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Associations between inflammatory markers and well-being during 12 weeks of basic military training

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Abstract

Purpose Stress, anxiety and physical exertion are all closely linked to well-being, and each can alter immune function. Diminished well-being has been observed during military training, however there is mixed evidence regarding whether concomitant changes in inflammatory markers occur, with these phenomena indicating potential maladaptive responses to imposed training loads. The aims of this project were (1) assess changes in inflammation and subjective well-being across a 12-week basic military training (BMT) program, and (2) evaluate relationships between circulating inflammatory markers and well-being.

Methods A total of 37 men and women undergoing 12 weeks of BMT in Australia were recruited. Well-being was assessed via questionnaire (DASS-21), and plasma samples were collected for the analysis of inflammatory cytokines [interleukin (IL)-4, IL-6, IL-1β, IL-8, IL-10, and tumor necrosis factor (TNF)-α] at weeks 1, 4, 8 and 12. Data were analysed using general linear mixed models.

Results Depression, anxiety and stress subscale scores all significantly improved (all $P \leq 0.001$), and TNF-α decreased ($P = 0.031$) across time. Compared to baseline (week 1), significant decreases in associations between depression and IL-10, anxiety and IL-10, and stress and IL-10, IL-4 IL-6 and TNF-α (all $P < 0.05$), were detected across BMT.

Conclusion The BMT program appears to support improved well-being over the 12 weeks, with minimal perturbation to inflammatory markers. Biomarkers and well-being displayed consistent associations and may have utility as psychophysiological indicators of health status in military research, however for now, subjective measures may represent more cost-effective proxies for ongoing monitoring of military personnel.

Keywords Inflammation · Self-rated health · Cytokines · Stress · Physical training · Monitoring

Abbreviations

ANOVA	Analysis of variance
BMI	Body mass index
BMT	Basic military training
CV	Coefficient of variation
DASS-21	Depression Anxiety Stress Scale—21-item version
GLMMs	General Linear Mixed Models
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
MSST	Multi-stage shuttle test
NF-OR	Non-functional overreaching
PTSD	Post traumatic stress disorder
rev·min^{-1}	Revolutions per minute
SEM	Standard error of the mean
SD	Standard deviation
TNF-α	Tumor necrosis factor alpha
VO$_2$ max	Maximal oxygen uptake

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Introduction

Military personnel are often exposed to a range of stressors including prolonged and strenuous physical exercise (Pihlainen et al. 2014), psychological stress, sleep deprivation, as well as energy and fluid restrictions (Margolis et al. 2014; Nindl et al. 2002). Collectively, these stressors can impose physiological strain and impair occupational performance and well-being of a soldier (Hamarsland et al. 2018; Nindl et al. 2002, 2007). Basic military training (BMT) prepares military recruits for these occupational demands, by exposing them to physically and cognitively demanding activities through training simulations and drills (Nindl et al. 2007). The Australian Army BMT program involves 12 weeks of continuous training that is both physically and psychologically demanding, with most days commencing at 6:00 a.m. and finishing at 10:00 p.m. Given these demands, it is important that across BMT exposure to these stressors is balanced with adequate recovery to maintain performance in recruits, and promote positive training adaptations (Kellmann et al. 2018; Nindl et al. 2002). Inadequate recovery can lead to fatigue, and nonfunctional overreaching (NF-OR) (Kellmann et al. 2018; Lehmann et al. 1993), which if not addressed can progress to 'overtraining', which has previously been observed in military training (Booth et al. 2006; Hamarsland et al. 2018; Nindl et al. 2002, 2007; Tanskanen et al. 2011). Overtraining is associated with a cascade of maladaptive responses including negative psychological symptoms, hormonal alterations and decreased performance (Meeusen et al. 2013). Overtraining and insufficient recovery may also increase injury rates in military organisations, particularly during BMT (Schram et al. 2019b), which translates into decreased operational capability, increased need for health care, and medical compensation costs (Schram et al. 2019a). At present, monitoring of recovery status and the development of monitoring systems for soldier wellbeing and performance is inadequate (Nindl et al. 2015; Schlenoff et al. 2007). Therefore, understanding the signs of inadequate recovery or maladaptation during BMT will improve personnel management, and thereby enhance military training outcomes and workforce readiness (Caravalho Jr 2015).

Alterations in circulating hormonal and inflammatory levels are often indicative of inadequate recovery or NF-OR (Jürimäe et al. 2011; Main et al. 2010; Meeusen et al. 2013). Increases in interleukin (IL-6) has also been identified as an indicator of muscle damage, as a consequence of prolonged exercise and overtraining (Gleeson 2002; Smith 2000), while elevated levels of tumor necrosis factor (TNF)-α have been associated with increased training stress and inadequate recovery in athletes (Jürimäe et al. 2011). Markers of inflammation may be useful in understanding the physiological and psychological demands of BMT. There is some evidence that pro-inflammatory cytokine levels (e.g., IL-6, IL-1β, and TNF-α) may increase during BMT (Booth et al. 2006; Gomez-Merino et al. 2003; McClung et al. 2013; Ojanen et al. 2018), however this is not consistent, with concentrations of IL-6 remaining unchanged (Chester et al. 2013; Nindl et al. 2012; Ojanen et al. 2018) or increasing following military training (Gomez-Merino et al. 2003; McClung et al. 2013). These mixed findings may be ascribed to the considerable training differences between studies (e.g., BMT vs survival simulation, with training lengths ranging from 7 to 45 days), group characteristics (e.g., single sex vs both, physical characteristics and training status), and the frequency of sampling. Given these inconsistencies, tracking the inflammatory response during a relatively longer 12-week BMT period, over multiple time points, will provide a greater understanding of the physiological strain imposed on recruits during BMT.

The transition to military life from the civilian environment can be a confronting experience for new recruits. Most recruits adapt to the training environment (Lerew et al. 1999; Martin et al. 2006), however BMT can also increase psychological distress (Booth et al. 2006; Martin et al. 2006), as it requires discipline, extensive physical training, and meeting training standards to progress to subsequent training phases (Lieberman et al. 2012, 2014). These elements can collectively impair well-being in recruits, which broadly includes subjective evaluations of psychological well-being and positive and negative affect (i.e., mood and emotions) (Lundqvist 2011). The impact of BMT on recruit well-being has primarily been evaluated through assessments of mood, with positive changes (Lieberman et al. 2008, 2012, 2014, 2016), and also negative psychological symptoms associated with overtraining (e.g., confusion), observed over the course of training (Booth et al. 2006). Maintaining well-being is important as psychological factors have been associated with performance, decision-making and health composites in military contexts (Bardwell et al. 2005; Burr et al. 1993). Validated and reliable metrics of overtraining and recovery are yet to be established in a military context, and therefore an initial assessment of the impact of Australian Army BMT on well-being is required to inform these indications, which may optimise the preparation of recruits for a military career.

Immune function has been linked to well-being in adults (Kiecolt-Glaser et al. 2010), as acute psychological stress and anxiety can upregulate pro-inflammatory cytokine production (Kiecolt-Glaser et al. 2002). As such, an emerging body of literature has outlined the links between chronic stress and depression and increased inflammation and impaired immune function (Kiecolt-Glaser et al. 2015; Miller and Raison 2016; Morey et al. 2015), which could

potentially impair individual readiness (Andersson et al. 2015). In healthy adults, there is evidence for an inverse relationship between circulating markers of inflammation with global measures of self-rated health and well-being (Hamer and Chida 2011; Lekander et al. 2004; Tait et al. 2019; Warnoff et al. 2016). These relationships remain unclear in the military context, however a limited number of nutritional, metabolic and hormonal markers predicted a substantial proportion of variability in mood in female recruits (Lieberman et al. 2012). Elevated levels of inflammation may therefore represent a risk factor for reduced well-being, and vice versa, with this relationship potentially mediated via sickness behaviours and/or physical health (Unden et al. 2007). However, it remains unclear whether inflammatory status can be used as a surrogate marker of subjective well-being in recruits. By taking a dual approach of both measuring changes in subjective and objective measures in response to BMT, we will be better positioned to make an informed decision about which measures have greatest utility in monitoring and managing soldiers, and by extension predicting military readiness. Therefore, the aims of the current project were to (1) assess changes in inflammation and subjective well-being across a 12-week BMT program, and (2) evaluate relationships between circulating inflammatory markers and well-being across BMT.

Material and methods

Study design

This study collected data during a 12-week BMT program and was part of a larger project designed to quantify training loads and monitor the physiological and psychological responses of recruits during BMT. All well-being and inflammatory data were collected at the same time of day at four time points across BMT, at weeks 1, 4, 8, and 12. Week 1 represents baseline data, and were collected 3 days after recruits arrived at the barracks, before recruits had engaged in any physical training or drills.

Participants

Participants had voluntarily enlisted into the Australian Army and were undertaking a 12-week BMT program at the Army Recruit Training Centre (Blamey Barracks, Kapooka). Of the $n = 48$ that consented for the larger study, 37 recruits (33 men and 4 women; aged 24.8 ± 7.6 years; body mass: 77.0 ± 14.8 kg, height: 1.78 ± 0.1 m; and body mass index; 24.2 ± 3.4 kg m^{-2}) provided the required ≥ 3 finger prick blood samples and had well-being data for ≥ 3 time points for the current analysis. Recruits were briefed on the investigation at the commencement of BMT, prior to

providing voluntary written informed consent to participate in the study. This study was approved by the Department of Defence and Veteran's Affairs Human Research Ethics Committee (021–17).

Anthropometry and body composition

Height was measured to the nearest 0.1 cm and body weight was measured to the nearest 0.01 kg using standard techniques (stadiometer and a metric scale, respectively). Body mass index (BMI) was calculated [weight (kg)/height (m^2)].

Inflammatory markers

Fasted morning finger prick blood samples (0600–0645 h) were collected at the four time points across BMT, at the recruits' accommodation blocks. Samples (≥ 350 µL) were collected in a 500-µL micro-test-tube coated with K$_2$EDTA (Becton–Dickinson, Franklin Lakes, NJ), and immediately stored on ice until the end of the sampling period. Samples were then centrifuged at 5000 rev min^{-1} (Sigma 203, Sigma, Osterode am Harz, Germany) for 10 min to separate plasma, and stored at -80 °C until analysis. All biomarkers were analysed in duplicate. Plasma IL-4, IL-6, IL-1β, IL-8, TNF-α and IL-10 were measured using the Milliplex™ T Cell high-sensitivity human cytokine panel (Merck Millipore, Australia) as per manufacturers' recommendations. All analyses recorded an intra-assay % coefficient of variation (%CV) of $< 11\%$ and an inter-assay %CV of $< 19\%$. Inflammatory markers falling below the detection threshold were assigned the value for the assay's lowest detectable limit (all pg/mL, IL-4: 1.0; IL-6: 0.36; IL-1β: 0.1; IL-8: 0.06; TNF-α: 0.07; IL-10: 0.4).

Well-being: depression anxiety and stress scale (DASS-21)

Symptoms of depression, anxiety and stress over the past week were assessed at the four time points using a 21-item self-report measure, the Depression Anxiety and Stress Scale (DASS)-21 (Lovibond and Lovibond 1995b). Seven items on a four-point Likert scale measure each factor of depression, anxiety and stress. Scores range from 0 to 21 on each subscale, with scores multiplied by two, and higher scores indicating greater anxiety, depression or stress (Lovibond and Lovibond 1995a). Subscale totals were classified as; Depression [normal: 0–9; mild: 10–13, moderate: 14–20; severe: 21–27; extremely severe: ≥ 28], Anxiety [normal: 0–7; mild: 8–9; moderate: 10–14; severe: 15–19; extremely severe: ≥ 20], Stress [normal: 0–14; mild: 15–18; moderate: 19–25; severe: 26–33; extremely severe: ≥ 34] (Paukert et al. 2010).

Physical fitness

Physical fitness was assessed via maximum number of push-ups in 2 min completed according to an audio cadence track (maximum 100), and multi-stage shuttle test (MSST) performance during weeks 2 and 8 of BMT. MSST performance was used to predict VO_2 max according to Ramsbottom et al. (Ramsbottom et al. [1988]). Changes in physical fitness were used as a covariate due to its potential effect on the outcome measures (Crowley et al. [2015]; Lavie et al. [2011]).

Statistical analyses

All statistical analyses were conducted using STATA statistical software release 15.0 (STATA, College Station, TX, USA). All data and their residuals were checked for normality and the following biomarkers were log transformed prior to analysis; IL-1β, IL-8, IL-10. Fourteen data points for IL-1β ($n = 12$), IL-10 ($n = 1$) and IL-8 ($n = 1$) were removed (15–40 SDs above the mean). Effects of BMT on inflammation and well-being were analysed using General Linear Mixed Models with random effects, adjusting for the variability within a cluster (participants). GLMMs also account for the possibility of autocorrelation in the repeated cytokine measurements (i.e., samples and/or days) on each individual, or serial correlation of data points over time, by including a model for the covariance structure, and are increasingly preferred over repeated-measures ANOVA for these types of data (Molenberghs and Verbeke [2001]). Time point was the fixed effect, and the unit of analysis (participants), was included as random effects to account for the clustered design. The effect of BMT on the primary outcomes was initially assessed, before adjusting for physical fitness and baseline levels of BMI, due to their potential effect on the outcome measures (Crowley et al. [2015]). No gender comparisons were undertaken due to the small sample size. Changes in the relationships between inflammatory markers and well-being across training were assessed using general linear mixed models with random effects, adjusting for participants. For the main analysis, associations between inflammatory markers and well-being were analysed using the same statistical method, however time point was used as a continuous variable to interact with each variable, and provided an indication as to whether global changes in the strength of associations had occurred over the four time points. Associations between inflammation and well-being at weeks 4, 8, and 12 were also compared to those at week 1, with changes in these relationships analysed with time point specified as discrete levels. In this model, the STATA lincom command was used to estimate the beta (β)-coefficients at each of the four time points. Changes in well-being subscale scores were additionally assessed with linear mixed models with gamma distribution. All data are presented as means $\pm$ SEM or $\pm$ SD, or β-coefficients with 95% CI. The significance level was set at $P < 0.05$.

Results

Physical fitness

At week 2, predicted VO_2max of participants was 43.1 ± 4.4 (ml/kg min^{-1}). Multi-stage fitness tests conducted at weeks 2 and 8 indicted a significant increase in predicted VO_2max [4.3 (95% CI 1.8–6.7) mL·kg^{-1} min^{-1}; $P = 0.001$]. Push-ups performed also increased between weeks 2 and 8 [5.3 (95% CI 1.5–9.0); $P = 0.008$].

Circulating inflammatory markers

For pro- and anti- inflammatory markers, there were no significant changes over time with the exception of TNF-α which decreased by 10% ($P = 0.031$) across the 12 weeks (Fig. [1]). Concentrations of IL-1β at week 12 were significantly different to those at week 4. Adjusting for change in predicted VO_2 max or BMI did not alter this finding.

Well-being

Across the 12-week BMT program, there were significant decreases in the depression (β = − 0.45; $P < 0.001$), anxiety (β = − 0.54; $P < 0.001$) and stress (β = − 0.52; $P < 0.001$) subscales of the DASS-21 (Fig. [2]). Mean depression subscale scores significantly decreased between weeks 1 and 8 [− 3.2 points (− 6.2, − 0.25); $P = 0.034$], 1 and 12 [-5.1 points (− 8.1, − 2.1); $P = 0.001$], and 4 and 12 [− 4.2 (− 7.1, − 1.2); $P = 0.006$]. Compared to anxiety subscale scores in week 1, lower scores were seen at weeks 8 [− 3.8 points (− 6.3, − 1.2); $P = 0.004$] and 12 [− 5.7 points (− 8.3, − 3.1); $P < 0.001$]. Lower scores were also seen at weeks 8 [− 3.0 points (− 5.5, − 0.5); $P = 0.018$] and 12 [− 4.9 points (− 7.5, − 2.4); $P < 0.001$], compared to those recorded in week 4. Compared to stress subscale scores in week 1, lower scores were seen at weeks 8 [− 4.2 points (− 7.5, − 0.9); $P = 0.013$] and 12 [− 5.9 points (− 9.3, − 2.6); $P = 0.001$]. Lower scores were also seen at week 12 [− 4.0 points (− 7.3, − 0.6); $P = 0.020$], compared to those recorded in week 4. Proportion of recruits recording scores within the 'normal' categorisation for each subscale increased over the training period ($P < 0.05$) (Fig. [3]). Adjusting for change in predicted fitness did not attenuate any significant findings.
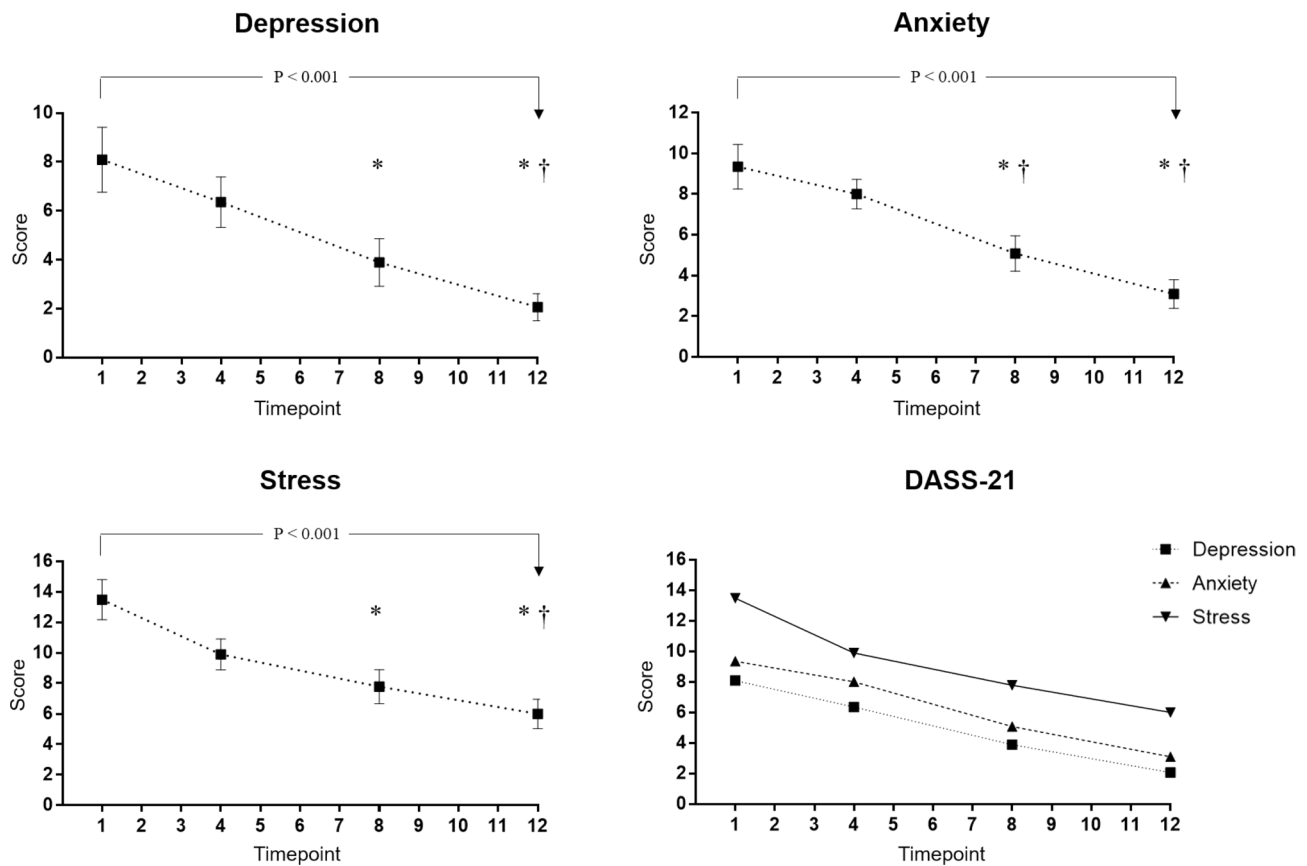
Associations between well-being, and biomarkers

Higher concentrations of IL-10 were associated with higher scores in the Depression subscale of the DASS-21
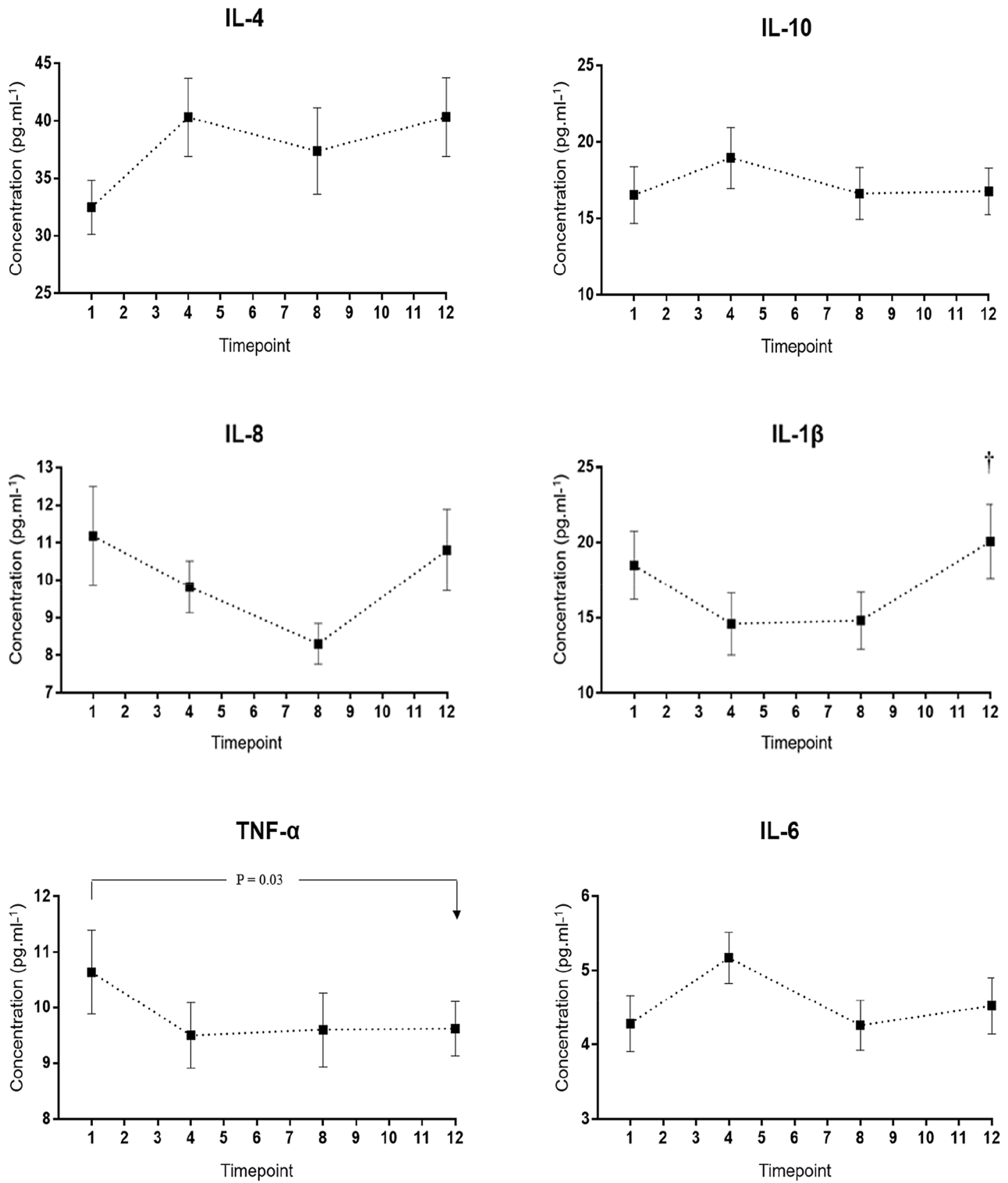
Fig. 1 Mean (± SEM) changes in interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-8 (IL-8), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α) and interleuklin-6 (IL-6), following a 12-week basic military training program. $^{\dagger}P < 0.05$ change relative to time point (week) 4. NB: For IL-10, IL-8 and IL-1β data were log-transformed prior to analysis, but raw data are presented for all inflammatory markers

Fig. 2 Mean (± SEM) changes in Depression, Anxiety and Stress subscale scores from the DASS-21 after 12-week basic military training program. *$P < 0.05$ change relative to time point (week) 1, †$P < 0.05$ change relative to time point (week) 4
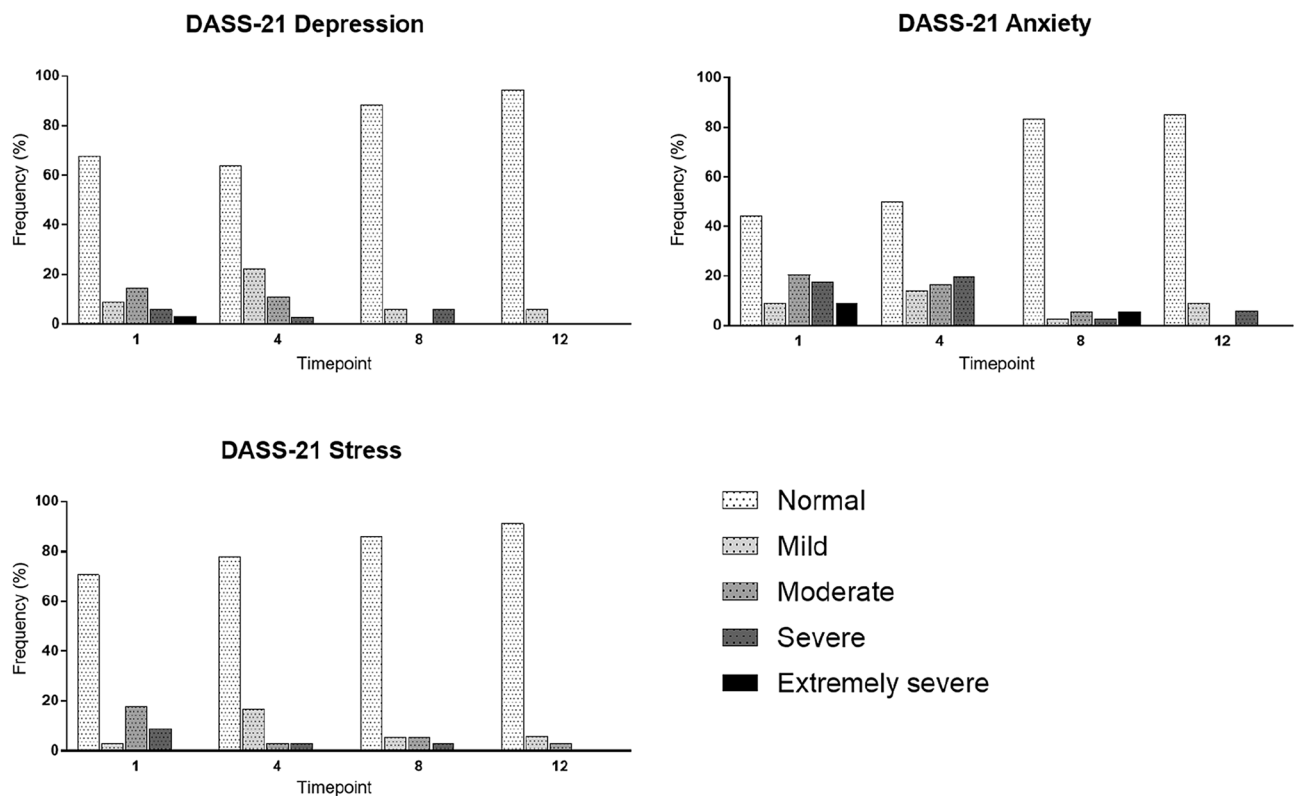
at week 1 (β = 3.60, $P = 0.018$). The strength of association between IL-10 concentrations and Depression scores decreased over training (β = − 0.38, $P = 0.021$; Table 1).

Higher concentrations of IL-10 were associated with higher scores in the Anxiety subscale of the DASS-21 at week 1 (β = 4.12, $P = 0.003$), while decreased concentrations of IL-1β were associated with higher anxiety scores at week 8 (β = − 2.87, $P = 0.032$). The strength of association between IL-10 concentrations and Anxiety scores decreased over training (β = − 0.39, $P = 0.012$; Table 1).

In week 1, higher concentrations of IL-6 (β = 1.72, $P < 0.001$), TNF-α (β = 0.68, $P = 0.005$) and IL-10 (β = 4.94, $P = 0.004$) were associated with higher scores in the stress subscale of the DASS-21 at week 1. The strength of association between IL-10 concentrations and stress scores decreased over training (β = − 0.48, $P = 0.010$; Table 1). The strength of association between IL-6 concentrations and stress scores decreased over training (β = − 0.112, $P = 0.026$; Table 1). Decreases in the magnitude of association were also observed between weeks 1 and 12 for TNF-α and stress (β = − 1.33, $P = 0.001$), and IL-4 and stress (β = − 0.18, $P = 0.044$).

Discussion

The main findings from this study were that the recently revised Australian Army BMT program appears to enable improvements in well-being over the 12 weeks, with observed reductions in subjective ratings of depression, anxiety and stress. BMT also appears to exert minimal perturbation on inflammatory markers, with only a decrease in the pro-inflammatory cytokine TNF-α observed across training. Associations between inflammatory markers and well-being were observed, with higher levels of IL-10 associated with improvements in subscales of well-being. The magnitude of relationships between pro-inflammatory cytokines and well-being decreased over BMT, which may also be reflective of improvements in well-being, and the ability of the recruits to adjust to the military training environment.

DASS-21 Depression

DASS-21 Anxiety

DASS-21 Stress

- Normal
- Mild
- Moderate
- Severe
- Extremely severe

Fig. 3 Mean proportional (%) changes in categorization of severity of depression, anxiety and stress subscales from DASS-21 following a 12-week basic military training program

Effect of BMT on circulating inflammatory levels

The lack of change in inflammatory biomarkers observed in the current study may be explained by several factors, including moderate training loads over the 12 weeks, and the time points of sampling. BMT primarily involves low-moderate intensity physical activity, with intermittent periods of high-intensity exercise, during training exercises that are physically and cognitively challenging (Drain et al. 2017; Burley et al. 2020). Previous research has indicated that increased cytokine levels during training periods may be indicative of NF-OR and overtraining, along with muscle damage and fatigue (Gleeson 2002; Smith 2000). Thus, the current results suggest that the cumulative loads of the activities undertaken during BMT did not evoke a significant inflammatory response. In contrast to our findings, Gomez-Merino and colleagues (Gomez-Merino et al. 2003) observed an increase in IL-6 in male soldiers following a 3-week moderate-intensity-based training program that concluded with an intensive 5-day course involving heavy physical training and sleep deprivation. However, this course was designed to be more intense than BMT, and so it is difficult to compare the findings to the current 12-week study. Consistent with our findings, levels of

IL-6 were unchanged following a 15-day survival training course, comprising mostly low-moderate intensity activity (Chester et al. 2013), while a 4-month BMT course also failed to elicit changes in IL-6 and IL-1β concentrations, with only decreases in TNF-α observed (Nindl et al. 2012). It is unclear as to whether the reductions in plasma TNF-α that were observed are indicative of a reduction in physical strain throughout the program, or the by-product of an exercise-induced anti-inflammatory environment, or a combination of both (Pedersen 2011). Alternatively, the 'shock of capture' and immediate advent of both physical and psychological stressors when beginning BMT may have elicited a stress response which elicited increases in TNF-α (Kiecolt-Glaser et al. 2002; Marsland et al. 2017), which then abated as the recruits grew accustomed to the demands of training. Moreover, increases in the production of pro-inflammatory cytokines have been observed in response to psychological stress alone (Segerstrom and Miller 2004). Nonetheless, our findings suggest that plasma TNF-α concentration may be useful to evaluate recruits' physical state and recovery when technological advances allow affordable, real-time monitoring.

The current study employed a sampling protocol of approximately once every 4 weeks, however it is uncertain

Table 1 Associations between well-being and circulating inflammatory markers across 12 weeks of training

	IL-6 β-value (95% CI)	TNF-α β-value (95% CI)	IL-1β β-value (95% CI)	IL-10 β-value (95% CI)	IL-4 β-value (95% CI)	IL-8 β-value (95% CI)
Depression						
T1	0.79 (− 0.02, 1.60)	0.33 (− 0.11, 0.77)	− 0.27 (− 3.58, 3.05)	3.60 (0.62, 6.59)[†]	0.06 (− 0.09, 0.21)	1.00 (− 2.05, 4.05)
T2	0.33 (− 0.57, 1.23)	0.02 (− 0.51, 0.58)	− 0.55 (− 2.89, 1.79)	0.46 (− 2.50, 3.42)	0.001 (− 0.01, 0.01)	2.69 (− 1.52, 6.90)
T3	− 0.19 (− 1.14, 0.77)	− 0.20 (− 0.69, 0.30)	− 1.87 (− 4.87, 1.13)	− 1.32 (− 4.64, 2.00)[*]	− 0.01 (− 0.10, 0.08)	2.36 (− 2.28, 7.00)
T4	− 0.08 (− 0.91, 0.75)	− 0.24 (− 0.91, 0.43)	− 0.97 (− 3.43, 1.49)	− 0.67 (− 4.01, 2.66)[*]	0.002 (− 0.10, 0.11)	− 1.42 (− 4.97, 2.13)
Anxiety						
T1	0.75 (− 0.001, 1.50)	0.19 (− 0.21, 0.59)	− 1.49 (− 4.57, 1.59)	4.12 (1.43, 6.80)[†]	0.07 (− 0.06, 0.20)	0.51 (− 2.45, 3.47)
T2	0.53 (− 0.28, 1.35)	0.29 (− 0.20, 0.78)	0.18 (− 1.95, 2.31)	0.42 (− 2.22, 3.05)[*]	0.01 (− 0.08, 0.09)	1.22 (− 2.83, 5.26)
T3	0.30 (− 0.54, 1.15)	− 0.08 (− 0.52, 0.37)	− 2.87 (− 5.51, − 0.24)[*]	0.29 (− 2.64, 3.22)[*]	0.03 (− 0.05, 0.12)	1.74 (− 2.55, 6.03)
T4	0.38 (− 0.37, 1.14)	− 0.30 (− 0.92, 0.31)	0.39 (− 1.88, 2.66)	− 0.88 (− 3.87, 2.11)[*]	− 0.03 (− 0.12, 0.06)	0.88 (− 2.55, 4.32)
Stress						
T1	1.72 (0.84, 2.60)[†]	0.68 (0.20, 1.16)[†]	− 1.17 (− 4.61, 2.28)	4.94 (1.62, 8.27)[†]	0.15 (− 0.02, 0.31)	1.28 (-2.22, 4.78)
T2	0.11 (− 0.86, 1.08)[*]	0.04 (− 0.54, 0.62)	1.05 (− 1.39, 3.49)	− 0.33 (− 3.63, 2.97)[*]	− 0.01 (− 0.12, 0.10)	1.41 (− 3.41, 6.22)
T3	0.39 (− 0.59, 1.38)[*]	0.09 (− 0.44, 0.62)	− 2.52 (− 5.49, 0.44)	-0.66 (− 4.31, 2.99)[*]	− 0.001 (− 0.11, 0.10)	3.70 (− 1.40, 8.79)
T4	0.09 (− 0.80, 0.98)[*]	− 0.65 (− 1.38, 0.08)[*]	− 0.41 (− 2.97, 2.15)	− 1.42 (− 5.14, 2.29)[*]	− 0.03 (− 0.15, 0.08)[*]	0.20 (− 3.86, 4.27)

All values represent β values and 95% confidence interval (CI). Outliers removed for IL-1β (n=12), IL-10 (n=1) and IL-8 (n=1). All models adjusted for within-participant variation. [*]$P < 0.05$ change in association compared to T1. [†]$P < 0.05$ at T1. IL-1β: interleukin-1 beta; IL-4: interleukin-4; IL-6: interleukin-6; IL-8: interleukin-8; IL-10: interleukin-10; TNF-α: tumor-necrosis factor alpha. N.B: IL-1β, IL-10 and IL-8 markers were log-transformed

whether any training-induced changes in cytokines may have washed out in this time. Studies that have detected increases in cytokines such as IL-6 have utilised shorter training programs and closer time points (i.e., 7 days) (Gomez-Merino et al. 2003; McClung et al. 2013), or only reported transient and acute changes in inflammatory levels (Ojanen et al. 2018). Booth and colleagues (Booth et al. 2006) observed no changes in inflammatory levels between baseline and week 6 of BMT, but showed an increase during the final week of the 7-week course, which involved a field training exercise with sleep deprivation. This increase following field training may reflect a short-term physiological state indicative of overreaching or overtraining (Smith 2000). The current BMT program included a similar field phase between weeks 10–11, although the final blood collection time point was 9 days after this exercise had concluded. Therefore, it is unclear as to whether training load, sampling time points, or both, may have influenced circulating inflammatory levels. Collectively, our findings suggest that the present BMT program was largely well tolerated with little effect on circulating inflammatory levels, despite the combined physical, psychological and cognitive stressors. This may in part be attributable to the recently revised Australian Army BMT training syllabus which provides a more progressive and controlled exposure to stressors, and the physical training regimen shifting bias from prolonged moderate-intensity cardiorespiratory and muscular endurance sessions towards higher-intensity lower volume strength and interval training (Burley et al. 2020). Thus, exposure to multifactorial stressful conditions during BMT does not appear to produce biological signals of maladaptation or overtraining.

Effect of BMT on well-being

Previous research has indicated that BMT is a psychological stressor (Booth et al. 2006; Martin et al. 2006), which can produce behavioural symptoms of overtraining in recruits (e.g., confusion, depression, fatigue) (Booth et al. 2006). However, we observed significant improvements in depression, anxiety and stress subscales of the DASS-21 across the 12-week period, indicating that the current training program facilitated improved recruit well-being. There are several factors that may explain these positive findings, including successful integration into the training program, and adequate recovery opportunities. The most likely explanation for the positive changes in well-being is that participants

successfully and progressively adapted to their new environment, which was punctuated by the completion of phases of training that may have previously been regarded as daunting. Moreover, it is possible that baseline levels of depression, anxiety and stress may have been elevated by the transition from civilian life to unfamiliar and challenging conditions of military training, which gradually subsided over the 12-week period. This aligns with previous accounts of recruits feeling confused, anxious and depressed at the start of BMT, when self-doubt is high and confidence is lacking (Lieberman et al. 2008, 2014). Our findings support positive changes in mood that have been reported in recruits across BMT (Lieberman et al. 2016), particularly depression/dejection and tension/anxiety (Booth et al. 2006), which have been attributed to the provision of regular bed times and waking times, training conducted in a team-oriented environment, and a distraction from existing life issues (Lieberman et al. 2016).

Previous research has proposed that well-being or perceptions of stress during BMT may be influenced or predicted by factors such as fitness levels, resiliency, and hardiness (Crowley et al. 2015; Jones et al. 2019; Skomorovsky and Sudom 2011). These psychological traits were not explored in our study, however increased maximal aerobic power (predicted) and upper-body local muscular endurance were observed during BMT. It is therefore possible that the training-induced improvements in physical fitness may have contributed to our improvements in well-being. Research in civilian populations (Conn 2010) suggests that physical fitness may have a protective role in reducing the adverse psychological effects of stress (Dishman et al. 2012; Sui et al. 2009), while recruits with high levels of baseline fitness are less likely to report depressive symptoms (Crowley et al. 2015). In addition, exercise activates therapeutic pathways that facilitate actual or perceived benefits for mental health and well-being, including increased serotonin and neurotransmitter release and availability which exerts antidepressant effects, the regulation and attenuation of stress hormone release (Barbour et al. 2007; Strohle 2009; Tsatsoulis and Fountoulakis 2006), and increased self-efficacy and self-esteem (White et al. 2009). Minimising depressive mood and enhancing psychological well-being during BMT (e.g., through stress resistance strategies) is therefore crucial in preparing recruits for the stressors encountered during a military career, while the early identification of adverse well-being could reduce mental health-related BMT attrition (Gold and Friedman 2000; Knapik et al. 2004).

Associations between inflammatory levels and recruit well-being

To our knowledge, no studies have evaluated associations between circulating inflammatory markers and well-being across a 12-week BMT course. Relationships between lipid and nutritional markers with mood have been previously reported over the course of BMT (Lieberman et al. 2012), however inflammatory markers were not assessed. In the current study, significant positive associations between the anti-inflammatory cytokine IL-10 were observed with depression, anxiety and stress subscale scores in week 1, and thereafter the strength of these associations significantly decreased over time to be inversely related in weeks 8–12. While the mechanisms underpinning these changes are unclear, the most likely explanation is that higher depression, anxiety and stress subscale scores at baseline may have contributed to the positive association with IL-10, which then diminished over training as a function of significant improvements in well-being, and unchanging levels of IL-10. This may also explain our observations that higher concentrations of cytokines IL-6 and TNF-α were associated with higher scores on the stress subscale in week 1, with significant reductions in the magnitude of these associations occurring thereafter. Higher levels of acute and chronic psychological stress have been previously shown to correlate with elevated circulating inflammatory levels (Hänsel et al. 2010; Steptoe et al. 2007), with pro-inflammatory levels in particular displaying inverse relationships with global measures of quality of life, self-rated health and well-being (Hamer and Chida 2011; Lekander et al. 2004; Nowakowski 2014; Tait et al. 2019; Warnoff et al. 2016). Mechanistically, multiple links exist between inflammation and depression (Slavich and Irwin 2014); experimental induction of inflammatory processes depresses mood and increases activation of brain sites that regulate positive and negative affect (Eisenberger et al. 2009, 2010). Given the links between higher levels of pro-inflammatory markers IL-6, IL-1 β and TNF-α with PTSD and chronic anxiety (Passos et al. 2015; Renna et al. 2018), the risk of which is elevated in military personnel (Hoge et al. 2004; Smith et al. 2009), our findings suggest that recruits mentally adjusted to the military training environment. Collectively, our results reinforce relationships between inflammatory markers and well-being, and suggest that various inflammatory markers may have utility in monitoring readiness in recruits, and health status in broader military research (Gimeno et al. 2009). It could be argued that subjective measures may represent more cost-effective proxies to monitor recruits, however psychoneuroimmunological relationships may in time provide a more in-depth assessment of interactions between indicators of fatigue, illness, mood disturbance, and performance.

There are several strengths associated with this study. To our knowledge, this is the first study to evaluate relationships between an array of inflammatory markers with well-being in a military context, to identify candidate markers for the longitudinal monitoring of well-being. Further, the BMT program employed was longer than

other programs in the literature (Booth et al. 2006; Lieberman et al. 2016; Ojanen et al. 2018), and the training format and cohort provide a point of difference to survival simulation training studies (Chester et al. 2013; Gomez-Merino et al. 2003; McClung et al. 2013). There are some limitations which must be considered when interpreting the results. First, a potential limitation may be an inability to determine whether the associations we observed persist in BMT programs where improvements in mood do not occur, and whether other markers may be more useful in predicting the well-being of recruits who exhibit deteriorations in well-being. Similarly, it is unclear as to how generalizable our findings are to civilian populations whereby mood may be more susceptible to fluctuations under life or clinical events. Second, due to the larger aims of the study, factors that influence circulating levels of inflammatory cytokines were not measured or included in this study (e.g., training background/history, nutritional status), but may have influenced the findings (Irwin et al. 2008; Kraemer and Ratamess 2005). Nonetheless, sleep opportunities and meals were regulated and consistent across training and between participants, so the potential impact of these factors was likely mitigated.

Conclusions

In conclusion, a 12-week BMT program supported improvements in perceived depression, anxiety and stress. BMT also appears to impart minimal perturbation to inflammatory markers, with a decrease in the pro-inflammatory cytokine TNF-α observed across the training period. The prescribed training loads and/or recovery opportunities within BMT appear to be sufficient to promote recruit readiness for the next stages of their military career. Higher levels of IL-10 were associated with improvements in several subscales of well-being, while the magnitude of relationships between pro-inflammatory cytokines and well-being decreased over BMT, which may be reflective of improvements in well-being. Inflammatory markers may be useful indicators of health status in the military context, however subjective measures may represent more cost-effective proxies that can be administered quickly and easily for practical, ongoing monitoring of recruits.

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Data availability All data generated or analysed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest None declared.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. This study was approved by the Department of Defence and Veteran's Affairs Human Research Ethics Committee (021–17).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent to publish The authors affirm that human research participants provided informed consent for publication of the data contained within the research.

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