Exercise Intensity and Duration Effects on In Vivo Immunity

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ABSTRACT

DIMENT, B. C., M. B. FORTES, J. P. EDWARDS, H. G. HANSTOCK, M. D. WARD, H. M. DUNSTALL, P. S. FRIEDMANN, and N. P. WALSH. Exercise Intensity and Duration Effects on In Vivo Immunity. Med. Sci. Sports Exerc., Vol. 47, No. 7, pp. 1390–1398, 2015. Purpose: To examine the effects of intensity and duration of exercise stress on induction of in vivo immunity in humans using experimental contact hypersensitivity (CHS) with the novel antigen diphenylcyclopropenone (DPCP). Methods: Sixty-four healthy males completed either 30 min running at 60% VO2peak (30MI), 30 min running at 80% VO2peak (30HI), 120 min running at 60% VO2peak (120MI), or seated rest (CON). Twenty min later, the subjects received a sensitizing dose of DPCP, and 2 wk later, the strength of immune reactivity was quantified by measuring the cutaneous responses to a low dose-series challenge with DPCP on the upper inner arm. Circulating epinephrine, norepinephrine and cortisol were measured before, after, and 1 h after exercise or CON. Next, to understand better whether the decrease in CHS response on 120MI was due to local inflammatory or T-cell–mediated processes, in a crossover design, 11 healthy males performed 120MI and CON, and cutaneous responses to a dose series of the irritant, croton oil (CO), were assessed on the upper inner arm. Results: Immune induction by DPCP was impaired by 120MI (skinfold thickness ~67% vs CON; P < 0.05). However, immune induction was unaffected by 30MI and 30HI despite elevated circulating catecholamines (30HI vs pre: P < 0.01) and greater circulating cortisol post 30HI (vs CON; P < 0.01). There was no effect of 120MI on skin irritant responses to CO. Conclusions: Prolonged moderate-intensity exercise, but not short-lasting high- or short-lasting moderate-intensity exercise, decreases the induction of in vivo immunity. No effect of prolonged moderate-intensity exercise on the skin’s response to irritant challenge points toward a suppression of cell-mediated immunity in the observed decrease in CHS. Diphenylcyclopropenone provides an attractive tool to assess the effect of exercise on in vivo immunity. Key Words: STRESS, RUNNING, IMMUNE, CONTACT HYPERSENSITIVITY, DIPHENCYPRONE, IRRITANT

The skin constitutes the body’s largest immunological organ, providing the first line of defense against pathogenic and environmental assaults (8). Measures of in vivo immunity at the skin include delayed type hypersensitivity (DTH) responses to intradermal injection of antigens, or the less invasive contact hypersensitivity (CHS) responses to epicutaneous application of antigens. These in vivo measures are considered more informative than the commonly used in vitro measures where immune cells, typically from peripheral blood, are extracted from their normal environment and analyzed in artificial cultures (2). Isolated measures of immune function may react differently to a whole-body immune challenge because they lack the highly integrated neural and hormonal components within the specific tissue environment in which immune responses usually take place (1). Studies using in vivo cutaneous immune measures have shown impaired responses in individuals exposed to psychological stress (3), physical stress (17), during acute infectious illness, e.g., Epstein-Barr virus (5), and in diabetes and psoriasis (4). Furthermore, in vivo cutaneous immune measures have been shown to predict mortality in critically ill HIV-infected patients (12) and in patients with surgical infections (31). There is a need to better understand in vivo cutaneous immune measures for investigators examining the influence of exercise stress on immunity in humans.

Physical exercise provides a well-controlled model to study the effects of stress on immune responses. Given the obvious ethical constraints of studying experimental infection in humans, animal models have provided valuable insight into the effects of exercise on clinically relevant responses to viral infection. The work in animals indicates that prolonged and high-intensity exercise is associated with higher mortality rates, whereas short moderate-intensity exercise lowers mortality rates, compared with controls (21). The research evidence on immune responses after short moderate-intensity exercise in humans is not definitive and tends to indicate immune enhancement only in individuals with suboptimal immune status.
Work in humans indicating that a single bout of short-duration high-intensity exercise and prolonged-duration moderate-intensity exercise decreases immunity is largely based on results of studies examining in vitro immune measures (26,32). Little is known about the impact of a single bout of exercise on cutaneous measures of in vivo immunity in humans. One such study showed that after an acute bout of prolonged continuous exercise (lasting ~6.5 h), DTH reactions to common recall antigens in the Méribius CMI Multitest were reduced, but this test is no longer commercially available (6). Moreover, the use of common recall antigens does not permit the assessment of the effects of stress on the induction of new immune memory, and findings may be confounded by the lack of control over immunological memory: both the sensitizing dose and time elapsed since sensitization influence immunological memory. To the best of our knowledge, no study has investigated the impact of the intensity and duration of continuous exercise stress on in vivo immunity in humans. Challenging the skin using novel antigens such as keyhole limpet hemocyanin (KLH) (35) or diphenylcyclopropenone (DPCP) (17) permits the investigation of the influence of stressors on in vivo immunity and allows rigorous control of both the dose and timing of sensitization. Using topical DPCP, we have recently shown that 2 h of moderate-intensity exercise decreases both the induction of immunity (~53%) in those with no prior exposure to DPCP and elicitation of immunity (~19%) in those who received repeated monthly DPCP exposures to boost responses to a reproducible plateau (17). Possible mechanisms include the activation of the hypothalamic-pituitary-adrenal axis and sympathetic-adrenal-medullary axis, which is widely acknowledged to occur after prolonged stress (typically lasting hours) and in turn increases glucocorticoids and catecholamines, previously shown to decrease the induction of CHS in mice (10,33). It has yet to be determined whether the inhibitory effects of prolonged exercise on immune responses to DPCP are due to systemic effects on the dendritic cell/T-cell axis between the skin and lymph nodes or whether they involve local effects on cutaneous inflammatory processes mediated principally via innate immune mechanisms. The levels of local cutaneous cytokines known to facilitate (e.g., IL-1β) and inhibit (e.g., IL-10) dendritic cell (DC) migration are considered to play a central role in the early DC-dependent events of CHS induction, namely, antigen processing and DC trafficking (38). One experimental approach to this problem is to investigate the effect of prolonged moderate-intensity exercise on cutaneous responses to a topically applied irritant such as croton oil (CO). Unlike DPCP, which ultimately stimulates an antigen-specific, T-cell–mediated immune response, CO is an irritant, which stimulates a non–T-cell–mediated inflammatory response after a single exposure (27). Croton oil has no sensitizing properties but is capable of producing similar cutaneous erythema responses to those seen after CHS challenge (40).

Here, we present the findings from two studies, starting with the effects of intensity and duration of exercise stress on in vivo immune induction by DPCP. We hypothesized that a prolonged moderate-intensity exercise bout (120 min at 60% \( \dot{V}\text{O}_{2\text{peak}} \)) and a short high-intensity exercise bout (30 min at 80% \( \dot{V}\text{O}_{2\text{peak}} \)) would decrease the CHS responses to DPCP compared with a short moderate-intensity exercise bout (30 min at 60% \( \dot{V}\text{O}_{2\text{peak}} \)) and seated rest. Then, to examine whether exercise-related effects on local cutaneous inflammatory processes play a role in the inhibitory effect of prolonged moderate-intensity exercise on the CHS response, we investigated irritant responses to a CO patch test.

**METHODS**

**Subjects**

All subjects were healthy, nonsmoking, recreationally active males with no previous history of exposure to DPCP. Subjects were excluded if they were taking any medication or dietary supplements, had a history of atopy or any other immune-related or inflammatory dermatological conditions. Subjects were required to abstain from caffeine, alcohol, and exercise for 24 h before and 48 h after the experimental trials. All subjects gave written informed consent to participate after being fully briefed and informed of the study’s procedures. The study received local university ethics committee approval and was conducted in accordance with the Declaration of Helsinki principles.

**The Effect of Exercise Intensity and Duration on Induction of DPCP Immune Memory**

Subjects were matched for age and aerobic fitness (gas exchange threshold (GET) and \( \dot{V}\text{O}_{2\text{peak}} \)) before being randomly assigned to one of four experimental groups: 1) 120 min of seated rest (CON); 2) 30 min of moderate intensity (60% \( \dot{V}\text{O}_{2\text{peak}} \)) exercise (30MI); 3) 30 min of high-intensity (80% \( \dot{V}\text{O}_{2\text{peak}} \)) exercise (30HI); or 4) 120 min moderate-intensity (60% \( \dot{V}\text{O}_{2\text{peak}} \)) exercise (120MI) (Fig. 1). These exercise intensities and durations were chosen to allow comparison with the relevant literature (17), to assess the in vivo immune response to exercise recommended to healthy adults for fitness and health (e.g., the ACSM recommends 30-min moderate-intensity exercise most days), to best separate intensity and duration effects on in vivo immunity, and finally, with feasibility in mind (e.g., our subjects could complete 30 min at 80% \( \dot{V}\text{O}_{2\text{peak}} \)). There were no significant differences between groups for characteristics (Table 1). The study was performed between February 2011 and April 2012, and no data were taken from our previous investigation that also included 120MI and CON trials (17).

**Preliminary Measures and Familiarization**

Anthropometric measures were recorded on arrival at the laboratory. Body composition assessment was completed by whole-body dual-energy x-ray absorptiometry (DEXA; Hologic...
QDR Series-4500, USA). After this, \( \dot{V}O_{2peak} \) was estimated by means of a ramped exercise test on a treadmill (h/p/cosmos Mercury 4.0, Nussdorf-Traunstein, Germany). After 3 min of walking at 5 km·h\(^{-1} \) with an incline of 1%, speed increased at a rate of 1 km·h\(^{-1} \)·min\(^{-1} \) to a maximum of 18 km·h\(^{-1} \), after which the incline increased at a rate of 1%·min\(^{-1} \) until volitional exhaustion. Pulmonary gas exchange was measured breath-by-breath for the duration of the test (Cortex Metalyser 3B, Biophysik, Leipzig, Germany). The \( \dot{V}O_{2peak} \) was taken as the highest 30-s average value before the subject’s volitional exhaustion, and the speed equivalent to 60% or 80% of the \( \dot{V}O_{2peak} \) was calculated. The GET was also determined from the ramped exercise test using the V-slope method.

At least 24 h after the preliminary test, each subject’s calculated exercise intensity was verified by running for 50% of their allocated exercise duration, and all subjects were familiarized with laboratory equipment.

**Experimental Procedures**

Dietary intake was controlled during the 24 h before the main experimental trial by providing subjects with their estimated daily energy requirement using DEXA-determined fat-free mass as described (mean ± SD, 11.2 ± 1.1 MJ·day\(^{-1} \)) (9), multiplied by a physical activity factor, and water proportional to 35 mL·kg\(^{-1} \)·d\(^{-1} \) body mass (37).

Within 3 wk of the preliminary testing, on the day of the exercise trial, all subjects were transported to the laboratory at 0730 h and provided with a standard breakfast (0.03 MJ·kg\(^{-1} \)). Subjects were permitted to perform light activity before commencing the intervention. Nude body mass (NBM) was recorded before and after exercise on a digital platform scale to determine water allowance (Model 705; Seca, Hamburg, Germany). Exercising subjects received 5 mL·kg\(^{-1} \) NBM of water immediately before and after the exercise, 2 mL·kg\(^{-1} \) NBM at 15-min intervals throughout, and any additional exercise fluid loss was replaced after exercise. Subjects 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 subjects completed the exercise at the same time of day (1300; Fig. 1). Immediately after the trial, exercising subjects showered and returned to the laboratory within 15 min of completion.

The CON, nonstress condition, consisted of 2 h of seated, passive rest in the same laboratory, in the same ambient conditions of 20°C, at the same time of day, with a fluid intake proportional to 35 mL·kg\(^{-1} \)·d\(^{-1} \) body mass.

**Induction of Contact Sensitivity**

Subjects were sensitized to DPCP at 1320, exactly 20 min after exercise cessation or equivalent seated rest, as described previously (17). This sensitization time was chosen to allow cutaneous blood flow to return to baseline (19). The sensitizing exposure to the novel antigen DPCP involved application of an occluded patch, constituting a 12-mm aluminum Finn chamber (Epitest Oy, Tuusula, Finland) on scanpor hypoallergenic tape containing an 11-mm filter paper disc. 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 the same antigen (Fig. 1). Twenty eight days after the initial sensitization to DPCP, all subjects received a challenge with a low-concentration dose series of DPCP on individual patches, each comprising an 8-mm aluminum Finn chamber on scanpor hypoallergenic tape containing a 7-mm filter paper disc. Patches were applied to the inner 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\(^{-2} \);

**TABLE 1. Subject information.**

<table>
<thead>
<tr>
<th>CON</th>
<th>30MI</th>
<th>30HI</th>
<th>120MI</th>
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<tbody>
<tr>
<td>N</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23 ± 4</td>
<td>20 ± 2</td>
<td>22 ± 4</td>
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<td>Height, cm</td>
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<td>180 ± 5</td>
<td>179 ± 7</td>
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<td>Body mass, kg</td>
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<td>74.5 ± 10.1</td>
<td>76.3 ± 12.8</td>
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<tr>
<td>Body fat, %</td>
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<td>15.1 ± 4.5</td>
<td>15.0 ± 4.7</td>
</tr>
<tr>
<td>( \dot{V}O_{2peak} ), mL·kg(^{-1} )·min(^{-1} )</td>
<td>57 ± 7</td>
<td>58 ± 5</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>GET, L·min(^{-1} )</td>
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<td>3.09 ± 0.59</td>
<td>3.08 ± 0.60</td>
</tr>
<tr>
<td>Weekly exercise (h)</td>
<td>6 ± 4</td>
<td>6 ± 2</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD.
Blood Collection and Analysis

Blood samples (venepuncture from an antecubital vein) were collected into one K3EDTA-coated vacutainer, and one lithium heparin–coated vacutainer (Becton Dickinson, Oxford, UK) before, immediately after, and 1 h after exercise. The samples were spun at 1500g for 10 min in a refrigerated centrifuge. Plasma was aliquoted into Eppendorf tubes, and immediately frozen at −80°C for later analysis.

Plasma epinephrine and norepinephrine concentrations in K3EDTA plasma were determined using a commercially available CatCombi ELISA (IBL International, Hamburg, Germany). Aliquots of lithium heparin plasma were used to determine cortisol concentration by ELISA, performed according to the manufacturer’s instructions (DRG Instruments, Marburg, Germany). The intra-assay coefficients of variation for plasma epinephrine, norepinephrine, and cortisol were 4.1%, 4.1%, and 4.4%, respectively.

The Effect of Prolonged Moderate-Intensity Exercise on the Cutaneous Response to the Irritant, Croton Oil

To investigate the possibility that the inhibitory effect of 120MI on CHS induction was mediated via local effects on cutaneous inflammatory processes, 11 healthy males (age, 24 ± 5 yr; height, 179 ± 8 cm; body mass, 79.0 ± 9.9 kg; VO2peak: 53 ± 6 mL·kg⁻¹·min⁻¹) completed a follow-up study to investigate the cutaneous responses to the nonspecific irritant, CO.

In a randomized, counterbalanced, repeated-measures design, subjects performed 120MI-CO or CON-CO separated by 7–14 d. Subjects received a CO challenge at 1320, exactly 20 min after exercise cessation or seated rest. This involved the topical application of a dose series of CO on individual patches comprising 8-mm aluminum Finn chambers mounted on hypoallergenic scanpor tape and 7-mm filter paper discs. Patches were applied where necessary (skinfold dose-series response to DPCP, epinephrine, norepinephrine, and cortisol). All statistical analyses were conducted using SPSS software. The mean difference with 95% confidence intervals is presented as mean skinfold thickness from triplicate measurements at each elicitation site using modified spring-loaded skin calipers (Harpenden Skinfold Caliper, British Indicators, England, UK), as described (17). Skinfold thickness was recorded to the nearest 0.1 mm by the same investigator by placing the jaws of the caliper at the outer diameter of the response site and measuring skin thickness only (no subcutaneous fat).

Dermal thickness was determined at each patch site using a high-frequency ultrasound scanner (Episcan, Longport Inc, Reading, UK). The ultrasound probe was placed over the center of each patch site together with ultrasound gel. The mean of three measurements was taken from each 12-mm scan image by an independent investigator who was blinded to the trial assignment. Owing to a delay in the availability of this equipment, dermal thickness was assessed in a subpopulation of 50 subjects who completed the DPCP patch test (CON, 13; 30MI, 14; 30HI, 12; and 120MI, 11) and all subjects who completed the CO patch test.

Skin erythema is an objective measure of skin redness, which is considered the key measure of irritant responses (29). This was determined from triplicate measurement at each patch site using an erythema meter (ColorMeter DSM11, Cortex Technology, Hadsund, Denmark) as previously described (17).

Mean background values were determined from triplicate measurements at the vehicle-only patch site for thickness and redness. To determine the increase in thickness and redness in response to DPCP or CO, the value from the vehicle-only site was subtracted from each patch site value. The values for increase in skinfold thickness, dermal thickness and erythema over all the doses were summed, which gave an approximation of the area under the dose-response curve, representative of the overall reactivity of each subject to DPCP or CO, respectively.

Assessment of Cutaneous Responses

Skin edema (inflammatory swelling) is considered the key measure of CHS elicitation responses (17). This was assessed as mean skinfold thickness from triplicate measurements at each elicitation site using modified spring-loaded skin calipers (Harpenden Skinfold Caliper, British Indicators, England, UK), as described (17). Skinfold thickness was recorded to the nearest 0.1 mm by the same investigator by placing the jaws of the caliper at the outer diameter of the response site and measuring skin thickness only (no subcutaneous fat).

Statistical Analysis

Data in the results are presented as mean ± SD, unless otherwise stated and statistical significance was accepted at P < 0.05. Data were checked for normality and sphericity. Greenhouse-Geisser adjustments to the degrees of freedom were applied where necessary (skinfold dose-series response to DPCP, epinephrine, norepinephrine, and cortisol). All statistical analyses were conducted using SPSS software. The mean difference with 95% confidence intervals is presented for the main outcome measures.

Sample size was estimated using data from a previous study examining the effect of prior exercise stress on CHS responses to DPCP (17). The alpha (Type I error rate) was set at 0.05, and power was set at 0.95 (1 − Type II error rate) (G*Power software, version 3.1.2). For the CO element, a minimum important difference using biological variation data of the summed CO erythema response was used to estimate an effect size (0.91). A one-way ANOVA was used to assess differences between the groups in physical characteristics. The effect of exercise intensity and duration was analyzed.
using a one-way ANOVA to determine differences in the summed increase in responses to DPCP between the CON, 30MI, 30HI, and 120MI trials. A two-way, mixed-model ANOVA (DPCP data) or a repeated-measures ANOVA (CO data) was used to analyze the skinfold and dermal thickness responses across the full dose-series challenge (trial–dose). A two-way mixed-model ANOVA (trial–time) was used to compare the circulating stress hormone data. Significant differences were identified using post hoc Tukey HSD or Bonferroni corrected t-tests, where appropriate. To further investigate the differences between CON and 120MI, independent t-tests (DPCP data) or paired t-tests (CO data) were used to assess summed increases. Logarithmic transformation was performed on the DPCP data to allow for 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 positive response to DPCP was then calculated by back transformation (antilog). Simple linear regression and a calculation of the standard error of the estimate (SEE) were performed to assess the validity of skinfold measurement, using skinfold calipers, as a practical method to determine dermal thickening compared with the objective criterion, high-frequency ultrasound. This was performed on the sum of the five elicitation sites for a subpopulation with complete data sets at the 48-h time point (N = 50).

RESULTS
The Effect of Exercise Intensity and Duration on Induction of DPCP Immune Memory

Assessment of CHS responses. The skinfold response, summed from five challenge doses, was significantly different between the groups (F(3,60) = 3.6, P < 0.05). Tukey post hoc analysis revealed that skinfold thickness was reduced 67% by 120MI compared with CON (P < 0.05; Fig. 2A). The mean difference between 120MI and CON was 3.17 mm (95% confidence intervals, 0.31–6.03 mm). There was no significant difference between the short-duration 30MI or 30HI exercise groups compared with CON. The full dose-series response to DPCP for each group was also determined for the increase in skinfold thickness (Fig. 2B). The skinfold thickness responses from the five individual doses revealed a significant trial–dose interaction (F(7.3,145.1) = 3.0, P < 0.01). Post hoc analysis revealed that skinfold thickness was significantly lower in 120MI compared with CON at the 1.98 μg cm⁻² dose (P < 0.05), 5.08 μg cm⁻² and 8.12 doses (P < 0.01) and approached significance at the 3.17 μg cm⁻² dose (P = 0.058). To further investigate the differences between CON and 120MI, the threshold dose for a positive response to DPCP was calculated using the linear part of the dose–response curve, as 0.48 and 2.09 μg cm⁻² for the CON and 120MI groups, respectively. This suggests that to elicit a positive response, 120MI required a 4.4 times greater DPCP dose in comparison with CON. Skinfold thickness assessed using skinfold calipers was strongly related with high-frequency ultrasound readings of dermal thickness (r = 0.93, r² = 0.86, SEE = 1.3 mm; P < 0.01).

Circulating stress hormones. At baseline preexercise, there were no significant differences between the groups for circulating epinephrine, norepinephrine, or cortisol concentration. A significant trial–time interaction was observed for circulating epinephrine (F(4,68.85) = 7.0, P < 0.01; Fig. 3A), norepinephrine (F(3,47.67) = 24.0, P < 0.01; Fig. 3B) and cortisol concentration (F(4,69.06) = 7.0, P < 0.01; Fig. 3C). The raised circulating epinephrine and norepinephrine concentrations...
observed immediately after exercise on both 120MI and 30HI \((P < 0.01)\) had returned to pre-exercise levels by 1 h after exercise. Circulating epinephrine concentration was greater after exercise on 120MI compared with CON \((P < 0.01)\), and circulating norepinephrine concentration was greater after exercise on 30HI compared with CON \((P < 0.01)\). Circulating cortisol concentration was greater after exercise and 1 h after exercise on 120MI and after exercise on 30HI compared with CON \((P < 0.01)\). The typical diurnal response in circulating cortisol concentration is shown, whereby levels were lower after exercise (1300) and 1 h after exercise (1400) compared with pre-exercise (1100) on both 30MI and CON \((P < 0.01)\).

The Effect of Prolonged Moderate-Intensity Exercise on the Induction of DPCP Immune Memory and Cutaneous Responses to the Irritant, Croton Oil

The aim here was to examine whether the inhibitory effect of 120MI on CHS is due to local effects on cutaneous inflammatory processes mediated principally via innate immune mechanisms. To this end, Figure 4 shows the summed responses to all challenge doses for induction of DPCP immune memory (five doses) and irritant responses to CO (four doses). Results are presented as dermal thickness, considered a key measure of CHS responses (17), and erythema, considered a key measure of irritant responses (29). Here, we show that 120MI significantly decreased DPCP responses measured as dermal thickness \((t(22) = 3.5, P < 0.01; \text{Fig. 4B})\) and erythema \((t(30) = 2.1, P < 0.05; \text{Fig. 4A})\). The mean difference for dermal thickness was 3.17 mm (95% confidence intervals, 1.27–5.07) and that for erythema was 18.61 AU (95% confidence intervals, 0.41–36.82). No effect of 120MI-CO on irritant responses measured as erythema \((t(10) = 0.2, P = 0.826; \text{Fig. 4C})\) or dermal thickness \((t(10) = 1.2, P = 0.253; \text{Fig. 4D})\) points to an inhibitory effect of 120MI on cell-mediated...
processes rather than local inflammatory processes in the decrease in CHS. The erythematous response to the top challenge dose of CO was comparable to the erythematous response to the top dose of DPCP (mean ± SD, 11.75 ± 5.28 AU and 11.25 ± 4.84 AU, respectively). As would be expected, dermal thickening response to the dose series of the irritant, CO was small compared with DPCP (Fig. 4D). For visual comparison, the increase in erythema responses to the full dose series of CO is also presented (Fig. 5). There was no significant trial–dose interaction observed between 120MI-CO and CON-CO for erythema responses ($F(3,30) = 1.4, P = 0.267$).

**DISCUSSION**

The advantages of, and the need for further research using, *in vivo* immune measures in humans have recently been highlighted (1,39). The primary aim of this work was to determine the unknown effects of the intensity and duration of continuous exercise stress on the induction of *in vivo* immunity in humans. In line with our hypothesis, prolonged moderate-intensity exercise (120MI) decreased the induction of *in vivo* immunity; however, short-lasting moderate-intensity (30MI) or high-intensity (30HI) exercise did not influence this response despite elevated circulating catecholamines on 30HI and greater circulating cortisol on 30HI compared with CON. We then demonstrated that prolonged exercise had no effect on cutaneous responses to the irritant, CO. These findings support the notion that the observed decrease in *in vivo* immune induction to DPCP represents an effect on T-cell–mediated immune responses rather than exercise effects on local expression of inflammatory effector processes.

This is the first study to compare the effects of intensity and duration of continuous exercise stress on *in vivo* immunity assessed by use of an experimental CHS model in humans. In keeping with our previous findings, we observed that 120MI had a significant inhibitory effect on the induction of new immunity via the skin (17). Our finding that 30MI had no effect on *in vivo* immune induction is at odds with one hypothesis underpinning the J-shaped model (25), whereby a moderate dose of exercise is proposed to be immune enhancing but in accordance with recent research showing no effect of a moderate dose of exercise on the response to vaccination in young, healthy adults (20). Whereas other studies have shown that a moderate dose of exercise can enhance antibody responses to vaccination, thereby supporting one hypothesis underpinning the J-shaped model, this typically occurs in individuals with suboptimal immune status or when a half-dose of vaccine is administered (14,30). We also acknowledge that exercise might differentially affect CHS, a cutaneous T-cell–mediated response, and the antibody response to vaccination, a systemic B-cell–mediated response.

We hypothesized that 30HI would decrease *in vivo* immune induction to DPCP based on evidence from *in vitro* work showing that short-lasting high-intensity exercise decreases indicators of both lymphocyte and neutrophil functions (26,32). However, our results do not support this despite elevated circulating catecholamines on 30HI and greater circulating cortisol on 30HI compared with CON. These findings provide little support for an involvement of circulating stress hormones in the mechanisms associated with altered *in vivo* immune responses to DPCP at the skin. For example, circulating norepinephrine was highest after 30HI when there was no immunosuppression, suggesting that circulating norepinephrine has little immunosuppressive effect on the CHS system. Although circulating cortisol tended to be higher on 120MI compared with 30HI, this did not reach statistical significance. In addition, circulating cortisol exceeded the purported binding capacity (~552 nmol·L$^{-1}$) (22) after exercise before DPCP application in a similar proportion of subjects on 30HI (11 of 16) and 120MI (12 of 16), yet 30HI did not decrease immune induction by DPCP. There is clear evidence from murine models that high doses of these stress hormones can have significant immune-modulating effects. Intradermal injections of high-dose corticosterone or catecholamines, both locally or distant from the sensitization site, inhibit the antigen-presenting capability of cutaneous DCs, reduce the number of T cells in draining lymph nodes, and ultimately suppress DTH and CHS responses (11,15,33). Results from human studies are less consistent, with some authors reporting a lack of association between stress hormones and *in vivo* immune responses (3,13,28). One frequently proposed explanation is that human studies typically rely on individual snapshot assessments of circulating stress hormones, thus missing important information regarding the kinetics of these responses. In this regard, we acknowledge a limitation of the current study in that we applied the DPCP sensitization patch 20 min after exercise at a time when circulating cortisol likely reached a

![Graph showing erythema responses to irritant challenge](image-url)
peak but circulating catecholamines would likely have returned to preexercise levels. At the outset, we considered the strengths and weaknesses of DPCP sensitization at the cessation of exercise to coincide with the peak in circulating catecholamines. After careful consideration, we chose to delay sensitization until 20 min after exercise to avoid possible confounding due to raised skin blood flow and sweating. One might also argue that another limitation is that we only took blood samples to characterize circulating cortisol at immediately after and 1 h after exercise, yet the DPCP sensitizing patch remained in place for 48 h. Work in young adults showed that the inhibitory effect of stress on the development of immune memory is particularly evident when stress is experienced at, or close to, the time of sensitization: This supports our choice of sample timing to characterize the circulating cortisol response in close proximity to the exercise stress (35).

The findings from the current study show that 120MI had no impact on cutaneous inflammatory responses to CO. This suggests that the inhibitory effect of 120MI on CHS induction with DPCP is likely associated with cell-mediated events rather than exercise effects on local inflammatory processes. Further research is required to better understand the mechanisms associated with the inhibitory effect of 120MI on in vivo responses to DPCP. Research should target the interactions between DCs and T cells in antigen processing and presentation and activation of T cells and the subsequent balance between effector and regulatory T cells considered central to the successful induction of CHS (38). Additionally, the duration of the inhibitory effect of prolonged heavy exercise on CHS induction in humans remains unknown and could be determined in a study that manipulates the timing of DPCP sensitization after 120MI. Given the reported sex differences in immune responses to exercise (16), we recognize the limitation of using only men in this study and encourage the investigation of in vivo immune responses to exercise using this CHS model in women.

Experimental CHS provides an attractive measure of in vivo immunity not only because the skin is immediately accessible but because it also overcomes many of the limitations of commonly used in vitro measures, which are lacking in clinical significance and practicality. We recognize that there are limitations with using DPCP in the CHS model described. Given that DPCP is benign, determining the clinical significance of the response, 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 generalized 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 (5), diabetes, and psoriasis (4) and predict mortality in critically ill HIV-infected patients (12). An alternative viewpoint is that the benign characteristic of DPCP actually overcomes the ethical constraints associated with using live pathogens, such as rhinovirus, to assess in vivo immunity. We also recognize the limitation that experimental CHS requires purposefully inducing CHS; nevertheless, the selected doses we use are low and the mild elicitation responses are temporary.

Experimental CHS with DPCP is practical, safe, and can be administered without the need for expensive equipment, invasive injections, or blood sampling, making it a suitable immunological tool for both laboratory and field investigations. Moreover, the use of a novel antigen such as DPCP provides investigators with rigorous control over the timing and dose of sensitizing exposure, enabling the effects of various stressors on the primary immune response to be studied. The measurement of DTH responses to KLH is an alternative percutaneous in vivo method, also reported to represent a primary immune response (36). However, since KLH is derived from a shellfish, this may explain why some individuals exhibit significant responses to KLH before immunization (34). Experimental CHS with DPCP is not restricted to examining the effects of stress on the induction phase. Recently, we have shown that this approach can be used to assess the effect of exercise stress on the elicitation phase in subjects who, after repeated monthly DPCP skin challenges, achieved a reproducible plateau in responses (17). Furthermore, the standardized CHS model we describe overcomes some of the limitations of vaccine models of in vivo immunity including variable immunogenicity (e.g., hepatitis B (18)), annual changes in vaccine (e.g., influenza (7)) and difficulty when comparing the circulating antibody results from different studies using in-house ELISA. Nevertheless, a standard protocol for measuring CHS elicitation responses in humans has yet to be established. The use of erythema to quantify CHS elicitation has been questioned, particularly at sites of stronger responses, where yellow vesicles can interfere with the erythema (redness) readings (17,24). Erythema is typically the preferred measure of irritant responses which, as we show (Fig. 4D), induce less edema than CHS responses (29). Notwithstanding the degree of subjectivity, a particular strength of the current findings is that skin-fold thickness was strongly related with dermal thickness measured by a high-frequency ultrasound scanner and read by a blinded investigator (r = 0.93). Hence, we agree with the recommendation of others that skinfold calipers present a simple and cost-effective measure of CHS edema (24).

In conclusion, using experimental CHS with DPCP, these results demonstrate that prolonged moderate-intensity exercise, but not short-lasting high- or short-lasting moderate-intensity exercise, decreases the induction of in vivo immunity in healthy humans. No effect of prolonged moderate-intensity exercise on the skin’s response to the irritant, CO, points toward a suppression of cell-mediated immunity in the observed decrease in CHS response. The topical application of DPCP provides an attractive tool to assess the effect of exercise stress on in vivo immunity in humans.

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