Effect of exercise, heat stress, and hydration on immune cell number and function

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ABSTRACT

MITCHELL, J. B., J. P. DUGAS, B. K. MCFARLIN, and M. J. NELSON. Effect of exercise, heat stress, and hydration on immune cell number and function. Med. Sci. Sports Exerc., Vol. 34, No. 12, pp. 1941–1950, 2002. Purpose: The purpose of this study was to determine the effect of thermal stress and hydration status on immune function during exercise. Methods: Ten trained men completed four cycle ergometer rides at 55% VO_{peak} under the following conditions: EN (euthydrated neutral; 22°C, 30% RH), DN (dehydrated neutral), EH (euthydrated hot; 38°C, 45% RH), and DH (dehydrated hot). During EN and EH, a carbohydrate/electrolyte beverage was consumed at a rate matching sweat loss, and during DN and DH, no fluid was ingested. Blood samples were drawn pre- and postexercise, and at 2 and 24 h of recovery. Cell counts were determined by automated counting and flow cytometry. Neutrophil activity was assessed as superoxide production, lymphocyte function was determined via PHA-stimulated mitogenesis, and natural killer (NK) cell activity was measured with a ^{51}Cr-release assay. Cortisol was assayed via RIA. Results: Lymphocytes proliferation was depressed 2 h after exercise in all conditions (P < 0.05); however, when expressed on a per cell basis, function was greater in the DH and EH conditions. NK activity (V_{max} · 10^3 cells) was greater post exercise compared with preexercise in all conditions (EH = 25.5 ± 16.8, DH = 26.2 ± 10.5, EN = 19.3 ± 11.0, and DN = 16.5 ± 8.7) but was not different between conditions. Leukocyte, neutrophil, lymphocyte, and NK cell counts were also elevated postexercise with the former two remaining elevated 2 h postexercise in the EH and DH conditions. Cortisol was greater postexercise in EH (22.1 ± 1.3) and DH (27.7 ± 1.3) compared with EN (17.8 ± 2.1) and DN (18.9 ± 1.6 μg·dL^{-1}). Conclusion: Euhydration did not affect cell number or function when compared with a dehydrated state; however, the hot environment caused more severe disturbances in these measures compared with a neutral environment. Key Words: NEUTROPHILS, LYMPHOCYTES, NATURAL KILLER CELL ACTIVITY, CORTISOL.

It is well documented that the immune system is affected by a variety of physiological and psychological stressors (14,22). Physical activity has been shown to cause disturbances in circulating white blood cell number and function that appear to be dependent on the intensity and duration of the exercise, and the associated release of stress hormones (12). Heat exposure is a form of physical stress in which elevations in body core temperature occur with concomitant alterations in hormonal responses and immune system function (4–7,14,23,30). Exercise in a thermally stressful environment represents a combination of physical stimuli that appears to have an additive effect on the hormonal and immune system disturbances (5,6,27). The difficulty that researchers have encountered in this area is separating the independent effects of heat exposure and exercise, as many of the immune system changes elicited by these stimuli are similar (4,5,23,29).

Although severe stress is generally thought to be immunosuppressive, the alterations in immune responses elicited by exercise and/or heat exposure include temporally specific increases and decreases in circulating immune cell numbers, and similar, yet less consistent, bidirectional changes in immune cell function. Hyperthermia produces similar responses to those noted for exercise; however, postexposure responses are less consistent and/or have not been reported (4,15). The changes in numerical and functional responses typically return to normal levels within a 24-h period (11–13,17,18).

In addition to elevations in core temperature and stress hormone responses, exercise in a hot environment elicits a substantial sweat response that results in a pronounced dehydration. The loss of body fluids represents an additional homeostatic disturbance that may interact with heat and exercise to further alter immune system function (20). Exercise in a dehydrated state causes an elevation in heart rate that is due to the decreased stroke volume associated with blood volume reductions and increases in subcutaneous blood flow. If heart rate compensation is not adequate, cardiac output may be compromised. Dehydration also elicits an exaggerated stress hormone response compared with that seen during exercise in a euhydrated state (10). Because the redistribution of immune cells during exercise is influenced by both cardiac output and catecholamine responses (9), dehydration-induced reductions in cardiovascular function may contribute to the alterations in circulating cell numbers. Further, the elevated physical stress associated with dehydration may produce a more pronounced disturbance in immune cell function compared with exercise in a euhydrated state.
To our knowledge, the interaction between exercise in a hot environment and hydration status on immune function has not been systematically studied. This question is of practical importance because the detrimental effects of severe exercise in a thermally stressful environment may be partially avoided with knowledge of the individual effects of heat and hydration on immune function. The purpose of this investigation was to determine the effects of exercise in a hot environment, in combination with exercise-induced dehydration, on circulating immune cell responses and immune cell function. Specifically, leukocyte and leukocyte subset numbers, lymphocyte proliferation, natural killer cell activity (NKCA), and O$_2^-$ production by neutrophils were determined before and during recovery from 75 min of exercise in hot (38°C) and neutral (22°C) environments in both dehydrated and euhydrated conditions. It was hypothesized that the greatest disturbance in immune function would occur in the hot environment in combination with dehydration and that the other conditions would produce graded responses with the neutral environment in combination with euhydration eliciting the least disturbance.

**METHODS**

**Subjects and preliminary testing.** Ten moderately trained men between the ages of 19 and 42, capable of completing 75 min of cycling signed an institutionally approved informed consent form, and were screened for contraindications for vigorous exercise based on their medical history. Preliminary testing consisted of a test for peak aerobic power (VO$_{2peak}$) by using a graded cycle ergometer testing protocol. After the initial testing, each subject completed four 45- to 60-min rides in a warm environment (22°C, 30% RH) to ensure a level of heat tolerance adequate for completing the 75-min rides. During the heat acclimation rides, individual sweat rates were determined by body mass changes. Subject characteristics (mean ± SD) for age, body mass, % fat, and VO$_{2peak}$ were 24.7 ± 6.6 yr, 75.3 ± 9.5 kg, 8.2 ± 4.3%, and 3.6 ± 0.8 L·min$^{-1}$, respectively.

**Experimental design.** After preliminary testing, each subject completed four experimental tests in a randomized, counterbalanced order. The four conditions were 1) euhydration in a neutral environment (22°C, 30% RH) (EN), 2) dehydration in a neutral environment, 3) euhydration in a hot environment (38°C, 45% RH) (EH), and 4) dehydration in a hot environment (DH). During the EH and EN conditions, the subjects ingested a volume of a carbohydrate/electrolyte (CHO/EL) beverage (Gatorade™) equal to the sweat and respiratory water loss for each environmental condition. Because the fluid intake in the EN condition was less than in the EH condition, additional powdered CHO was added to the beverage in order to keep total CHO intake the same between conditions. In the DH and DN conditions, no fluid intake was allowed; however, the subjects consumed an amount of CHO in paste form equivalent to that consumed in the EH and EN conditions. A CHO/EL beverage was used to eliminate CHO depletion as an additional form of stress, which could alter the hormonal responses.

Each experimental trial was separated by at least 7 d during which the subjects resumed their normal physical activity. All testing was completed between 0600 h and 0900 h. The subjects refrained from physical activity during the 24 h before, and after, each trial. Further, the subjects were instructed to record their dietary intake during the 24 h before each trial and to duplicate that diet as closely as possible for each of the four conditions. The diets did not differ between conditions for caloric intake and the percentages of macronutrient intake.

**Experimental testing.** For each condition, the subject began by resting in a supine position for 15 min, after which a baseline blood sample was taken. Before beginning exercise, nude body weight was measured, and the subjects were fitted with a rectal thermistor inserted to a depth of 12 cm for determination of rectal temperature (T$_{rect}$). Subjects then completed 75 min of exercise at a target intensity of 55% VO$_{2peak}$. In the EH and EN conditions, subjects consumed the prescribed volume of CHO/EL beverage in equal increments every 15 min. In the DH and DN conditions, the CHO paste was consumed at a comparable rate. Fluid consumption for the entire ride in the EN trials was 868 ± 197 mL, and in the EH ride it was 1794 ± 240 mL. The CHO consumption was 140.0 ± 19.3, 142.1 ± 17.1, 134.0 ± 11.9, and 136.7 ± 17.3 g for the EN, DN, EH, and DH conditions, respectively. Heart rates and T$_{rect}$ were determined every 15 min throughout the ride. A physiological strain index (PSI), developed by Moran et al. (16), was calculated from the T$_{rect}$ and heart rate (HR) data according to the following formula:

\[
PSI = 5(T_{ref} - T_{rect})(39.5 - T_{ref})^{-1} + 5(HR_{i} - HR_{f})(180 - HR_{f})^{-1},
\]

where T$_{ref}$ is the final T$_{rect}$, T$_{rect}$ is the initial T$_{rect}$, HR$_{f}$ is the final HR, and HR$_{i}$ is the initial HR. Nude body weight was measured immediately after exercise to determine percent dehydration in the DH and DN conditions, and to verify euhydration in the EH and EN conditions. Blood samples were taken immediately postexercise and at 2 and 24 h of recovery (the latter two after 15 min of supine rest). Subjects consumed 5.0 mL·kg$^{-1}$ body mass of the CHO/electrolyte beverage within 15 min of completing the ride and were allowed water _ad libitum_ thereafter until the 2 h blood sample. They then resumed their normal dietary intake until the 24-h sample the next morning.

**Blood sample analysis.** At each time point, blood was drawn from an antecubital vein into three different evacuated collection tubes (Vacutainer, Becton Dickinson, Mountain View, CA): the first contained heparin and was used for the neutrophil function, lymphocyte proliferation, and NKCA procedures; the second contained EDTA and was used for the determination of leukocyte counts and lymphocyte subpopulations via flow cytometry; and the third was a plain tube to obtain serum for cortisol and blood glucose measurements.

Neutrophil O$_2^-$ production was determined as previously described by Pizza et al. (24). Briefly, neutrophils were
isolated using a double-gradient separation medium (Cardinal Associates, Santa Fe, NM), washed with Hanks balanced salt solution (HBSS), and resuspended at a concentration of 2.0·10^6 mL^{-1} in HBSS. An aliquot of the neutrophil suspension was placed in four sets of triplicate culture tubes, two of which also received the stimulant, N-formyl-methionyl-leucyl-phenylalanine (FMLP) at a concentration of 2.0·10^{-6} M in dimethyl sulfoxide. Superoxide dismutase (SOD) at a concentration of 4.0 mg·mL^{-1} was then added to one triplicate set of FMLP-containing tubes and one set of nonstimulated tubes. The other two sets of tubes received an equivalent amount of HBSS. Ferricytochrome c (6 mg·mL^{-1}) was added to all tubes, followed by a 10-min incubation at 37°C. The reaction was stopped by placing the tubes on ice, followed by centrifugation at -8°C. The production of O_2^- from the isolated neutrophils was determined by spectrophotometric measurement of the reduction of ferricytochrome c to ferrocytochrome c at a wavelength of 550 Nm. Quantification of O_2^- was determined by the difference in absorbance between the tubes with and without SOD based on the molar extinction coefficient of ferrocytochrome c. By using the known number of neutrophils in the preparation, O_2^- was expressed on a per cell basis.

Mitogen stimulated lymphocyte proliferation was determined in heparinized whole blood using the mitogen, phytohemagglutinin (PHA) (Difco Labs, Detroit, MI). Briefly, the blood was diluted 1:10 with RPMI 1640 tissue culture media (Sigma Chemical, St. Louis, MO) supplemented with 1.0 mmol·L^{-1} glutamine. Two sets of triplicates of 100 μL of the diluted blood were then plated into microtiter plate wells, and PHA (100 μL) at a concentration of 100 mmol·L^{-1} was added to one set of the triplicate wells. The second set of triplicate wells was used as a control to determine background levels of proliferation and did not receive PHA. The plates were then incubated for 72 h in a humidified atmosphere of 5% CO_2 at 37°C (Model 2310, Shellab, Portland, OR) and subsequently pulsed with 0.5 μCi tritiated thymidine (ICN Radiochemicals, Irvine, CA). After an additional 8-h incubation, the cells were harvested onto glass fiber filters using a cell harvester (Model 1-71757, Nalgene/Nunc, Roskilde, Denmark). Radiolabeled thymidine uptake was determined using liquid scintillation counting (Model LS 5801, Beckman Instruments, Irvine, CA).

A whole-blood chromium release cytotoxicity assay was used to determine NKCA. The target cells utilized were the NK-sensitive erythroleukemic K562 cell line (1,8). Briefly, 150 μL of heparinized whole blood was incubated in triplicate with 50 μL of ^51 Cr-labeled target cells at 2.0, 1.0, 0.5, and 0.25·10^8 cells·mL^{-1} in 250-μL microtiter plates. Wells were also set up for determination of spontaneous release (labeled target cells with NK medium) and total release (labeled target cells with 1% Triton-X). The microtiter plate was incubated for 4 h in a 37°C, 5% CO_2, humidified environment. After incubation, 50 μL of chilled NK cell culture medium was added to each well to stop the cell activity. The plates were then centrifuged, and 100 μL of cell-free supernatant was removed and dispensed into 4-mL scintillation fluid for counting. Data reduction initially involved the calculation of percent lysis as:

\[
\frac{\text{CPM}_{\text{SM}} - \text{CPM}_{\text{SR}}}{\text{CPM}_{\text{TR}} - \text{CPM}_{\text{SR}}}.
\]

where SM is sample, SR is spontaneous release, and TR is total release. By using the percent lysis data from all four concentrations of target cells, the number of targets killed was then determined based on the kinetic calculations described by Baron et al. (1). This procedure produces a single value for each time point that can then be expressed as V_{max}, or the number of targets killed if their number were infinite. This calculation also controls for the number of effector cells in the assay so that with a whole blood procedure, the results are not influenced by variations in the number of circulating NK cells.

The flow cytometry procedure involved preparation of the cells by using a Coulter Immunoprep Leukocyte Preparation System (Q-Prep, Coulter Electronics, Hialeah, FL). A 100-μL aliquot of the cell preparation from the Q-Prep was washed with phosphate-buffered saline (PBS) and incubated at room temperature for 10 min with 5 μL of a monoclonal antibody for lymphocyte subsets and interleukin-2 receptor expression (Becton Dickinson). The monoclonal antibodies were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), and peridinin-chlorophyll-a-protein (PERCP) (Becton Dickinson). The conjugated monoclonal antibodies were specific for T lymphocytes (CD3+ (PERCP)), helper/inducer lymphocytes (CD3+ (PERCP)/CD4+ (FITC)/CD8- (PE)), cytotoxic/suppressor lymphocytes (CD3+ (PERCP)/CD8+ (PE)/CD4- (FITC)), B lymphocytes (CD20+ (PERCP)), and NK cells (CD16+ (FITC)/CD56+ (PE)). The antibody-labeled cells were analyzed using a Cytorion Absolute flow cytometer (Ortho Diagnostics, Raritan, NJ). Complete and differential blood counts were also determined from the blood drawn into EDTA treated tubes using an automated analyzer (Sysmex K1000, TOA Electronics, Kobe, Japan).

From the serum samples, glucose was determined using a colorimetric method (procedure no. 510, Sigma Chemical) on a spectrophotometer (Spectronic 601, Spectronic Instruments Inc., Rochester, NY). Cortisol was determined using a RIA method (procedure no. 9002, ICN Radiochemicals, Costa Mesa, CA) followed by detection of counts per minute on a gamma counter (Beckman Instruments, Fullerton, CA).

**Statistical analysis.** All data based on blood sampling were analyzed using a three factor (2 × 2 × 4) analysis of variance (ANOVA) with repeated measures. The Greenhouse-Geisser epsilon was used in all analyses to correct for repeated sampling. The two levels of the first factor, fluid, were euhydric and dehydrated; the two levels of the second factor, environment, were neutral and hot; and the four levels of the third factor, time, were pre- and postexercise, and 2 h and 24 h after exercise. Heart rate and T_c were analyzed using a 2 × 2 × 6 ANOVA. Differences detected by the ANOVAs were located with a Newman-Keuls post hoc test. Significance was accepted at the P < 0.05 level. The effect sizes for the dependent variables.
analyzed range from approximately 0.50 to greater than 2.0. For the lower effect sizes, with \( N = 10 \), the power value would be approximately 0.4; however, the majority of the variables had effect sizes in excess of 0.70. With \( N = 10 \), the power values for the larger effect sizes were between 0.70 and 0.80.

RESULTS

**Fluid and metabolic data.** There was a significantly greater level of dehydration in the DH and DN conditions compared with EN and EH, and the level of dehydration in DH was greater than that in DN (Fig. 1A). The loss of plasma volume was greater in EH and DH compared with EN and DN (Fig. 1B). There was also a significantly greater decline in plasma volume in DH compared with EH.

Core temperature and heart responses in EH and DH were greater than those in EN and DN beginning at 45 and 15 min of exercise, respectively, and continuing until the end of exercise (Fig. 2, A and B). The physiological strain index (PSI) computed from these two variables showed significantly lower levels in the EN and DN conditions (3.17 ± 0.53 and 3.59 ± 0.54) compared with those in the EH and DH conditions (8.13 ± 2.41 and 8.32 ± 1.02). The EH and DH conditions were not different from each other for the PSI. The oxygen uptake responses were not different between conditions and corresponded to an actual intensity of approximately 50% \( \dot{V}O_2\text{peak} \) (1.83 ± 0.21, 1.78 ± 0.24, 1.77 ± 0.26, and 1.76 ± 0.20 L·min⁻¹ for EN, DN, EH, and DH, respectively).

Serum glucose and cortisol levels in EH and DH were significantly greater than those in EN and DN at the postexercise time point (Fig. 3, A and B). The postexercise serum glucose response was greater than preexercise in the DH condition, and the 2-h response was less than preexercise in all the conditions except DN. Cortisol levels at the 2-h time point were lower than preexercise in all conditions except DH.

**Neutrophil data.** All of the cytometric data were corrected for changes in plasma volume. Total leukocyte number was greater in EH and DH compared with EN and DN postexercise and at 2 h of recovery (Table 1). Circulating neutrophil numbers were significantly greater in the EH and DH conditions at the 2-h time point (Fig. 4A). These levels were also significantly elevated above preexercise at the postexercise and 2-h time points in all but the EN condition. Superoxide anion production from isolated neutrophils expressed on a per cell basis was greater in DH than DN.

![FIGURE 1—Percent dehydration (A) and percent change in plasma volume (B); ‡ indicates a difference from all other conditions; * indicates that EH and DH are different from EN and DN and that DH is different from EH at the postexercise time point (\( P < 0.05 \)).](image1)

![FIGURE 2—Core temperature (A) and heart rate responses (B): * indicates that EH and DH are different from EN and DN at the time points indicated (\( P < 0.05 \)).](image2)
postexercise, and EH was greater than EN at the 2-h time point (Fig. 4B). When superoxide anion production was expressed as a percent change from preexercise, DH was greater than EN and EH postexercise, and the level in the EN condition was less than all other conditions as the 2-h time point (Fig. 4C).

**Lymphocyte data.** The number of circulating lymphocytes was significantly higher in EH and DH compared with EN and DN at the postexercise time point (Fig. 5A). The levels at the 2-h time point were significantly less than preexercise in all conditions. Lymphocyte proliferation was significantly lower at the 2-h time point compared with preexercise for all conditions (Fig. 5B). When expressed as CPM per lymphocyte, lymphocyte proliferation at the 2-h time point in EH and DH was significantly greater than in EN and DN (Fig. 5C).

The number of NK cells was greater in EH and DH compared with EN and DN at the postexercise time point (Fig. 6A). Further, NK number at the postexercise time point was significantly greater than preexercise for all conditions. There were no differences between the levels observed preexercise, and at the 2-h and 24-h time points. NKCA, expressed as the percent cytotoxicity for a 1:1 effector to target ratio, was greater at the postexercise time point compared with preexercise for all conditions (Fig. 6B).

Lymphocyte subsets (CD3+, CD4+, and CD8+ cells) followed a pattern similar to that observed for NK cells (Table 2). T-cell numbers in EH and DH were greater than in the EN and DN conditions immediately postexercise. This was followed by a reversal at 2-h postexercise such that T-cell levels in EH and DH were below those in EN and DN. CD4+ and CD8+ responses demonstrated a similar response, although the differences between conditions at the 2-h time point were not consistent.

**DISCUSSION**

The primary findings of this investigation were that the fluid and environmental manipulations produced the expected differences between conditions in the body fluid balance, temperature, and cardiovascular responses; however, environment as an independent factor produced more pronounced differences in these variables than fluid manipulations. Further, there were metabolic and hormonal differences elicited by the experimental manipulations that were in proportion to the relative degree of stress imposed primarily by the differences in environment. The elevations in cell number after exercise were greatest in the hot environment for both levels of hydration; thus, hydration status did not influence the distribution of leukocytes, lymphocytes, or lymphocyte subsets. Elevations in cell function following exercise were also influenced primarily by the exposure to a hot environment with the only dehydration-induced effect being a postexercise elevation in superoxide production by neutrophils in the DH condition. The addition of a resting control condition in the hot environment would have helped in making more definitive conclusions regarding the separate effects of exercise and the hot environment.

**TABLE 1. Total leukocyte data.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time</th>
<th>Pre-Ex</th>
<th>Post-Ex</th>
<th>2 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN</td>
<td>5.71 ± 1.22</td>
<td>5.94 ± 1.24</td>
<td>5.62 ± 0.98</td>
<td>5.55 ± 1.34</td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>5.73 ± 1.58</td>
<td>6.07 ± 1.64</td>
<td>5.91 ± 1.32</td>
<td>6.09 ± 2.16</td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>5.95 ± 1.25</td>
<td>6.79* ± 1.07</td>
<td>6.68* ± 1.12</td>
<td>5.59 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>DH</td>
<td>5.73 ± 1.30</td>
<td>6.40* ± 1.40</td>
<td>6.82* ± 1.29</td>
<td>5.74 ± 1.23</td>
<td></td>
</tr>
</tbody>
</table>

* EH and DH different from EN and DN post exercise and at 2 h of recovery (P < 0.05).

All values are the mean ± SD.

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Fluid and metabolic data. The level of dehydration in the DN and DH conditions was of a magnitude that would be expected for exercise of this intensity and duration under the two environmental conditions. These levels correspond to sweat rates of approximately 10.00 and 23.00 mL·min⁻¹, for the DN and DH conditions, respectively. The greater loss of plasma in the DH condition shows the combined influence of sweat loss and the exercise-induced shift in fluid from the vascular space.

As indicated by the $T_c$ and heart rate responses, and based on the physiological strain index, the hot environmental conditions produced the greatest stress on the body. A pattern of environment by time interactions without a significant fluid effect appeared throughout the data with few exceptions. The serum glucose and cortisol responses show a similar pattern with the greatest postexercise levels observed in the EH and DH conditions. The greater serum glucose levels observed in the two hot conditions occurred despite an equal oral glucose intake in all conditions. This was apparently due to a greater hepatic release that was probably due to the elevated sympathoadrenal response observed in hot environments (26). The significantly lower
cortisol level measured at the 2-h time point in all conditions may be due to a diurnal effect because all the trials were conducted in the early morning and the 2-h blood sample corresponded to a time of day when cortisol levels are known to decline. It is also possible that the postexercise responses were also influenced by diurnal effects; however, they did appear to exhibit a graded response based on the relative stress imposed by each condition. Reductions in cortisol levels during short-term recovery have not been observed consistently in previous studies (12,18).

It was expected that fluid restriction would add an additional element of physiological strain that would manifest itself in at least the heart rate response; however, we did not observe this effect. The fairly low relative exercise intensity combined with the fitness level of the subjects may be responsible for this unexpected finding because the influence of graded dehydration on cardiovascular responses observed previously involved a higher exercise intensity. Regardless of the cause, the lack of a fluid effect on the cardiovascular system, and concomitantly, on a number of the primary variables of this investigation is important because we had hypothesized that there would be a greater stress imposed in the dehydrated conditions, especially the DH condition.

**Circulating cell number responses.** An exercise-induced elevation in circulating leukocytes was observed only in the hot conditions; thus, it appears that the relatively low exercise intensity and the associated stress of the exercise in the neutral environment were not adequate to elicit a mobilization of these cells. The elevation in circulating neutrophils at 2 h of recovery is typical of the response reported by a number of previous investigators (12,17,19). Circulating neutrophil elevations appeared to follow a pattern corresponding to the degree of stress imposed, with the hot environment providing an additive stimulus for neutrophil mobilization into the circulation. In studies where a resting control condition in a hot environment has been employed, heat exposure alone had little effect on circulating neutrophil number (6,27). Exercise in a hot environment has, however, produced greater mobilization of cells compared with exercise in a neutral environment or when \( T_c \) is clamped (6,26,27). This suggests that the additive effect is present even when individual stimuli may not be powerful enough to produce a measurable effect.

### TABLE 2. Lymphocyte subset data.

<table>
<thead>
<tr>
<th>Condition/Variable</th>
<th>Time→</th>
<th>Pre-Ex</th>
<th>Post-Ex</th>
<th>2 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD3+ cells (x10⁶ mL⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EN</td>
<td>1.40 ± 0.44</td>
<td>1.27 ± 0.31</td>
<td>1.10 ± 0.30</td>
<td>1.14 ± 0.24</td>
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<tr>
<td>DN</td>
<td>1.59 ± 0.45</td>
<td>1.26 ± 0.48</td>
<td>1.10 ± 0.45</td>
<td>1.26 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>1.49 ± 0.46</td>
<td>1.57* ± 0.35</td>
<td>1.05* ± 0.36</td>
<td>1.36 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>DH</td>
<td>1.63 ± 0.53</td>
<td>1.55* ± 0.63</td>
<td>0.88* ± 0.36</td>
<td>1.37 ± 0.37</td>
<td></td>
</tr>
<tr>
<td><strong>CD4+ cells (x10⁶ mL⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EN</td>
<td>0.80 ± 0.27</td>
<td>0.66 ± 0.16</td>
<td>0.60 ± 0.10</td>
<td>0.60† ± 0.24</td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>0.90 ± 0.26</td>
<td>0.66 ± 0.18</td>
<td>0.59 ± 0.19</td>
<td>0.72 ± 0.18</td>
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</tr>
<tr>
<td>EH</td>
<td>0.85 ± 0.32</td>
<td>0.82* ± 0.19</td>
<td>0.57 ± 0.23</td>
<td>0.76 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>DH</td>
<td>0.93 ± 0.32</td>
<td>0.78* ± 0.27</td>
<td>0.46± ± 0.19</td>
<td>0.76 ± 0.14</td>
<td></td>
</tr>
<tr>
<td><strong>CD8+ cells (x10⁶ mL⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EN</td>
<td>0.48 ± 0.18</td>
<td>0.45 ± 0.19</td>
<td>0.40 ± 0.21</td>
<td>0.38 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>0.54 ± 0.23</td>
<td>0.44 ± 0.26</td>
<td>0.40 ± 0.26</td>
<td>0.43 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>0.50 ± 0.19</td>
<td>0.56* ± 0.24</td>
<td>0.38 ± 0.20</td>
<td>0.47 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>DH</td>
<td>0.56 ± 0.23</td>
<td>0.55* ± 0.31</td>
<td>0.32 ± 0.20</td>
<td>0.48 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

* EH and DH different from EN and DN post-exercise.
† DH different from all others at 2-h and EN different from all others at 24-h.
All values are the mean ± SD; \( P < 0.05 \).
It has been reported that the initial exercise-induced elevation in neutrophils is due to demargination mediated by catecholamine release and that the delayed elevations are a result of plasma cortisol acting upon neutrophils from bone marrow (25). Given that heat exposure alone can produce elevations in catecholamine levels, and that there were significant differences in $T_c$, it is surprising that neutrophil number was not elevated to a greater extent immediately postexercise in the EH and DH conditions. The elevated cortisol levels in the DH and EH conditions would, however, correspond to the proposed role of cortisol as a mediator of the neutrophilia observed during recovery. The lack of separation between conditions in the immediate postexercise responses may be due to the low exercise intensity because many of the previous studies have employed intensities of 65–75% of $V_{O_2}^{max}$ or higher (6,12,26).

The 5–10% elevation in circulating lymphocytes in the hot conditions was small compared with previous reports in which circulating cell numbers have increased by almost twofold (12,18). Despite the high physiological strain index, the elevation in the EH and DH conditions was similar to that reported by Nieman et al. (18) after “moderate” (50% $V_{O_2}^{max}$) exercise in a neutral environment. The NK cell number responses mirrored those observed for lymphocyte number; however, the exercise-induced elevations were more pronounced, especially in the DH and EH conditions. This response is in keeping with the intensity-dependent, and therefore, the stress-dependent nature of NK cell recruitment into the circulation (14,18).

The current findings suggest that the combination of physical stressors stimulated the mobilization of all lymphocyte subsets to a greater extent than exercise alone. The additive effects of heat and exercise on circulating lymphocytes was also observed by Rhind et al. (26), and others who found that exertional hyperthermia affects cell distribution via the combination of exercise and thermally induced activation of the sympathetic nervous system and the elevation of hormones associated with a generalized stress response (6,27). The significant elevation of cortisol in the EH and DH conditions would support this conclusion. Again, there was no fluid interaction; thus, the hot environment appears to be the primary mediator of the additive response with exercise.

At 2 h postexercise, there was a significant depression in lymphocyte number that occurred in all conditions. This was driven primarily by the decreases in CD3+ cells. Although this has been reported previously (12,22), it is somewhat unexpected in the EN and DN conditions because no exercise-induced elevation was observed and it could be assumed that an exercise and/or stress-induced elevation is a prerequisite to the subsequent depression. This finding suggests that exercise-induced depressions are not always tied to a preceding exercise-induced lymphocytosis.

Cell function responses. Neutrophil function involves a variety of cellular responses, including phagocytic activity, adherence, and the respiratory burst (25,28). Previous research on the effects of exercise on these processes has produced results suggesting intensity-dependent effects, with moderate exercise enhancing function and more severe exercise leading to suppression of some or all of the responses (25,28). In the current study, the superoxide data were more variable than any of the other functional responses. When expressed as superoxide production on a per cell basis, a three-way interaction showed exercise, heat, and fluid effects that indicated enhanced function. The percent change data for this measurement showed a cleaner pattern suggesting that the mild exercise in a neutral environment did not produce an adequate stimulus for enhanced function whereas either the addition of heat stress or dehydration stimulated greater activity in the oxidative burst pathway. The more severe DH condition brought about an earlier and more prolonged activation.

When expressed as unadjusted counts per minute, the lymphocyte proliferation responses mirrored the cell number results, thus suggesting that the reductions observed at 2 h postexercise were due to fewer cells present in the assay. When adjusted for cell number, the exercise-induced depression was eliminated, and a significant elevation at 2 h of recovery in the EH and DH conditions appeared. When Neiman et al. (18) adjusted proliferative responses for CD3+ cell number, they observed that the suppression observed during recovery was eliminated. Although recovery responses increased above resting in their study, the elevations were not significant.

Cortisol, an intensity-dependent hormone, is known to regulate immune cell function. Specifically, it has been shown to reduce proliferation via the down-regulation of IL-1 receptors on the cell surface (5). Cortisol levels were the highest in the DH and EH conditions; thus, suppression of function in those conditions might be expected; however, the elevations in cortisol may not have been great enough to elicit this expected effect. Another consideration is the possible competing effects of enhanced proliferation produced by moderate elevations in tissue temperature versus the impairment mediated by cortisol. It has been reported that moderate increases in $T_c$ in response to relatively short periods of heat exposure bring about elevations in lymphocyte proliferation, whereas exposure of an hour or more or severe elevations in $T_c$ may reduce proliferation (4). Although there was considerable thermal strain in the DH and EH conditions of the current study, the elevation of approximately 2°C would not be considered severe, especially because values climbed throughout the exercise and did not reach 39°C until near the end of the exercise. Therefore, the enhanced proliferation observed at 2 h of recovery in the hot conditions, as a combination of exercise and temperature, or as a temperature effect alone, seems to be in agreement with previous findings.

The NKCA responses paralleled those observed for cell number; however, the lack of an environment effect suggests that cell function was not altered by the addition of heat exposure. Previous work has produced both the elevations observed in the current study and also a postexercise suppression usually present at 1–2 h of recovery (2,19,21). We have reported the cytotoxicity using a method that accounts for the number of effectors and targets in the assay; thus, variations in
circulating cells would not influence the NKCA results (1). It is of interest to note that significant condition and time differences were eliminated when Neiman et al. (19) expressed lytic units per NK cell number. Pedersen et al. (21) reported data suggesting that the exercise-induced impairment of NKCA was due to elevated prostaglandin release. Neither the current study nor those in which adjustments for cell number were made support this finding (3). The differences in in vitro assay procedures should be considered because cell isolation removes a variety of potential mediators of function from the assay environment. Despite the aforementioned differences in findings and possible methodological issues, in the current study, NKCA was not influenced by heat exposure and/or the fluid status of the individual. This finding is in agreement with that of Tate et al. (29), who reported no differences in NKCA between responses after exercise in hot and normal environments; however, it is not in agreement with previous studies in which heat alone or exercise alone produced alterations in NKCA (15,19,30). Although the data suggested a tendency of heat and exercise can be made.

Conclusion. In general, the numerous responses assessed in this investigation indicate that the combination of exercise and heat exposure produced an additive effect that was indicative of enhanced immune function. Based upon the general concept that severe stress is immunosuppressive, we initially hypothesized that the more extreme conditions would produce greater disturbances that would indicate depressed function. Our data do not support this hypothesis, and from a practical standpoint, they suggest that the level of physical stress imposed in this study was not immunosuppressive.

The primary limitation of this study was the absence of resting control conditions, particularly in the hot environment. The addition of this condition would have allowed a more precise separation of exercise and environmental effects, thus allowing more definitive conclusions regarding additive responses. A second issue that should be addressed is the interaction of the subjects’ training status and the exercise intensity. As has been postulated by previous investigators, the critical exercise variables that determine whether a bout of exercise will cause disturbances in immune function are intensity and duration (12,18,22). Despite the superimposition of heat stress and dehydration, it is probable that, in particular, the intensity used in this study was not high enough to elicit more severe disturbances in this population of subjects. Future studies should address the possible interaction of heat, fluid status, and exercise by using higher intensity or longer duration exercise and a resting control condition in a hot environment.

This work was funded by the TCU Research and Creative Activities Fund, grant number S-23619, and a grant from the Gatorade Sport Science Institute.

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