Anxiety and perceived psychological stress play an important role in the immune response after exercise

RUNNING HEAD: Psychological stress, exercise and immunity

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ABSTRACT

There are common pathways by which psychological stress and exercise stress alter immunity. However, it remains unknown whether psychological stress plays a role in the *in vivo* immune response to exercise. We examined the relationship between anxiety and perceived psychological stress reported before exercise and *in vivo* immunity after exercise using skin sensitisation with Diphenylcyclopropenone (DPCP). In a randomised design, sixty four, thoroughly familiarised, males completed widely used psychological instruments to assess state-anxiety and perceived psychological stress before exercise, and ran either 30 minutes at 60% (30MI) or 80% (30HI) $\dot{V}O_{2peak}$, 120 minutes at 60% (120MI) $\dot{V}O_{2peak}$ or rested (CON) before DPCP sensitisation. Cutaneous recall to DPCP was measured as the dermal thickening response to a low-dose series DPCP challenge 4-weeks after sensitisation. After accounting for exercise (R^2 = 0.20; P < 0.01), multiple-regression showed that pre-exercise state-anxiety (STAI-S; ΔR^2 = 0.19; P < 0.01) and perceived psychological stress (ΔR^2 = 0.13; P < 0.05) were moderately associated with the DPCP response after exercise. The STAI-S scores before exercise were considered low-to-moderate in these familiarised individuals (median split; mean STAI-S of low 25 and moderate 34). Further examination showed that the DPCP response after exercise (30MI, 30HI or 120MI) was 62% lower in those reporting low *vs*. moderate state-anxiety before exercise (mean difference in dermal thickening: -2.6 mm; 95% CI: -0.8 to -4.4 mm; P < 0.01). As such, the results indicate a beneficial effect of moderate (*vs*. low) state-anxiety and perceived psychological stress on *in vivo* immunity after exercise. Moreover, correlations were of comparable strength for the relationship between physiological stress (heart rate training impulse) and the summed dermal response to DPCP (r = -0.37; 95% CI: -0.05 to -0.62; P = 0.01), and state-anxiety and the summed dermal response to DPCP (r = 0.39; 95% CI: 0.08 to 0.63; P < 0.01). In conclusion, state-anxiety and perceived psychological stress levels before exercise play an

important role in determining the strength of the *in vivo* immune response after exercise. These findings indicate a similar strength relationship for the level of state-anxiety prior to exercise and the level of physiological stress during exercise with the *in vivo* immune response after exercise. Future research is required to investigate exercise-immune responses in athletes, military personnel and others in physically demanding occupations experiencing higher levels of psychological stress than those reported in this study e.g. related to important competition, military operations and major life events. Nevertheless, the present findings support the recommendation that exercise scientists should account for anxiety and psychological stress when examining the immune response to exercise.

KEYWORDS: Running, Immunity, In vivo, Diphencyprone, STAI

INTRODUCTION

Numerous studies report an increase in upper respiratory tract infection (URTI) symptoms following a bout of strenuous exercise and during periods of heavy training in athletes (25, 33, 37), and there is widespread agreement that a transient suppression of immune function is at least partly responsible (48). A multitude of training and lifestyle stressors are thought to be involved in the observed decrease in immune function in athletes and military personnel; including, prolonged training sessions, exposure to environmental extremes (e.g. heat, cold and high altitude), poor nutrition and poor sleep (41-43, 47, 48). For example, prolonged heavy exercise ($\geq$ 2 h) transiently decreases *in vitro* measures of immunity in isolated blood samples (48) and more clinically meaningful *in vivo* measures of immunity instigated at the skin, including delayed type hypersensitivity (DTH) and contact hypersensitivity (CHS) (6, 16, 24). Indeed, recent work highlights the immunosuppressive effect of prolonged exercise (2 h) on the induction of CHS using the novel antigen Diphenylcyclopropenone (DPCP) (16, 24). Besides the immunosuppressive effects of prolonged heavy training sessions, the training environment and lifestyle stressors such as nutritional deficits (e.g. energy, macro- and micro- nutrients) and poor sleep (e.g. total deprivation and disruption) have long been implicated in the decrease in immune function in athletes and military personnel (41-43). Somewhat surprisingly, field studies (multi-stressor environment) and laboratory studies mimicking real-world athletic and military scenarios by exposing participants to these stressors, either separately or combined, demonstrate only subtle and short-lived modulation of immunity at rest and in response to exercise (5, 28, 31, 40). Rather than decrease immunity, some studies actually show a beneficial 'priming' effect of stressors such as short-term sleep disruption (1 night) (28), intermittent cold exposure (29) and intermittent hypoxic exposure on immunity (50). As such, there is a pressing need for research investigating other

likely behavioural, environmental and lifestyle candidates involved in the observed decrease in immune function in athletes and military personnel.

Given the well-known and marked influence of psychological stress on immunity and infection resistance (10, 13), and the likely shared mechanisms by which psychological stress and exercise stress alter immunity (36); i.e. principally through activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic-adrenal-medullary (SAM) axis and subsequent immunomodulatory hormones, it has been hypothesised that psychological stress can play a role in the decrease in immunity with prolonged heavy exercise and heavy training (8, 36, 49). Unfortunately, exercise immunologists rarely report measures of psychological stress in their studies and so there is little by way of empirical evidence to support this hypothesis (38). That there are striking similarities in the way acute and chronic psychological stress and acute and chronic exercise stress influence immunity provides indirect support for this hypothesis. For example, although chronic psychological stress is widely accepted to decrease immunity and increase infection risk (10, 13), short-lasting, moderate-intensity psychological stress can enhance *in vivo* immunity (21) and is considered a fundamental adaptive response to help us survive (13). Similarly, prolonged heavy exercise and heavy training are widely accepted to decrease immunity and increase infection risk (48), but short-lasting, moderate-intensity exercise stress can enhance *in vivo* immunity (34).

With this information in mind, using a multiple linear regression model, we tested, and provide evidence supporting our hypothesis that the level of anxiety and perceived psychological stress reported by an individual prior to exercise play an important role in determining the strength of the *in vivo* immune response to DPCP after exercise.

METHODS

Using the CHS responses to exercise from a previous study (16), here we present previously unpublished and novel insights regarding the influence of anxiety and perceived psychological stress on *in vivo* immunity after exercise.

Participants

Sixty four healthy, non-smoking, recreationally active males (age 22 ± 3 years; height 180 ± 6 cm; body mass 76.7 ± 11.5 kg; $\dot{V}O_{2peak}$ 57 ± 6 mL/kg/min) gave written informed consent to participate in the study. Participants had no previous history of exposure to DPCP and were excluded if they were taking any medication or dietary supplements, or had a history of atopy or any other immune-related or inflammatory dermatological condition. Participants were required to abstain from alcohol and exercise for 24 h before and 48 h after the experimental trials. The study received local ethics committee approval, and all protocols were conducted in accordance with the Declaration of Helsinki (2013).

Participants were matched for age and aerobic fitness (gas exchange threshold and $\dot{V}O_{2peak}$) before being randomly assigned to one of four groups. Groups were 1) 120 min of seated rest (CON); 2) 30 min of moderate-intensity (60% $\dot{V}O_{2peak}$) exercise (30MI); 3) 30 min of high-intensity (80% $\dot{V}O_{2peak}$) exercise (30HI); or 4) 120 min of moderate-intensity (60% $\dot{V}O_{2peak}$) exercise (120MI).

Preliminary measures and familiarisation

$\dot{V}O_{2peak}$ was estimated by means of a ramped exercise test on a treadmill (h/p/cosmos Mercury 4.0, Nussdorf-Traunstein, Germany) as described (16). At least 24 h after the preliminary measures and approximately 7 days before the experimental trial, participants

were informed of their group allocation and attended the laboratory for familiarisation. For exercising participants, the calculated exercise intensity was verified, and the participant was familiarised by running for 50% of their allocated exercise duration. During this visit, all participants were familiarised with blood sampling and other relevant procedures.

Experimental procedures

On the day of the experimental trial, participants were transported to the laboratory at 0730 h and provided with a standard breakfast (0.03 MJ/kg) before completing widely used, validated psychological instruments. The level of anxiety was assessed using the state aspect of the State Trait Anxiety Inventory (STAI-S): the STAI-S is one of the most commonly used scales to measure anxiety, which has been defined as an unpleasant emotional state that exists at a given moment in time and at a particular level of intensity, and is characterised by subjective feelings of tension, apprehension, nervousness, and worry (45). The STAI-S consists of 20-items, with responses being measured on a four-point Likert scale (from 1 ‘not at all’ to 4 ‘very much so’) and a range of scores from 20−80 (composite reliability = 0.94). Perceived psychological stress was assessed using the Perceived Stress Scale (PSS): the PSS is a widely used psychological instrument for measuring the perception of stress, and measures the degree to which life situations are considered stressful by the individual during the previous month (11). The PSS is a 14-item inventory, with responses measured on a five-point Likert scale (from 0 ‘never’ to 4 ‘very often’) and a range of scores from 0−56 (composite reliability = 0.73). Average PSS score for young adults has been reported as 21 ± 7 and high PSS score in posttraumatic stress disorder patients as 34 ± 8 (11, 26). Participants assigned to 120MI began running on a treadmill at 1100 h, and those assigned to 30HI and 30MI began at 1230 h, so that all participants completed the exercise at the same time of day (1300 h). Heart rate was monitored continuously during the experimental trials (Polar FT1,

Polar Electro, Kempele, Finland). Immediately after the exercise, participants showered and returned to the laboratory within 15 min of completion before being sensitised to DPCP at 1320 h, exactly 20 min after exercise cessation. This short standardised delay in sensitisation allowed cutaneous blood flow to return to baseline (16).

Blood collection and analysis

Blood samples were collected before, immediately after, and 1 h after exercise or seated rest by venepuncture into two separate vacutainer tubes (Becton Dickinson, Oxford, UK), one containing K_3EDTA, and one containing lithium heparin. The samples were spun at 1500 *g* for 10 minutes in a refrigerated centrifuge. Plasma was aliquoted into Eppendorf tubes, and immediately frozen at -80 °C for later analysis. Plasma epinephrine and norepinephrine were determined on K_3EDTA plasma, and plasma cortisol was determined on lithium heparin plasma using commercially available ELISA kits (CatCombi, IBL International, Hamburg, Germany and DRG Instruments, Marburg, Germany, respectively). The intra-assay coefficient of variation for plasma epinephrine, norepinephrine and cortisol was 4.1%, 4.1% and 4.4%, respectively.

Induction of CHS

The sensitising exposure to the novel antigen DPCP involved application of an occluded patch, constituting a 12-mm aluminium Finn chamber (Epitest Oy, Tuusula, Finland) on scanpor hypoallergenic tape containing an 11-mm filter paper disc (16). The paper disc was soaked in 22.8 μL of 0.125% DPCP in acetone (patch = 30 μg/cm^2 DPCP) and allowed to dry for 5 min before being applied to the skin on the lower back for exactly 48 h.

Elicitation

The magnitude of *in vivo* immune responsiveness was quantified by measuring the responses elicited by secondary exposure to DPCP. Twenty eight days after the initial sensitisation to DPCP, all participants received a challenge with a low-concentration dose-series of DPCP on individual patches, each comprising an 8-mm aluminium Finn chamber on scanpor hypoallergenic tape containing a 7-mm filter paper disc. Patches were applied to the volar aspect of the upper arm in the following concentrations: 10 μL of DPCP: 0.0048%, 1.24 μg/cm^2; 0.0076%, 1.98 μg/cm^2; 0.0122%, 3.17 μg/cm^2; 0.0195%, 5.08 μg/cm^2; 0.0313%, 8.12 μg/cm^2; and, 10 μL of 100% acetone served as a control patch for background subtraction. Patches were applied in randomly allocated order at the local site to minimise any anatomical variability in responses. Elicitation patches were removed after 6 h, and the strength of immune reactivity was assessed as the cutaneous responses 48 h after application (16).

Assessment of CHS responses

Dermal thickness was determined at each elicitation site using a high-frequency ultrasound scanner (Episcan, Longport Inc, Reading, UK). The ultrasound probe was placed over the centre of each patch site together with ultrasound gel. The mean of three measurements was taken from each 12-mm scan image assessed at a later time by a blinded investigator. Mean skinfold thickness was determined from triplicate measurements at each elicitation site using modified spring-loaded skin callipers (Harpenden Skinfold Calliper, British Indicators, England, UK). As previously described (24), this method provides an objective measure of skin oedema (inflammatory swelling). Skinfold thickness was recorded to the nearest 0.1 mm by placing the jaws of the calliper at the outer diameter of the response site and measuring skin thickness only (no subcutaneous fat). Skinfold thickness assessed using skinfold

callipers has previously been shown to be strongly related (r = 0.93) with high-frequency ultrasound readings of dermal thickness (16). Mean skin erythema was determined from triplicate measurements at each elicitation site using an erythema meter (ColorMeter DSM11, Cortex Technology, Hadsund, Denmark) which provides an objective measure of skin redness (24). Mean background values were determined from triplicate measurements at the acetone patch site for both thickness and redness. To determine the increase in thickness and redness, the value from the acetone-only site was subtracted from each elicitation site value. The values for increase in dermal thickness, skinfold thickness and erythema over all the doses were summed to give an approximation of the area under the dose–response curve, representative of the overall reactivity of each participant to DPCP (24).

Statistical analyses

Hierarchical linear regression analysis was used to examine the relationship between STAI-S and PSS (in 2 separate models) and *in vivo* immunity after exercise. In step 1 of each model, the influence of exercise on the summed dermal thickening response to DPCP was accounted for by calculating the training impulse (TRIMP) to reflect the level of physiological stress, as described (2). In step 2, the influence of each psychological measure on the summed dermal thickening response to DPCP was assessed. Sample size was deemed appropriate for the multiple linear regression analysis with 2 steps, in line with recommendations (46). To further illustrate the influence of anxiety on *in vivo* immunity after exercise, we performed additional analyses by categorising the population based on STAI-S scores using a median split; whereby, the levels before exercise were defined as low anxiety (LOW: STAI-S $\leq$ 29; mean 25) and moderate anxiety (MOD: STAI-S $\geq$ 30; mean 34): the STAI-S ranges for LOW and MOD are in line with those reported in the literature (30, 45). Independent *t*-tests were used to compare the summed dermal responses to DPCP in LOW and MOD in each group

(30MI, 30HI, 120MI and CON). Comparisons of psychological measures between groups (30MI, 30HI, 120MI and CON) were made using one-way ANOVA. A two-way, mixed-model ANOVA was used to analyse DPCP responses across the full dose-series challenge (anxiety level x dose) and circulating stress hormones (anxiety level x time) with significant differences identified using *post hoc* Tukey HSD, where appropriate. Pearson correlation coefficients were also calculated between physiological stress (TRIMP) and the DPCP response, and anxiety and the DPCP response. To determine the influence of anxiety on the threshold DPCP dose that elicits a response, logarithmic transformation was performed on the DPCP data (LOW *vs*. MOD). This enabled the calculation of the *x*-intercept when $y = 0$, using linear regression on the linear portion of the dose-response curve. A threshold dose for a response to DPCP was then calculated by back transformation (antilog). Data are presented as mean ± SD, unless otherwise stated and statistical significance was accepted at $P < 0.05$. Data were checked for normality and where appropriate natural log transformation was performed before analysis. Statistical analyses were performed using common statistical software packages (SPSS 22; IBM, Chicago, IL, and GraphPad Prism 5.0, San Diego, CA). Cohen's *d* effect sizes (*d*) are presented to indicate the meaningfulness of group differences for DPCP responses; whereby, values greater than 0.2, 0.5, and 0.8 represent small, medium, and large effects, respectively (9).

RESULTS

STAI-S Anxiety

Prior to exercise, there were no differences in psychological measures between groups (e.g. STAI-S scores for 30MI, 30HI, 120MI and CON) and participants reported low-to-moderate STAI-S scores (Fig. 1A). In step 1 of the regression model (Table 1), exercise (TRIMP; 78 ± 60 AU) was a significant predictor accounting for 20% of the variance in the summed dermal thickening response to DPCP ($P < 0.01$); whereby, greater physiological stress was associated with a lower DPCP response following exercise. In step 2, STAI-S score was a significant predictor over and above exercise, accounting for an additional 19% of the variance in DPCP response ($P < 0.01$); together, exercise and anxiety accounted for 39% of the variance in the dermal thickening response to DPCP (Table 1). Pearson correlation coefficients were of comparable, moderate strength for the relationship between physiological stress and the summed dermal response to DPCP (TRIMP; $r = -0.37$, $R^2 = 0.13$, $P = 0.01$), and anxiety and the summed dermal response to DPCP (STAI-S score; $r = 0.39$, $R^2 = 0.15$, $P < 0.01$). This association between anxiety before exercise and *in vivo* immunity after an exercise challenge indicates that LOW were more likely to have a lower DPCP response following exercise stress than MOD (Fig. 1B). When reported as the summed response to the five DPCP challenge doses, dermal thickening response was 62% lower in LOW than MOD (LOW 1.6 ± 2.3 and MOD 4.2 ± 3.1 mm; $P < 0.01$; $d = 1.0$).

Table 1 near here

Fig. 1 near here

The ubiquitous influence of anxiety on *in vivo* immunity after exercise challenge (but not rested CON) is further illustrated in the comparisons between LOW and MOD in each group

(30MI, 30HI, 120MI and CON; Fig. 2A-D). Responses to DPCP assessed as skinfold thickness and erythema (data not shown for brevity), were smaller in LOW *vs*. MOD for 30MI ($P < 0.01$) and 30HI ($P < 0.05$; Fig. 2A-B), but not CON. The suppressive effect of LOW *vs*. MOD was also apparent in 120MI ($P = 0.05$; $d = 0.9$; Fig. 2C) which is particularly striking given that the suppressive effect of prolonged exercise on the induction of DPCP immune memory has been reported (16). The lower CHS response to exercise in LOW *vs*. MOD is also illustrated in the smaller dermal thickening response across the full dose-series of DPCP in LOW *vs*. MOD ($F(1, 35) = 11.1$, $P < 0.01$; Fig. 1B for 30MI and 30HI). Furthermore, the threshold dose for a positive response to DPCP was calculated using the linear part of the dose-response curves. Compared with MOD, LOW required a 4-times greater DPCP dose (1.5 $\mu g/cm^2$) to elicit a positive response.

Fig. 2 near here

Perceived Stress Scale

Participants reported low-to-moderate PSS scores (16.5 ± 5.3). After accounting for the influence of exercise in step 1 of the regression model (Table 1), PSS score was a significant, moderate predictor (in step 2), accounting for an additional 13% of the variance in DPCP response ($P < 0.05$); together, exercise and PSS score accounted for 33% of the variance in the dermal thickening response to DPCP (Table 1). This association between the perception of psychological stress in the last month (i.e. the degree to which life situations are considered stressful) and *in vivo* immunity after exercise challenge indicates that participants reporting lower life stress were more likely to have a lower DPCP response following an exercise challenge than participants reporting moderate life stress.

Circulating stress hormones

When comparing LOW and MOD, a significant anxiety level x time interaction was observed for circulating epinephrine concentration ($F(2, 88) = 5.9$; $P < 0.01$); whereby, epinephrine was lower in LOW than MOD at pre-exercise (LOW 0.25 ± 0.17 *vs.* MOD 0.58 ± 0.46 nmol/L; $P < 0.01$), but not different at post or 1 h post-exercise. Similarly, an independent *t*-test showed that circulating cortisol concentration was also lower pre-exercise in LOW than MOD (LOW 545 ± 190 *vs.* MOD 699 ± 289 nmol/L; $P < 0.05$); albeit, there was no significant interaction. Nevertheless, the lower circulating epinephrine and cortisol concentration in LOW than MOD before exercise represent large ($d = 0.94$) and medium ($d = 0.63$) effects, respectively. Circulating norepinephrine was not different between LOW and MOD.

DISCUSSION

The aim of this work was to investigate the influence of anxiety and perceived psychological stress on the *in vivo* immune response after exercise. The findings support our hypothesis that the level of anxiety and perceived psychological stress reported by the individual prior to exercise play an important role in determining the strength of the subsequent *in vivo* immune response after exercise (Table 1 and Fig. 1): *in vivo* immunity was assessed by DPCP sensitisation after exercise and recall responses measured 28 d later. Moreover, the findings indicate a similar, moderate strength relationship for the level of anxiety prior to exercise (STAI-S; $r = 0.39$) and the level of physiological stress during exercise (TRIMP; $r = -0.37$) with the *in vivo* immune response after exercise challenge. The ubiquitous influence of anxiety on the immune response after exercise is further evidenced by a lower *in vivo* immune response to DPCP in individuals reporting low compared with moderate anxiety, regardless of the intensity and duration of the exercise challenge (30MI, 30HI and 120MI, Fig. 2A−C). These findings support the recommendation that exercise scientists should account for anxiety and psychological stress when examining the immune response to exercise.

The findings of the present study demonstrate an important interaction between the a priori level of anxiety and perceived psychological stress and the subsequent immune response after an exercise challenge. We previously showed no significant influence of 30MI or 30HI on *in vivo* immunity (16), but these new insights show a lower *in vivo* immune response in individuals reporting low compared with moderate anxiety in 30MI and 30HI (Fig. 2A−B). Moreover, although we have previously shown a suppressive effect of 120MI compared with rested control on *in vivo* immunity (16), particularly striking is the 50% lower *in vivo* immune

response in individuals reporting low compared with moderate anxiety on 120MI (Fig. 2C). Given that DPCP is benign, determining the clinical significance of these findings, with specific regard to infection (skin and other) is an important avenue for future research. Preferably, the strength of the cutaneous recall response to DPCP could be generalised beyond skin immunity to indicate the immune system's general ability to respond to an infectious challenge. The available evidence in this regard is supportive as cutaneous immune measures are impaired in individuals with acute infectious illness (3, 22), diabetes and psoriasis (1) and predict mortality in critically ill HIV-infected patients (17). That we show lower pre-exercise circulating cortisol and epinephrine in the low compared with moderate anxiety group raises the possibility that stress hormones may modulate the immune response to subsequent exercise; indeed, stress hormones are considered to play important roles in preparing the immune system for challenge (13, 15). For example, administration of physiological doses of corticosterone and epinephrine increased T-cell drainage away from the site of DTH challenge to lymph nodes, which in-turn enhanced the DTH response in rats (15). In addition, adrenalectomy has been shown to eliminate stress-induced immune-enhancement in rats, likely by reducing the glucocorticoid and epinephrine response (15). Nevertheless, post-exercise circulating cortisol and epinephrine were not different between individuals reporting low and moderate anxiety in the present study; as such, further research is required into the underlying mechanisms.

Regarding the timing of the psychological measurements, the findings were unlikely due to an acute anticipatory effect prior to exercise as our participants underwent thorough familiarisation to all procedures, including running 50% of their allocated exercise duration; indeed, the success of familiarisation is shown as similar STAI-S scores prior to exercise and rested CON (Fig. 1A). In addition, our findings for the relationship between STAI-S score

and the *in vivo* immune response after exercise are further supported by the relationship between PSS score and the *in vivo* immune response after exercise: PSS assesses the perception of stress, and measures the degree to which life situations spanning the last month are considered stressful (whereas STAI-S provides an acute measure of anxiety) (11). As such, the PSS findings provide added confidence regarding the observed association between psychological stress and the *in vivo* immune response after exercise challenge. It remains to be shown whether individuals are predisposed to respond to stressful situations, such as competitive sport or military scenarios, in a predictable manner with regards to neuro-endocrine-immune responses. In support of this notion, there is some evidence that personality traits predict endocrine-stress-reactivity (4, 19); nevertheless, further research is required to investigate this novel concept in exercise immunology, and to establish whether the findings of the present study extend to other immune measures e.g. vaccination responses (7) and mucosal immunity (23). Further research is also required to disentangle the influence of psychological and physiological strain during prolonged exercise (e.g. during endurance and ultra-endurance events) on *in vivo* immunity. Psychological stress measurements were made before exercise in the present study and it is reasonable to assume that psychological stress during more prolonged exercise (e.g. 120MI) might also play a role in the observed decrease in the *in vivo* immune response (Fig. 2C).

Bridging the gap between exercise immunology and psycho-neuro-immunology

Research investigators have long since acknowledged a role for psychological stress in the decrease in immunity associated with heavy exercise and training but there is little empirical research to support this hypothesis (8, 36). Since Clow and Hucklebridge's Exercise Immunology Review article highlighting this working hypothesis in 2001 (8) there have been

> 3,000 peer-reviewed publications in exercise immunology (using the search terms 'exercise' and 'immune', Web of Science^{TM}) yet < 5% of these publications include the search terms 'psychological stress' or 'anxiety'. Closer inspection of this small subset of exercise immunology publications reveals that the large majority mention a putative role for psychological stress or anxiety in exercise-immune modulation; however, only a small handful of original investigations either attempt to manipulate psychological stress or include objective measures of psychological stress (27, 32, 38, 39). The present study answers the recent calls to physiologists (51) and exercise immunologists (49) to incorporate objective psychological measurements in their human studies.

The findings herein support the recommendation that exercise immunologists should include aspects of mental health (e.g. psychological stress and others), in a broader conceptual framework of exercise-immune interactions alongside other factors thought to decrease immunity in athletes and military personnel (e.g. prolonged training sessions, poor nutrition etc.). This will inform and direct research questions and experimental designs with the aim of improving our understanding of the complicated exercise-immune interactions and with the potential to provide effective countermeasures to immune impairment in those concerned. To this end, the exercise immunologist's toolkit will be enhanced by joining forces with experts in the ever expanding field of psycho-neuro-immunology to begin to disentangle the psychosocial and physiological underpinning of decreased immunity and increased infection risk in high level athletes, military personnel and others in physically demanding occupations. Our finding that pre-exercise anxiety and perceived psychological stress accounted for additional variance in post-exercise *in vivo* immunity after accounting for exercise (using TRIMP) emphasises the importance of incorporating psychological measurements in studies investigating the immune response to exercise. As do the similar strength correlations for pre-

exercise anxiety (STAI-S; $r = 0.39$) and physiological stress during exercise (TRIMP; $r = -0.37$) with *in vivo* immunity after exercise. These findings indicate a beneficial effect of moderate (*vs*. low) anxiety and perceived psychological stress on *in vivo* immunity after exercise (Fig. 2A and D); as such, the findings accord with the immune-enhancement theory of moderate stress (13, 20, 21). Further research is required to investigate exercise-immune responses in athletes, military personnel and others in physically demanding occupations (e.g. firefighters and mountain rescue workers) experiencing higher levels of psychological stress than those reported in this study e.g. as might occur in relation to important competition, major life events etc. The immuno-suppressive effects of chronic high stress in rats (3 weeks of restraint and shaking stress) (14) and humans (examination period) (44) are widely acknowledged (13). As such, research is required to test the hypothesis that chronic high levels of psychological stress exacerbate the decrease in *in vivo* immunity after exercise. Irrespective, the present findings support the recommendation that exercise scientists should account for anxiety and psychological stress when examining the immune response to exercise, and for coaches and support staff to monitor anxiety and psychological stress alongside more traditional physiological measures of training stress. Accordingly, recent evidence highlights that aspects of mental health such as psychological stress and depression are important risk factors for illness in Olympic athletes (18). In time, studies may demonstrate the utility of interventions to alter psychological stress in order to optimise immunity and host defence in athletes, military personnel and those in physically demanding occupations. There is good reason for optimism as an 8-week mindfulness meditation programme increased the antibody response to influenza vaccine in employees working in a highly stressful environment (*vs*. waiting-list controls) (12). Also, although somewhat limited methodologically, preliminary work in competitive athletes showed that a 3-week stress management intervention reduced the number of days out due to illness and injury (35).

CONCLUSIONS

In conclusion, these findings show that anxiety and perceived psychological stress levels prior to exercise play an important role in determining the strength of the *in vivo* immune response after exercise. Moreover, these findings indicate a similar, moderate strength relationship for the level of state-anxiety prior to exercise and the level of physiological stress during exercise with the *in vivo* immune response after exercise. Future research is required to investigate exercise-immune responses in athletes and others in physically demanding occupations experiencing higher levels of psychological stress than those reported in this study e.g. related to important competition and major life events. Nevertheless, these findings support the recommendation that exercise scientists should account for anxiety and psychological stress when examining the immune response to exercise.

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FIGURE LEGENDS

FIGURE 1. Effect of state-anxiety prior to exercise on the *in vivo* immune response after exercise. (A) Low (LOW) and moderate (MOD) levels of anxiety. Data are Mean ± SD. (B) Contact hypersensitivity (CHS) assessed as elicitation challenge 28 d after DPCP induction. Dermal thickening response to the full dose-series challenge with DPCP is shown (30MI and 30HI). Data are Mean ± SEM for clarity. [1]Shown for comparison.

FIGURE 2. Effect of state-anxiety prior to exercise on the *in vivo* immune response after exercise of varying intensity and duration. (A−D) Summed increase in skinfold thickening response to DPCP challenge for each exercise group (30MI, 30HI and 120MI) and rested CON. Data are Mean ± SD.

TABLE 1. Multiple linear regression analysis examining the influence of state-anxiety and perceived psychological stress level prior to exercise on the subsequent *in vivo* immune response after exercise. Contact hypersensitivity (CHS) assessed as the summed dermal thickening response to the full dose-series elicitation challenge with DPCP 28 d after DPCP induction. After accounting for the negative influence of exercise in step 1, separate models show the positive influence of anxiety (from low to moderate levels), assessed using STAI-S in step 2 (A) and perceived psychological stress (from low to moderate levels) over the last month, assessed using PSS in step 2 (B), respectively.

Dependent variable: CHS	**B**	**SE**	***β***	***t***	**Δ*F***	***R*^{2}**	**Δ*R*^{2}**
A. Step 1							
Exercise (TRIMP)[1]	-0.005	0.002	-0.44	-2.93	8.56	0.20**	0.20**
Step 2							
STAI-S	0.06	0.02	0.44	3.24	10.50	0.39**	0.19**
B. Step 1							
Exercise (TRIMP)[1]	-0.005	0.002	-0.44	-2.93	8.56	0.20**	0.20**
Step 2							
PSS	0.06	0.02	0.36	2.54	6.45	0.33**	0.13*

[1]TRIMP = training impulse; STAI-S = State Trait Anxiety Inventory; PSS = Perceived Stress Scale;* $P < 0.05$; ** $P < 0.01$.

FIGURE 1.

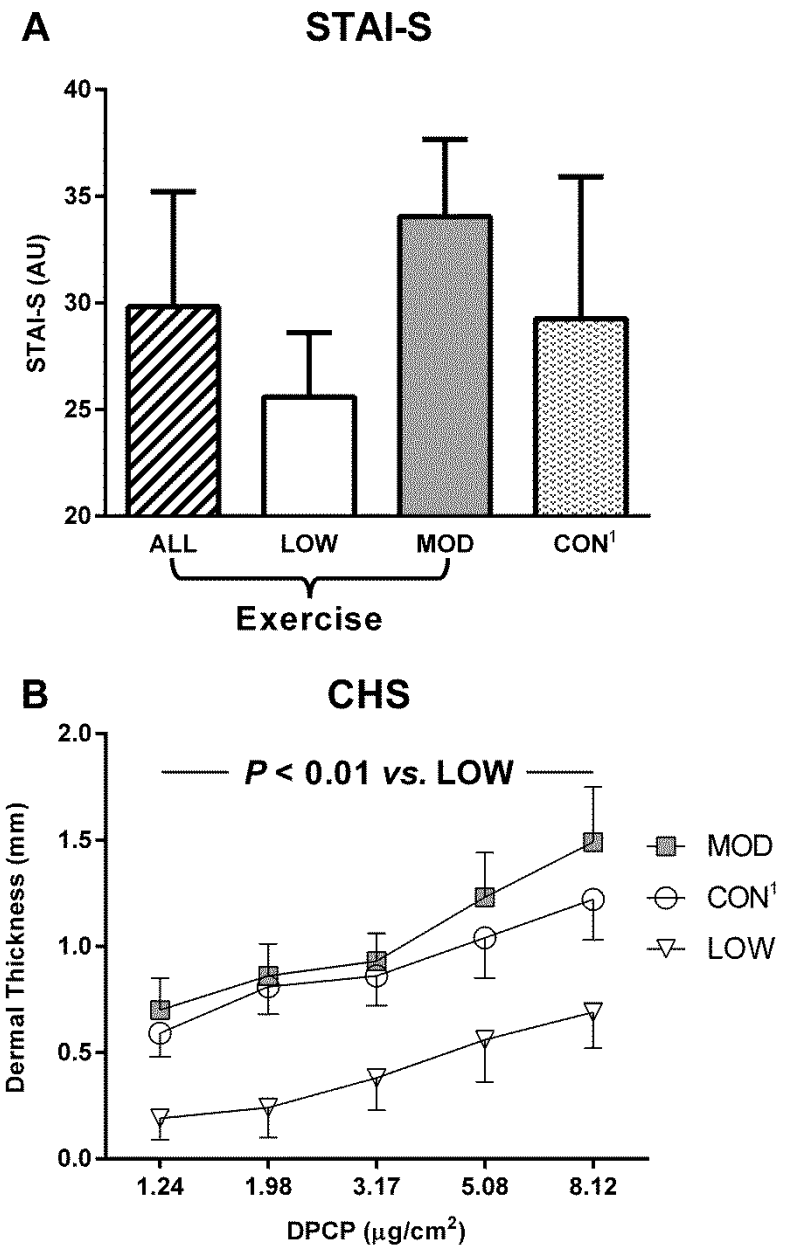
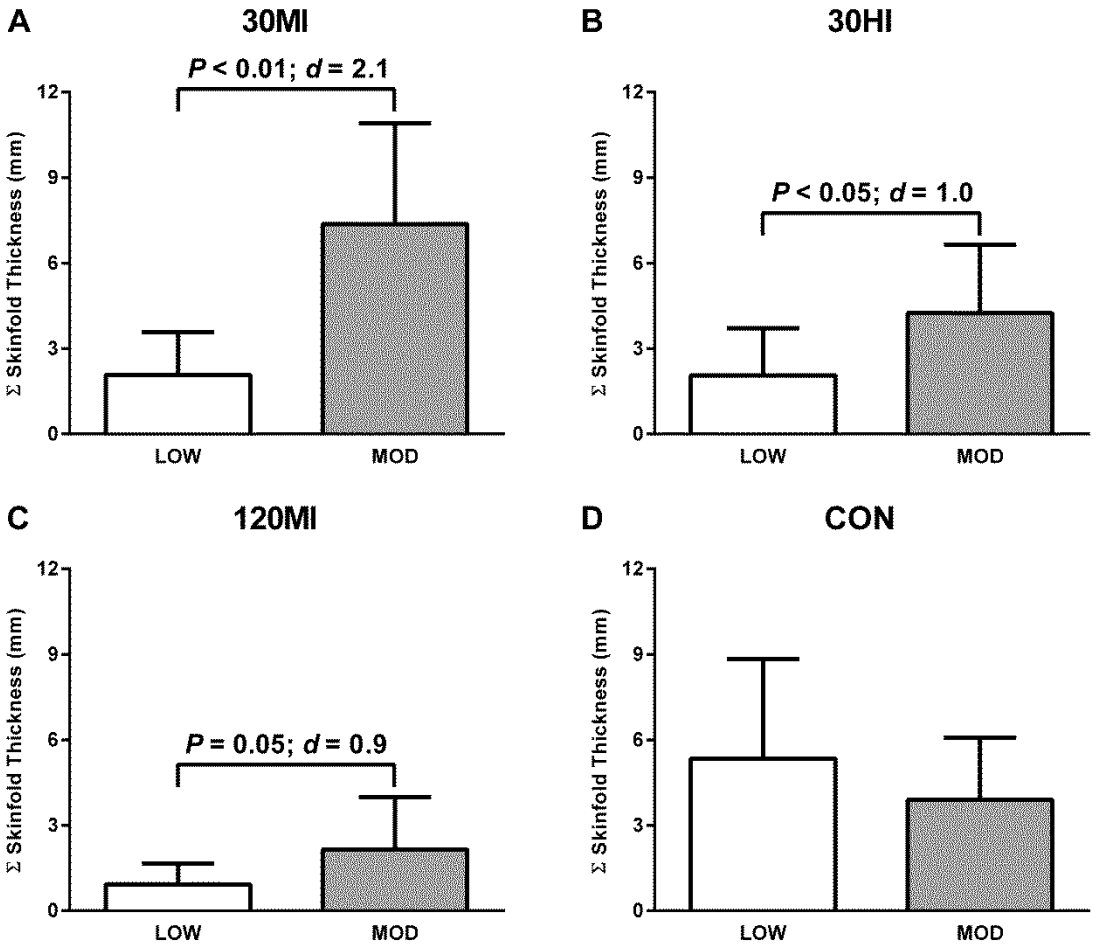

A
STAI-S
STAI-S (AU)
40
35
30
25
20
ALL
LOW
MOD
CON1
Exercise
B
CHS
P < 0.01 vs. LOW
Dermal Thickness (mm)
2.0
1.5
1.0
0.5
0.0
1.24
1.98
3.17
5.08
8.12
DPCP (μg/cm2)
MOD
CON1
LOW

FIGURE 2.


A
30MI
P < 0.01; d = 2.1
B
30HI
P < 0.05; d = 1.0
C
120MI
P = 0.05; d = 0.9
D
CON
Σ Skinfold Thickness (mm)
0
3
6
9
12
LOW
MOD