

# Medicine & Science IN Sports & Exercise

The Official Journal of the American College of Sports Medicine

www.acsm-msse.org

**. . . Published ahead of Print**

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Accepted for Publication: 14 November 2019

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# Smoking and Biochemical, Performance, and Muscle Adaptation to Military Training

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This research was funded by the Army Recruiting and Training Division (UK Ministry of Defence: Army). All authors declare that they have no conflict of interest. The results of the present study do not constitute endorsement by ACSM. The results of this study are presented clearly, honestly and without fabrication, falsification or inappropriate data manipulation.

## ABSTRACT

**Purpose:** To determine whether physical performance adaptation is impaired in smokers during early stages of military training, and to examine some of the putative mechanistic candidates that could explain any impairment. **Methods:** We examined measures of oxidative stress (malondialdehyde (MDA), lipid hydroperoxides), inflammation (C-reactive protein (CRP), interleukin-6), antioxidants (Vitamins A, E and carotenes) and hormones (cortisol, testosterone, insulin-like growth factor-1) in 65 male British Army Infantry recruits (mean  $\pm$  SD age:  $21 \pm 3$  yr; mass:  $75.5 \pm 8.4$  kg; height:  $1.78 \pm 0.07$  m) at week 1, week 5 and week 10 of basic training. Physical performance (static lift, grip strength, jump height, 2.4 km run time and two-minute press up and sit up scores) was examined and lower-leg muscle and adipose cross-sectional area (CSA) and density measured by peripheral Quantitative Computed Tomography. **Results:** Basic Military training, irrespective of smoking status, elicited improvement in all physical performance parameters (main time effect;  $P < 0.05$ ) except grip strength and jump height, and resulted in increased muscle area and decreased fat area in the lower leg ( $P < 0.05$ ). MDA was higher in smokers at baseline, and both MDA and CRP were greater in smokers during training (main group effect;  $P < 0.05$ ), than non-smokers. Absolute performance measures, muscle characteristics of the lower leg and other oxidative stress, antioxidant, endocrine and inflammatory markers were similar in the two groups. **Conclusions:** Oxidative stress and inflammation were elevated in habitual smokers during basic military training, but there was no clear evidence that this was detrimental to physical adaptation in this population over the timescale studied.

**Key words:** SMOKING, OXIDATIVE STRESS, INFLAMMATION, HORMONES, ARMY, FITNESS.

## INTRODUCTION

Basic military training is an intense process of physical fitness development involving arduous and often unaccustomed exercise, and results in a high rate of drop-out (1). Cigarette smoking is widely reported as an independent risk factor for training-related injury (2–4) and is associated with poorer physical fitness and training outcomes in military populations (5–7). Importantly, smoking prevalence in the military is typically higher than in the general population (8, 9). To date several studies have examined rate of physical fitness development in smokers and non-smokers, which have suggested both similar (10) and poorer (11) improvement in performance in smokers in a military training population. The mechanisms that might be responsible for attenuated adaptation are unclear.

Cigarette smoking can influence an array of physiological functions and processes, whereby habitual smokers typically possess chronic elevations in oxidative stress and depleted antioxidant reserve or capacity (12–14), low-grade systemic inflammation (15) and altered immune and endocrine function (16). Collectively, this could hinder a pro-adaptive response during a physical training programme. Oxidative stress is an imbalance in the cellular environment that favours oxidant production which can be harmful to cell membranes, DNA and functional components of cells via lipid peroxidation, and is induced in lung tissue by the constituents of tobacco smoke (17). This results in the circulatory appearance of end-products of lipid peroxidation, such as malondialdehyde (MDA), and downstream effects on further markers of oxidative stress and pro-inflammatory mediators interleukin (IL)-6 and the acute phase protein CRP (C-Reactive Protein) (14, 18–20). Oxidative stress and inflammation exacerbate one another (14) and prolonged systemic levels have been associated with chronic inflammatory

disease and mechanisms of muscle atrophy (20). In skeletal muscle, mediation of oxidative stress and inflammation at transient low levels can be beneficial as part of adaptive and/or homeostatic processes and are key for optimal muscle function and cell signalling (21, 22). Prolonged elevation, however, has the potential for maladaptive effects on muscle via inhibition of anabolic signalling and muscle protein synthesis (20, 23) and from oxidative damage via restricted modulation of redox balance (17, 24).

Through the indirect actions of nicotine on endocrine glands, and via signalling from elevated inflammation, habitual smoking also modulates secretion and suppression of several stress hormones and circulatory growth factors. Prolonged elevation of IL-6 has been associated with elevated cortisol and decreased insulin-like growth factor (IGF)-1, which is a key initiator in the signalling pathways for muscle protein synthesis (20, 23, 25). Moreover, the presence of nicotinic binding sites in the hypothalamus has been implicated as a mechanism by which smoking (via corticotrophin releasing hormone and adrenocorticotrophic hormone) might directly increase cortisol secretion from the adrenal gland (16). Basal concentrations of circulating hormones, by contributing to the mediation of physiological and metabolic processes, have been suggested to impact upon growth and development such as maturation, and physical recovery during consecutive days of exercise training (26, 27). Based on these observations, it is proposed that chronic oxidative stress, inflammation and hormone dysfunction normally observed in habitual smokers might disrupt the adaptive response to long-term training. This could also, at least partially, explain the poorer physical fitness and increased risk of injury previously observed in smokers in military training environments.

To prepare recruits for the physically demanding role of a soldier, British Army initial training is arduous and contains a large variety of fitness training. Tasks essential to occupational performance of a soldier include moving quickly over varied terrain while wearing heavy loads, dragging casualties and manually carrying equipment (28). These occupational capabilities require a balance of strength, power, and cardiorespiratory and muscular endurance leading to their correlation with performance on a variety of practicable tests such as jump tests, grip- and lift- strength tests and timed-runs (28). Alongside improvement in fitness, the high volume of running, load carriage and physical work inherent to this training would be expected to result in reduced whole-body adiposity, skeletal muscle development and greater fat-free mass, particularly of the lower limbs.

We hypothesised that, in comparison to non-smokers, smokers would exhibit a) less performance improvement and lower leg muscle adaptation and b) increased markers of oxidative stress and systemic inflammation, decreased markers of antioxidant availability and altered hormone concentrations during the initial phase of British Army infantry training.

## **MATERIALS AND METHODS**

**Participants.** Participants were recruited from three platoons commencing the Combat Infantryman's Course (CIC) at the Infantry Training Centre, Catterick (ITC(C)), UK. The platoons were selected from the same training regiment in consecutive intakes to ensure training schedules were identical and would fit within research timescales. Each platoon of prospective participants were given a full written and verbal brief without the presence of military staff or a member of chain of command. Participants were informed that participation in the research was

voluntary and would not in any way affect their military careers. Both of these measures were taken in order to reduce the likelihood of exerting undue pressure to participate. Inclusion criteria were that participants were commencing week 1 of the CIC, and therefore had already passed British Army selection and medical screening, and that they completed military training up until the end of the data collection period. Participants gave written informed consent to take part in the study. During the investigation, participants followed the standard line-infantry training syllabus, which was not affected by data collection. The study was approved by the Ministry of Defence Research Ethics Committee (Protocol Ref - 0824/179).

**Military Training.** The CIC is the 26-week training course for entry into the British Army Infantry. British Army recruits are housed at ITC(C) and are not permitted to leave camp except on military business until the end of the first 6 weeks where they have one weekend off-site. Recruits are permitted to smoke during the CIC and on-site at ITC(C), within the normal restrictions of the UK-wide smoking ban applying to all enclosed work places.

Physical training during the CIC typically consists of sessions of between one and three hours, three-four times per week, containing endurance-based running or marching on variable outdoor terrain while carrying external loads, military drill tasks and/or high-intensity circuit training. One of the key aims of the first 10 weeks of the CIC syllabus is to develop physical fitness of recruits in preparation for later phases of the course and, as such, contains the highest frequency of progressive physical training. For this reason, it was hypothesised that examining this 10-week period would capture the greatest magnitude of adaptive change in recruits. As such, excluding performance in the British Army physical fitness test, which is completed at

weeks 1, 14 and 24, all variables were monitored up to week 10 of basic training. It was not possible to schedule time in the CIC syllabus for our physical performance testing in week 14 but since the British Army physical fitness test is not completed at week 10, the week 14 time-point is included solely for this fitness parameter.

**Military Pre-training Lifestyle Questionnaire.** Participants completed the Military Pre-training Questionnaire (MPQ) in week 1. The MPQ has previously been tested for reliability and validity (29), and recorded details on current smoking status, smoking history and smoking behaviour prior to joining the army. Respondents were also asked to rate their physical activity prior to entry to training relative to men of the same age from 1 (much less active) to 5 (much more active). Current smokers were defined as those who smoked at least one cigarette or “roll-up” per day and non-smokers were defined as those who had either never smoked a cigarette or who did not smoke currently and had never smoked cigarettes regularly (where ‘regularly’ is defined as  $\geq 1$  cigarette/roll-up per day). Exclusion criteria were if respondents were defined as “occasional smokers” ( $< 1$  cigarette/roll-up per day) or “former smokers” (previously a regular smoker). A shortened version of the MPQ, with only the smoking-related questions, was administered at week 10 of training to confirm that participants’ smoking status had not altered during the study. Participants who failed to answer all appropriate questions, gave conflicting answers or altered smoking status during training could not be characterised into a group and were not included in analysis.

**Anthropometric and Physical Performance Testing.** In weeks 1 and 10 of training, anthropometric and performance data were collected. Body mass and height were measured

using a set of weighing scales (Seca, Hamburg, Germany) and a stadiometer (Leicester, UK), respectively, in participants wearing shorts and a t-shirt. Body fat percentage was estimated using measurements of skin fold thickness (30) on four sites of the upper body (Biceps brachii, triceps brachii, sub-scapular and supra-iliac) using callipers (Holtain LTD. Crymych, UK). To assess changes in localised body composition, muscle and fat cross sectional area (CSA), density and fat-to-muscle CSA ratio of the dominant lower leg for each individual was measured in weeks 1 and 10 using peripheral quantitative computed tomography (pQCT; XCT2000L, Stratec Pforzheim, Germany). Participants were seated comfortably and asked to remain still with their lower leg placed inside the scanning cylinder for the duration of the scan (~10 min). Muscle and fat CSA and density were determined at 66% of tibial length (distance from the distal aspect of medial malleolus to the medial joint line) and analysed using manufacturer's software (Stratec, Pforzheim, Germany).

As indicators of maximal strength, peak isometric hand-grip and static lift strength (SLS) were measured using portable dynamometers (Takei, Japan). For SLS, the dynamometer is integrated into a baseplate with a height-adjustable handle. Participants were required to take a double overhand grip on the handle and position themselves with a hip-width stance and bent knees in a "power" position, similar to the second pull of a clean. A researcher adjusted the height of the handle to be above the knee and below mid-thigh. Participants were then instructed, while maintaining a straight back, to pull upwards as forcefully as possible for approximately five seconds. For hand-grip, participants were instructed to adjust the hand dynamometer to their hand size and, with their arm extended down by their side, grip as forcefully as possible for approximately five seconds. For SLS, two attempts were completed and for hand-grip strength

two attempts were completed with each hand. Participants were given three attempts to record maximum vertical counter-movement jump performance which was measured using a jump mat that calculated jump height from flight time using integrated software (FSL, UK). Participants were instructed to take a shoulder-width stance on the mat and, while keeping their hands on their hips, squat down and immediately extend the legs to jump as high as possible. The British Army physical fitness test consisted of a timed best-effort 2.4 km run and the maximum number of press ups and sit ups completed in 2 minutes for each exercise. Participants who did not complete every test within each stage of physical performance data collection were excluded from analysis for that exercise.

**Blood Samples.** Fasted blood samples were obtained by venepuncture from an antecubital vein using a needle and Vacutainer system (BD Diagnostics, Becton, Dickinson & Co.) upon waking (0500-0600) in weeks 1, 5 and 10 of training. Participants abstained from smoking overnight until after blood sample collection. Blood samples (20 mL) were collected at rest within 30 minutes of waking using plain untreated tubes and tubes containing EDTA to collect serum and plasma, respectively. After centrifugation (10 mins, 2000 RPM, 4°C), all samples were stored at -80°C until subsequent analysis.

**Sample analysis.** For oxidative stress, systemic marker MDA was determined in serum following the HPLC method described previously (31). This method was based on the derivatisation of MDA using 2-thiobarbituric acid (TBA), leading to the formation of the fluorescent MDA-TBA complex. Lipid hydroperoxides (LOOH) were determined in serum

following the method described previously (32) based on the measure of the ferric-xylenol orange complex in a perchloric acid medium (PCA-FOX assay).

Antioxidant parameters (Vitamin A, Vitamin E ( $\alpha$ -tocopherol) and carotenes) were determined in serum. After deproteinisation with ethanol containing 0.2% BHT, liposoluble vitamins A and E, and carotenes were extracted using n-hexane (33). The n-hexane extract was dried under a nitrogen current and re-dissolved in ethanol. An aliquot of the ethanolic solution was injected in the HPLC system with a diode array detector and a Nova Pak, C18, 3.9x150 mm column. The mobile phase consisted of 550:370:80 acetonitrile:tetrahydrofuran:H<sub>2</sub>O. Vitamin A was determined at 330 nm,  $\alpha$ -tocopherol at 280 nm, and  $\beta$ -carotene, lycopene, cryptoxanthin and lutein/zeaxanthin were determined at 460 nm.

For inflammatory markers, commercially-available enzyme immunoassays were used to measure serum concentrations of inflammatory cytokine IL-6 (Sensitivity 0.04 pg.mL<sup>-1</sup>; CV 7.4%; R&D Systems Inc., Abingdon, UK) and CRP (Sensitivity 1.6 ng.mL<sup>-1</sup>; CV 2.8%; Diagnostic Systems Laboratories Inc., Webster, Texas, USA). Serum Alanine Transaminase (ALT) was measured by commercial assay (Sensitivity 3.44 U.L<sup>-1</sup>; CV 1.59%; Randox Laboratories, NI) using an automated spectrophotometer (COBAS, Roche Diagnostics Limited) to assess liver health which could alter the production of inflammatory markers.

Endocrine markers total testosterone (Sensitivity 0.030 ng.mL<sup>-1</sup>; CV 3.3%; R&D Systems Inc., Abingdon, UK), IGF-1 (Sensitivity 0.01 ng.mL<sup>-1</sup>; CV 6.5%; Diagnostic Systems Laboratories Inc., Webster, Texas, USA) and cortisol (Sensitivity 2.46 ng.mL<sup>-1</sup>; CV 2.57%; IBL

International, Hamburg, Germany) were determined by enzyme immunoassay in plasma. All standards and samples were analysed in duplicate apart from LOOH which were analysed in triplicate. Samples that were measured to be outside of assay standard curve range on first analysis were diluted and reanalysed to verify values.

**Statistical Analysis.** An a priori power calculation was performed (G\*Power: Version 3.0.10) for a two-group, repeated measures design assuming a small-moderate effect of smoking or time ( $f = 0.25$ ). A requirement for 18 participants per group was estimated to achieve sufficient power with statistical significance defined as  $P \leq 0.05$ . Statistical analyses were performed using SPSS software (SPSS for Windows: Version 23.0). To account for the possible influence of body size on several physical performance measures (SLS, jump height, run time) these were normalised by body mass and both absolute and normalised data are presented. To identify whether differences were present between groups before training commenced, independent t-tests were performed on all baseline measurements. Unlike a randomised group design with pre-intervention tests, smokers and non-smokers are not randomly assigned and many characteristics and lifestyle behaviours in habitual smokers will be influenced (an unknown magnitude), directly or indirectly, by smoking prior to training. This means that any variance at baseline maintains the ecological validity of examining recruits entering the training environment, and it would be inappropriate to include any adjustment using pre-values during analysis. As such, to detect statistical differences between smokers and non-smokers across all time points, all biochemical and performance variables were analysed by two-way mixed model analysis of variance (ANOVA). Post-hoc analysis with bonferroni adjustment was used to determine the location of variance in the event of a significant group, interaction or training

effect when analysing more than two time points. An alpha level of  $P \leq 0.05$  was used to define the statistical significance of within- or between- subject effects. In addition, where effect sizes would be of interest, Hedge's G effect sizes ( $g$ ) were also calculated, where small, moderate and large effects were defined as 0.3, 0.5 and 0.8, respectively. Population characteristics are presented as mean  $\pm$  SD. Unless otherwise stated, all other data are presented as mean  $\pm$  SE.

## RESULTS

**Participants.** Sixty-five male recruits (mean ( $\pm$ SD) age:  $21 \pm 3$  yr; mass:  $75.5 \pm 8.4$  kg; height:  $1.78 \pm 0.07$  m) completed the study, and comprised 24 non-smokers and 41 smokers. Cigarette consumption in the smoking group was a mean of  $13 \pm 6$  cigarettes per day for an average of  $7 \pm 5$  years and all participants remained the same smoking status throughout the study. Rating of physical activity relative to peers prior to training was not significantly different between non-smokers ( $3.74 \pm 1.29$ ) and smokers ( $3.31 \pm 1.20$ ) in this population ( $P = 0.19$ ). Blood samples were obtained for all 65 recruits and complete anthropometric and performance data were obtained for 46 recruits (22 non-smokers, 24 smokers). The reduced sample size of  $n=46$  for these outcomes was the product of a specific platoon of participants being unable to attend the physical performance testing in week 10 of training and not from drop out from the study or from military training.

**Anthropometric and Physical Performance Data.** No significant baseline differences, main group or interaction effects were observed between non-smokers and smokers in any anthropometric variable or lower leg muscle characteristic ( $P > 0.05$ ; Table 1). Irrespective of smoking status, estimated body fat percentage ( $P < 0.001$ ) decreased from baseline during

training while body mass remained unchanged ( $P = 0.9$ ). In both groups, between weeks 1 and 10, lower leg mean muscle CSA and total density of muscle and fat increased ( $P < 0.001$ ), fat and muscle CSA ratio decreased ( $P = 0.01$ ) and fat CSA did not change ( $P = 0.1$ ; Table 1).

Irrespective of group, performance in static lift strength, press ups, sit ups and 2.4 km run improved from baseline ( $P < 0.001$ ; Table 2), and these effects remained present for parameters also normalised for body mass ( $P < 0.001$ ). Smoking status had no effect on baseline performance, or improvement in absolute performance, in any physical performance test ( $P > 0.05$ ; Table 2). Though not significant, there was a moderate effect of smoking on run performance where non-smokers tended to exhibit a greater improvement than smokers over 14 weeks ( $P = 0.067$ ,  $g = 0.60$ ) which, when normalised to body mass, resulted in a significant interaction term ( $P = 0.023$ ; Table 2).

**Blood Biochemistry.** MDA concentrations were higher in smokers than non-smokers both at baseline ( $P = 0.02$ ) and overall (Main group effect:  $P = 0.03$ ; Figure 1). Independent of smoking status, MDA was significantly lower in week 10 relative to baseline ( $P = 0.01$ ; Figure 1) but no significant training or group effects were observed on LOOH ( $P > 0.05$ ). Antioxidant variables did not differ between groups ( $P > 0.05$ ) but temporal changes in Vitamin A, Lycopene,  $\beta$ -Carotene, and Lutein and Zeaxanthin occurred, irrespective of smoking status, and are presented in the supplemental table ( $P < 0.05$ ; see Table, Supplemental Digital Content, Antioxidants in weeks 1, 5 and 10 (n=61) organised by smoking status, <http://links.lww.com/MSS/B841>).

Serum CRP concentrations were not significantly different between groups at baseline ( $P = 0.6$ ) but were higher in smokers overall (Main group effect:  $P = 0.047$ ; Figure 1). However, this did not result in an interaction effect and CRP, irrespective of smoking status, was not significantly affected by training ( $P > 0.05$ ). ALT activity did not differ by group ( $P = 0.08$ ), but was increased in week 5 ( $31 \pm 2 \text{ U}\cdot\text{L}^{-1}$ ) compared to weeks 1 ( $22 \pm 1 \text{ U}\cdot\text{L}^{-1}$ ) and 10 ( $25 \pm 2 \text{ U}\cdot\text{L}^{-1}$ ;  $P < 0.001$ ). However, there were no significant between, or within, group effects on IL-6 concentrations or during training ( $P > 0.05$ ; Figure 1).

There were no baseline or between-group differences in hormone concentrations ( $P > 0.05$ ), but training elicited significant temporal effects independent of smoking status ( $P < 0.05$ ; Table 3). This was such that, across groups, IGF-1 declined from baseline ( $268 \pm 10 \text{ ng}\cdot\text{mL}^{-1}$ ) to week 5 ( $246 \pm 8 \text{ ng}\cdot\text{mL}^{-1}$ ;  $P = 0.006$ ) and cortisol displayed lower concentrations in week 10 ( $133 \pm 6 \text{ ng}\cdot\text{mL}^{-1}$ ) than in week 5 ( $146 \pm 4 \text{ ng}\cdot\text{mL}^{-1}$ ;  $P = 0.005$ ). Testosterone, however, remained unchanged by training ( $P = 0.06$ ).

## DISCUSSION

This study was the first to investigate differences in biochemical markers between smokers and non-smokers during arduous military training alongside measures of physical performance and objective measures of changes in muscle size and density. The main findings of the study were that, as hypothesised, oxidative stress marker MDA was higher in smokers than non-smokers and, during training, coincided with elevated CRP, but surprisingly there was little evidence that this adversely impacted performance improvement. Smoking status had no effect on baseline concentrations of the other measured endocrine, antioxidant or inflammatory

parameters, and temporal changes during training were similar in both smokers and non-smokers. Furthermore, basic military training elicited significant favourable alterations in body composition, physical performance and lower-leg muscle adaptation, irrespective of smoking status.

Basic military training improved press up, sit up, 2.4 km run performance and static lift strength. Habitual smokers have been reported to have poorer physical fitness (34) and run performance in military training (5, 6) compared to non-smokers but was not observed in this study. This may have been partly explained by similar physical characteristics and self-reported physical activity level, relative to peers, at baseline. Studies that have examined changes in physical performance in response to a standardised training programme in smokers and non-smokers, have shown both similar improvement between groups undertaking the same training as the current study (10), or poorer improvement in smokers compared to non-smokers over six months of Army officer training (11). The training duration was longer in the latter study and participants were older, which in addition to differences in training environment and the training program, might explain these differences in findings. While only statistically significant once normalised to body mass, the current study supported the previous finding of a moderate effect of smoking status on poorer improvement in run performance. It should be noted here that smoking status, as opposed to the direct actions of smoking *per se*, carries with it inherent behavioural and physical effects prior to- and during- training. A field-based observational study design maintains the ecological validity of examining a real-world population sample of recruits entering military training and, unlike a randomised controlled trial (as smokers cannot be

randomised), means the isolation of any one of a number of multifaceted impacts of smoking on participants would be challenging or, indeed, inadvisable.

While energy expenditure and training load were not measured in this study, previous physical demands analyses of similar British Army training environments have shown a substantial daily energy demand of approximately 4100-4500 kcal·day<sup>-1</sup> (35). Since performance indices can be confounded by fatigue, motivation and effort, objective measures of body composition and muscle adaptation to training were also examined. Whole body anthropometric changes, increased muscle area and density, and therefore decreased fat-to-muscle ratio in the lower leg confirmed an overall increase in lean mass from training. These whole-body and localised morphological changes correspond both with the programmed physical training for development of aerobic capacity and the high volume of sustained load carriage that is customary in military training. The positive health impacts of reduced adiposity, especially abdominal, and the development and maintenance of fat-free mass are well-recognised. In addition, improved muscle health (high density and low fat-to-muscle ratio) and strength have been inversely related to metabolic disease risk in young adulthood (36). Generally, lower muscle density indicates muscle fat infiltration which has adverse implications for muscle function (37) but whether muscle health or density is, itself, an indicator of injury risk or training outcome has not been researched. Nevertheless, with regard to the present study outcomes, muscle composition data supported that physical adaptation to training was not discernibly impaired in smokers and does not specifically support the notion that habitual smoking attenuates muscle hypertrophy in response to training.

The concentration of MDA, a stable end-product of lipid peroxidation, was significantly higher in smokers at baseline, and both MDA and the acute-phase protein CRP were higher in smokers than non-smokers across the study period, characterised by main group effects (irrespective of time). This indicates higher oxidative stress and low-grade inflammation were present in smokers during the training period, both of which have been reported in habitual smokers (12, 19). Although CRP did not differ between groups prior to training, the lack of interaction effect means there is no evidence that a greater inflammatory response to training occurred in smokers relative to non-smokers. Interestingly, average CRP in smokers exceeded expected values for the age and activity level of participants (38) and, in week 10, was almost two-fold greater than non-smokers. Since systemic inflammation and oxidative stress can exacerbate each other (14, 39), it is possible that increased oxidative stress and the typical low-grade immune-inflammatory elevation in smokers coupled with the stimulus of training maintained higher than expected inflammation in smokers. Smoking-induced oxidative stress and associated oxidative damage (14, 39), coupled with other factors associated with military training environments such as reduced sleep duration (40), high incidence of minor infections (1) and intense or unaccustomed exercise training, could all contribute to heightened inflammation. Andrade et al (2001) suggested that an upper limit for oxidative stress exists in muscle (24), above which the beneficial effects of redox signalling on muscle contractility and adaptation to exercise would be disrupted during training (24). Equally, impairment of muscle development from inflammation has also been observed (23). Thus, elevated concentrations of CRP and MDA are indicative of negative physiological changes that are typically induced by smoking which, while not impairing performance or adaptation in the young participants recruited to the current

study over the relatively short time period, would likely be detrimental to performance, physical fitness development and health in the longer term.

Significant temporal changes in several biochemical markers were evident during training. To our knowledge, only one other study has observed reduced concentrations of MDA in response to long-term training (41). This is generally accepted to be an adaptive response of endogenous, mainly enzymatic, antioxidant defences which, if examined in the current study, may have provided some explanation. Research has observed no additional downstream impact of endogenous increases in ostensibly anabolic hormones on muscle mass regulation in resistance training compared to control (42, 43), which has recently brought the role of hormones in long-term exercise adaptation into question. However, changes in resting hormone concentrations may still reflect the presence and/or severity of recent training periods (26, 44). In the current study, the training syllabus in week 5 contained more bouts of physical training (a 10-mile loaded march and combat training) than week 1 or week 10. This coincided with significant decreases in IGF-1, vitamin A, lycopene and  $\beta$ -Carotene, and a peak in cortisol. The observed hormonal responses are similar to those previously observed during periods of energy deficit, intense physical activity and poor sleep quality and/or sleep restriction during both long and short term military training (27, 40, 45). The inclusion of an array of antioxidant variables in this study was predominantly to assess ostensible differences between habitual smokers and non-smokers. The specific impact of temporal changes in these markers is therefore beyond the scope of the initial research question but the lack of difference between groups suggests smokers have no greater antioxidant protection for the additional oxidative insult that daily smoking provides. Taken together, since changes and absolute concentrations of these markers were similar within

smokers and non-smokers the findings indicate that, specifically in the markers we measured, smokers and non-smokers responded to training in a similar manner.

Several biochemical parameters that were hypothesised to differ between smokers and non-smokers remained similar between groups, but it should be acknowledged that only waking morning blood samples were collected. For markers influenced by circadian variation and/or acute environmental stimuli, our blood samples likely reflect early morning peak or nadir concentrations. Specifically, the testosterone levels observed in this study are at the high end of normative population values but the combination of blood sample timing and the age and sex of the study participants correspond with typical testosterone peaks both in diurnal variation in the early morning and in the lifespan in early adulthood. In contrast, it is plausible that low observed concentrations of LOOH and antioxidant activity are explained by their acute reactive variation with oxidative processes such as in immediate response to smoking or exercise (18, 46), which would be negligible upon waking. Therefore, acute within- and between- group variations in these markers, and their subsequent downstream effects, may have been missed with the current study design. For instance, IGF-1 and cortisol can be mediated via smoking by the indirect action of nicotine (16) and transient increases in circulating IL-6 (25). These parameters, while not different between smoking groups at waking, may still have contributed to the group differences observed in MDA and CRP. Hypothetically, an elevation in both IL-6 and LOOH in response to individual bouts of exercise or smoking could contribute to increased CRP and MDA, respectively, but would not have been evident in morning samples due to shorter systemic half-lives, and particularly following overnight abstinence from smoking or other inciting stimuli.

It is possible that the duration of basic military training may not be long enough to identify differing responses to exercise training between smoking groups. Any adverse outcomes from biochemical differences observed in smokers may only be evident over a larger time scale, especially given the relatively young age of the current sample. The current sample of military recruits are younger and have a lower life-time smoking exposure ( $7 \pm 5$  y) than the 20-40-year exposure typically observed in populations studied in smoking-related health research. In addition, the combination of limited variation in smoking behaviour and sample size meant statistical power was not sufficient to examine groups of different smoking exposures (i.e. light, moderate, heavy) which may have been beneficial. Further related work is warranted with the recent advent of tobacco variations and substitutes (such as e-cigarettes and 'vaping'). A limitation of the study protocol meant it was not possible to examine acute / daily biochemical changes during training since only one morning blood sample was collected at each time-point. The collection of additional/repeat blood samples without impacting upon military training is challenging in this environment. These important considerations may have influenced the effect of smoking on development of physical fitness and muscle adaptation, as well as biochemical markers that were assessed. Future research should aim to capture acute daily variation in systemic biomarkers, including enzymatic antioxidant parameters, to investigate the potential that smoking disrupts total daily secretion and/or production, bioavailability or circadian rhythm (16). In the current study, missing performance data caused a discrepancy in sample size between data for blood biochemistry (n=65) and physical performance (n=46) from participants missing data collections. This highlights a challenge of completing research in a field-based setting, and in a military environment with the population of interest. Importantly though, this specific discrepancy was not caused by drop out from military training or individuals being deemed

unsuitable for service, meaning performance data was unlikely to contain sample bias by including only recruits who adapted more positively to training.

Young, relatively active smokers showed elevated oxidative stress and systemic inflammation during basic military training, similar to levels associated with poor health observed in older, longer-term smokers. While this did not translate into any discernible impairment in physical performance improvement or muscle adaptation during basic training, this would likely have negative implications on health, occupational performance and physical fitness development in the longer term and during a military career. This is particularly noteworthy given that, unlike non-smokers, improved physical fitness in smokers does not appear to be protective against training-related injury (7). Thus, alongside the already well-established links to injury risk, the cumulative impacts of smoking in military populations remain an ongoing concern where further investigation would be valuable.

## **ACKNOWLEDGMENTS**

This research was funded by the Army Recruiting and Training Division (UK Ministry of Defence: Army). The authors would like to acknowledge the staff at the Infantry Training Centre, Catterick, and the study volunteers.

## **CONFLICTS OF INTEREST**

All authors declare that they have no conflict of interest. The results of the present study do not constitute endorsement by ACSM. The results of this study are presented clearly, honestly and without fabrication, falsification or inappropriate data manipulation.

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ACCEPTED

## TABLE CAPTIONS

**TABLE 1.** Anthropometric characteristics across training weeks organised by smoking status.

Values are means  $\pm$  SD.

*\*denotes a significant effect of training ( $P \leq 0.05$ ), irrespective of group*

**TABLE 2.** Performance data across training weeks organised by smoking status. Values are means  $\pm$  SD.

*\*denotes a significant effect of training ( $p \leq 0.05$ ), irrespective of group. † denotes a significant interaction effect ( $p \leq 0.05$ ).*

**TABLE 3.** Endocrine markers in weeks 1, 5 and 10 (n=65) organised by smoking status. Values are mean  $\pm$  SE.

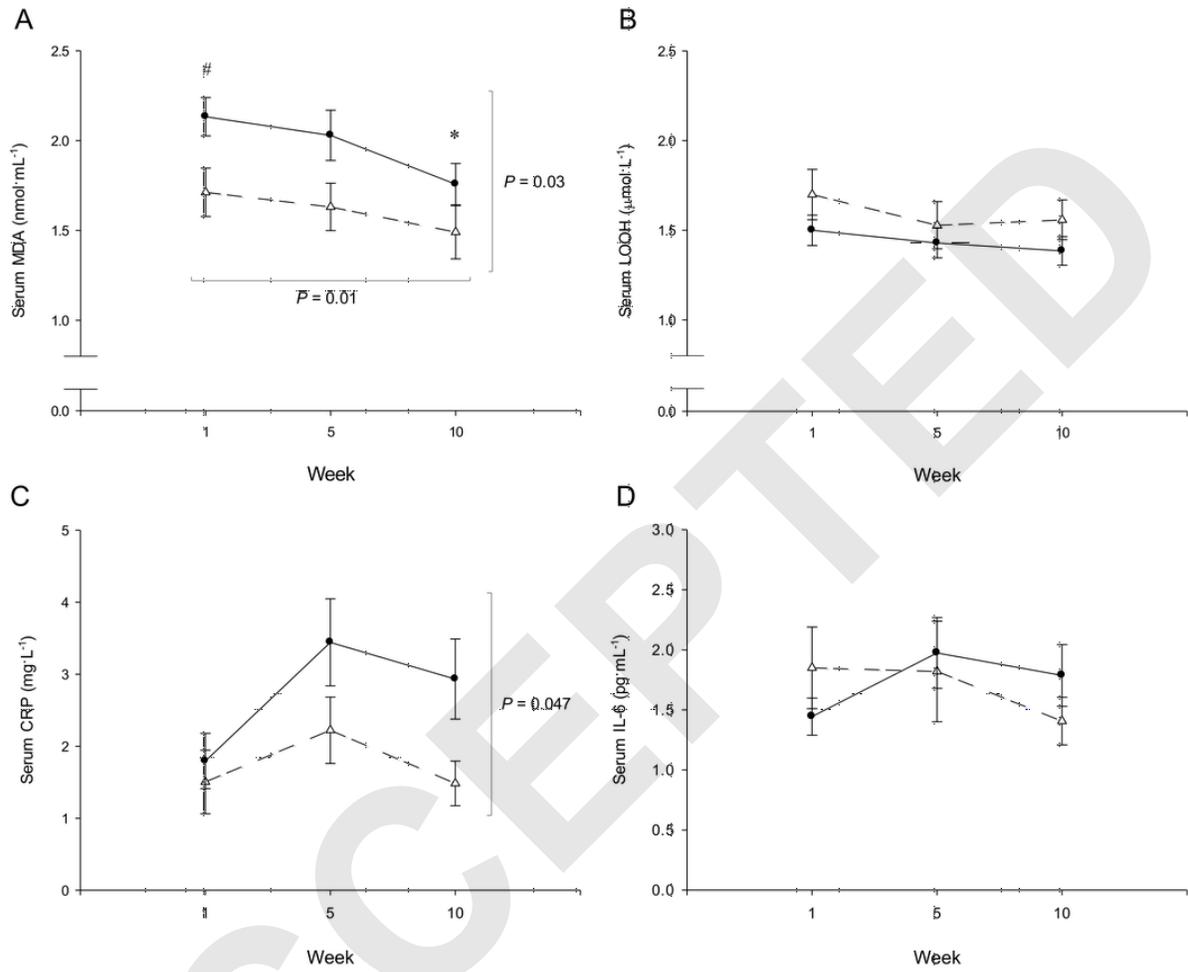
*Note: \*denotes a main group effect of training, irrespective of group. Post hoc analysis: <sup>a</sup>denotes week 1 is different from week 5. <sup>b</sup>denotes week 5 is different from 10.*

**SUPPLEMENT (TABLE A).** Antioxidants in weeks 1, 5 and 10 (n=61) organised by smoking status. Values are mean  $\pm$  SE.

## FIGURE CAPTIONS

**FIGURE 1.** Mean ( $\pm$ SE) data for MDA (A), LOOH (B), CRP (C) and IL-6 (D) for non-smokers (dashed line) and smokers (solid line) at week 1, 5 and 10 of training. Vertical parentheses and *P* values denote significant main group effect of smoking. Horizontal parentheses and *P* values denote significant main effect of training, irrespective of group. \*denotes significantly different from baseline ( $P = 0.01$ ). #denotes significantly different from non-smokers ( $P = 0.02$ )

Figure 1



**TABLE 1.** Anthropometric characteristics across training weeks organised by smoking status. Values are means  $\pm$  SD.

Variable	Smoking Status (n)	Week of training	
		1	10
Body Mass (kg)	NS (22)	75 $\pm$ 9	75 $\pm$ 9
	S (24)	78 $\pm$ 7	77 $\pm$ 6
Body Fat (%)*	NS (22)	16 $\pm$ 4	15 $\pm$ 4
	S (24)	16 $\pm$ 4	14 $\pm$ 3
Muscle Cross Sectional Area (mm <sup>2</sup> )*	NS (22)	8110 $\pm$ 895	8510 $\pm$ 1023
	S (23)	8354 $\pm$ 766	8698 $\pm$ 779
Fat Area (mm <sup>2</sup> )	NS (22)	1753 $\pm$ 631	1646 $\pm$ 496
	S (23)	1857 $\pm$ 584	1791 $\pm$ 576
Fat/Muscle Cross Sectional Area Ratio (%)*	NS (22)	22 $\pm$ 9	20 $\pm$ 7
	S (23)	22 $\pm$ 7	21 $\pm$ 7
Muscle Density (mg·cm <sup>-3</sup> )	NS (22)	76 $\pm$ 2	76 $\pm$ 2
	S (23)	76 $\pm$ 1	76 $\pm$ 2
Total Density (mg·cm <sup>-3</sup> )*	NS (22)	67 $\pm$ 5	67 $\pm$ 4
	S (23)	66 $\pm$ 4	67 $\pm$ 4

\*denotes a significant effect of training ( $P \leq 0.05$ ), irrespective of group

**TABLE 2.** Performance data across training weeks organised by smoking status. Values are means  $\pm$  SD.

Variable	Smoking Status (n)	Week of training		
		1	10	14
Static Lift Strength (kg)*	NS (22)	149.4 $\pm$ 24.5	159.8 $\pm$ 28.2	
	S (24)	154.1 $\pm$ 23.8	169.7 $\pm$ 31.8	
Mass-normalised Static Lift Strength (kg·kg <sup>-1</sup> )*	NS (22)	2.0 $\pm$ 0.4	2.2 $\pm$ 0.45	
	S (24)	2.0 $\pm$ 0.4	2.2 $\pm$ 0.44	
Grip Right (kg)	NS (22)	48.0 $\pm$ 5.2	48.3 $\pm$ 4.8	
	S (23)	48.0 $\pm$ 5.2	50.1 $\pm$ 5.5	
Grip Left (kg)	NS (22)	47.8 $\pm$ 7.1	47.0 $\pm$ 6.2	
	S (24)	48.1 $\pm$ 6.8	49.3 $\pm$ 5.5	
Jump (m)	NS (21)	0.34 $\pm$ 0.05	0.35 $\pm$ 0.06	
	S (24)	0.34 $\pm$ 0.05	0.34 $\pm$ 0.04	
Mass-normalised Jump (cm·kg <sup>-1</sup> )	NS (21)	0.46 $\pm$ 0.10	0.48 $\pm$ 0.11	
	S (24)	0.45 $\pm$ 0.07	0.44 $\pm$ 0.07	
Press ups*	NS (20)	49.5 $\pm$ 14.8		62.0 $\pm$ 13.6
	S (21)	47.0 $\pm$ 16.0		61.3 $\pm$ 9.2
Sit ups*	NS (20)	61.8 $\pm$ 16.1		70.1 $\pm$ 11.6
	S (21)	55.0 $\pm$ 9.3		67.9 $\pm$ 8.5
2.4 km run time (min:sec)*	NS (19)	10:08 $\pm$ 00:59		9:26 $\pm$ 00:38
	S (21)	10:07 $\pm$ 00:46		9:48 $\pm$ 00:32
Mass-normalised 2.4 km run time (sec·kg <sup>-1</sup> )*†	NS (19)	8.3 $\pm$ 1.0		7.7 $\pm$ 1.0
	S (21)	8.0 $\pm$ 0.79		7.8 $\pm$ 0.6

\*denotes a significant effect of training ( $p \leq 0.05$ ), irrespective of group. †denotes a significant interaction effect ( $p \leq 0.05$ ).

**TABLE 3.** Endocrine markers in weeks 1, 5 and 10 (n=65) organised by smoking status. Values are mean  $\pm$  SE.

Markers	Smoking Status (n)	Week of Training			ANOVA P value (Effect of Training)
		1	5	10	
<i>Hormones</i>					
IGF-1 (ng·mL <sup>-1</sup> )*	NS (24)	270 $\pm$ 14	249 $\pm$ 10	264 $\pm$ 14	0.004 <sup>a</sup>
	S (41)	267 $\pm$ 12	242 $\pm$ 11	242 $\pm$ 10	
Testosterone (ng·mL <sup>-1</sup> )	NS (24)	10.6 $\pm$ 0.7	10.5 $\pm$ 0.6	11.4 $\pm$ 0.6	0.064
	S (41)	11.3 $\pm$ 0.6	10.4 $\pm$ 0.6	11.1 $\pm$ 0.6	
Cortisol (ng·mL <sup>-1</sup> )*	NS (24)	145 $\pm$ 7	144 $\pm$ 4	131 $\pm$ 6	0.022 <sup>b</sup>
	S (41)	143 $\pm$ 7	149 $\pm$ 6	136 $\pm$ 8	

Note: \*denotes a main group effect of training, irrespective of group. Post hoc analysis: <sup>a</sup>denotes week 1 is different from week 5. <sup>b</sup>denotes week 5 is different from 10.

## APPENDIX.

**SUPPLEMENT (TABLE A).** Antioxidants in weeks 1, 5 and 10 (n=61) organised by smoking status. Values are mean  $\pm$  SE.

Markers	Smoking Status (n)	Week of Training			ANOVA <i>P</i> value (Effect of Training)
		1	5	10	
<i>Antioxidants</i>					
Vitamin E ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	NS (17)	7.0 $\pm$ 0.3	6.8 $\pm$ 0.4	7.1 $\pm$ 0.3	0.437
	S (36)	6.6 $\pm$ 0.3	7.1 $\pm$ 0.3	6.9 $\pm$ 0.2	
Vitamin A ( $\mu\text{g}\cdot\text{mL}^{-1}$ )*	NS (17)	534 $\pm$ 19	425 $\pm$ 16	498 $\pm$ 17	< 0.001 <sup>abc</sup>
	S (36)	518 $\pm$ 15	428 $\pm$ 11	490 $\pm$ 15	
Lycopene ( $\mu\text{g}\cdot\text{L}^{-1}$ )*	NS (17)	147 $\pm$ 13	164 $\pm$ 11	162 $\pm$ 14	0.014 <sup>ab</sup>
	S (36)	149 $\pm$ 9	165 $\pm$ 9	139 $\pm$ 7	
$\beta$ -Carotene ( $\mu\text{g}\cdot\text{L}^{-1}$ )*	NS (17)	138 $\pm$ 26	174 $\pm$ 21	152 $\pm$ 20	0.002 <sup>ab</sup>
	S (36)	112 $\pm$ 8	138 $\pm$ 8	125 $\pm$ 8	
Cryptoxanthin ( $\mu\text{g}\cdot\text{L}^{-1}$ )	NS (17)	43.9 $\pm$ 6.1	47.4 $\pm$ 7.7	42.9 $\pm$ 4.2	0.327
	S (36)	37.7 $\pm$ 2.0	40.1 $\pm$ 2.6	39.4 $\pm$ 1.8	
Lutein and Zeaxanthin ( $\mu\text{g}\cdot\text{L}^{-1}$ )*	NS (17)	91.9 $\pm$ 4.0	98.2 $\pm$ 5.7	97.2 $\pm$ 3.9	0.003 <sup>bc</sup>
	S (36)	87.5 $\pm$ 2.4	95.1 $\pm$ 2.8	93.0 $\pm$ 2.9	

*Note:*\*denotes a significant effect of training, irrespective of group (*P* values given in ANOVA column). Post hoc analysis: <sup>a</sup>denotes week 1 is different from week 5. <sup>b</sup>denotes week 5 is different from 10. <sup>c</sup>denotes week 1 is different from week 10.