTS**

677 The authors declare that they have no competing interests.

678

679 **AUTHOR CONTRIBUTIONS**

680 Blood samples were analysed at the Centre for Sport, Exercise & Life Sciences, Coventry University and the  
681 Environmental Extremes Laboratory, University of Brighton. EW, MH, PW and AJ were involved in the  
682 conception and design of the work. EW, MH, PW, DR and AJ contributed towards the acquisition, analysis and  
683 interpretation of the data. EW, MH, PW, DR and AJ drafted the work or revised it critically for important  
684 intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all  
685 aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are  
686 appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those  
687 who qualify for authorship are listed.

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692

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696

697

698 **TABLES**

699

700 **Table 1** Demographic and work history details for firefighters and fire service instructors. <sup>a</sup> denotes values  
701 displayed are median  $\pm$  IQR. \* denotes a difference between the two groups,  $p < 0.05$ .

	<b>Fire Service Instructors</b>	<b>Firefighters</b>
<b>Age (yrs)</b>	45 $\pm$ 8	44 $\pm$ 7
<b>Body mass (kg)</b>	84.1 $\pm$ 13.8	84.4 $\pm$ 10.1
<b>BMI (kg.m<sup>2</sup>)</b>	26.5 $\pm$ 5.1 <sup>a</sup>	25.4 $\pm$ 3.5 <sup>a</sup>
<b>Time in Service (yrs)</b>	19 $\pm$ 8	17 $\pm$ 7
<b>Time in role (yrs)</b>	3 $\pm$ 8 <sup>a</sup> *	10 $\pm$ 10 <sup>a</sup>
<b>Weekly Exposures</b>	1 $\pm$ 3 <sup>a</sup> *	0 $\pm$ 0 <sup>a</sup>
<b>Monthly Exposures</b>	5 $\pm$ 8 <sup>a</sup> *	1 $\pm$ 1 <sup>a</sup>

702

703

704 **Table 2** Resting levels of haematological variables in FSI and FF. <sup>a</sup> denotes values displayed are median  $\pm$  IQR.  
705 Effect size is given as  $d_s$ , unless the variable is presented as median  $\pm$  IQR, whereby the corresponding effect  
706 size is  $r$ . \* denotes a difference between groups,  $p < 0.05$ . † denotes median value is greater than upper reference  
707 value. Upper limit for WBC, RVC, HGB, HCT, NEUT, LYMPH, MONO, EO and BASO from Bain et al.  
708 (2011), PLT and MPV from Briggs et al. (2007), NLR from Forget et al. (2017), cTnT from Zhelev et al.  
709 (2015), IL-6 from Ridker et al. (2000), TNF $\alpha$  from Todd et al. (2013), IL-1 $\beta$  from Di Iorio et al. (2003) and La  
710 Fratta et al. (2018), CRP from Pearson et al. (2003) and IgG from Fuggle (2017).

	<b>Fire Service Instructors</b>	<b>Firefighters</b>	<b>Effect Size</b>	<b>Upper Reference Limit</b>
<b>WBC</b> ( $\times 10^9.L^{-1}$ )	6.56 $\pm$ 2.10 <sup>a</sup>	6.47 $\pm$ 1.73 <sup>a</sup>	0.09	10.0
<b>PLT</b> ( $\times 10^9.L^{-1}$ )	234 $\pm$ 79 <sup>a</sup> *	190 $\pm$ 35 <sup>a</sup>	0.49	400
<b>MPV</b> (fL)	10.0 $\pm$ 0.9	10.3 $\pm$ 0.7	0.37	11.2
<b>NEUT</b> ( $\times 10^9.L^{-1}$ )	3.85 $\pm$ 1.20 <sup>a</sup> *	3.54 $\pm$ 1.18 <sup>a</sup>	0.18	7.0
<b>LYMPH</b> ( $\times 10^9.L^{-1}$ )	1.97 $\pm$ 0.52	1.97 $\pm$ 0.52	0	3.0
<b>MONO</b> ( $\times 10^9.L^{-1}$ )	0.59 $\pm$ 0.26 <sup>a</sup>	0.57 $\pm$ 0.19 <sup>a</sup>	0.01	1.0
<b>EO</b> ( $\times 10^9.L^{-1}$ )	0.13 $\pm$ 0.15 <sup>a</sup> *	0.18 $\pm$ 0.13 <sup>a</sup>	0.20	0.5
<b>BASO</b> ( $\times 10^9.L^{-1}$ )	0.05 $\pm$ 0.05 <sup>a</sup> *	0.03 $\pm$ 0.03 <sup>a</sup>	0.28	0.1
<b>NLR</b>	2.12 $\pm$ 0.84*	1.83 $\pm$ 0.63	0.19	3.53
<b>cTnT</b> (ng.L <sup>-1</sup> )	4.41 $\pm$ 2.68 <sup>a</sup> *	3.00 $\pm$ 1.32 <sup>a</sup>	0.40	14
<b>IL-6</b> (pg.mL <sup>-1</sup> )	1.66 $\pm$ 2.26 <sup>a</sup> *	0.93 $\pm$ 1.29 <sup>a</sup>	0.28	2.41
<b>TNF<math>\alpha</math></b> (pg.mL <sup>-1</sup> )	3.31 $\pm$ 5.42 <sup>a</sup>	2.56 $\pm$ 2.40 <sup>a</sup>	0.14	3.59
<b>IL-1<math>\beta</math></b> (pg.mL <sup>-1</sup> )	2.50 $\pm$ 9.99 <sup>a</sup> *†	0.00 $\pm$ 2.35 <sup>a</sup>	0.24	1.00
<b>CRP</b> (mg.L <sup>-1</sup> )	1.62 $\pm$ 2.40 <sup>a</sup> *	0.77 $\pm$ 0.88 <sup>a</sup>	0.13	3.35
<b>IgG</b> (mg.dL <sup>-1</sup> )	1649 $\pm$ 865 <sup>a</sup> *	835 $\pm$ 695 <sup>a</sup>	0.49	1696

711 *WBC = white blood cell count, PLT = platelet count, MPV = mean platelet volume, NEUT = neutrophil count,*  
712 *LYMPH = lymphocyte, MONO = monocyte, EOS = eosinophil, BASO = basophil, NLR = neutrophil*  
713 *lymphocyte ratio, cTnT = cardiac troponin T, IL-6 = interleukin-6, TNF $\alpha$  = tumour necrosis factor alpha, IL-1 $\beta$*   
714 *= interleukin-1 beta, CRP = C reactive protein, IgG = immunoglobulin G.*

715

716 **Table 3** Association between monthly wear limit ( $\leq$  or  $>$  9 wears per month) and participants IL-6, IL-1 $\beta$ , CRP  
 717 and IgG above or below upper reference levels. Odds ratios based on likelihood of those completing  $>$ 9 wears  
 718 per month exhibiting haematological levels above reference limits. \* denotes significant association ( $p < 0.05$ ).

	Chi squared	<i>p</i> value	Odds Ratio (95% CI)
IL-6	11.981	0.002 *	6.27 (2.04 – 19.3)
IL-1 $\beta$	7.784	0.004 *	7.00 (1.51 – 32.51)
CRP	21.023	0.001 *	12.43 (3.57 – 43.22)
IgG	14.563	0.001 *	7.55 (2.41 – 23.61)

719 *IL-6 = interleukin-6, IL-1 $\beta$  = interleukin-1 beta, CRP = C reactive protein, IgG = immunoglobulin G*

720

721 **Table 4** Association between haematological group (above or below reference limits) with the presence of  
722 symptoms of ill health. Odds ratios based on the likelihood of those above reference limits exhibiting symptoms  
723 of ill health. \* denotes significant association ( $p < 0.05$ ).

	Chi squared	<i>p</i> value	Odds Ratio (95% CI)
IL-6	11.695	0.002 *	5.63 (1.96 – 16.20)
IL-1 $\beta$	5.131	0.040 *	3.67 (1.13 – 11.90)
CRP	10.507	0.004 *	6.05 (1.86 – 19.72)
IgG	13.792	0.001 *	6.45 (2.24 – 18.58)

724 *IL-6 = interleukin-6, IL-1 $\beta$  = interleukin-1 beta, CRP = C reactive protein, IgG = immunoglobulin G*

725

726 **FIGURES**

727

728 **Figure 1** IgG (A), IL-1 $\beta$  (B), IL6 (C) and CRP (D) plotted against monthly wear number. Green zone  
729 represents the “healthy” range for each variable, as identified in Table 2. Vertical grey dashed line represents the  
730 9 exposure limit. IL-6 = interleukin-6, CRP = C reactive protein, IL-1 $\beta$  = interleukin-1 beta, IgG =  
731 immunoglobulin G.

732

