Human blood neutrophil responses to prolonged exercise with and without a thermal clamp

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Laing SJ, Jackson AR, Walters R, Lloyd-Jones E, Whitham M, Maassen N, Walsh NP. Human blood neutrophil responses to prolonged exercise with and without a thermal clamp. J Appl Physiol 104: 20–26, 2008. First published September 27, 2007; doi:10.1152/japplphysiol.00792.2007.—The purpose of this study was to investigate the effects of prolonged exercise with and without a thermal clamp on neutrophil trafficking, bacterial-stimulated neutrophil degranulation, stress hormones, and cytokine responses. Thirteen healthy male volunteers (means ± SE: age 21 ± 1 yr; mass 74.9 ± 2.1 kg; maximal oxygen uptake 58 ± 1 ml·kg⁻¹·min⁻¹) completed four randomly assigned, 2-h water-immersion trials separated by 7 days. Trials were exercise-induced heating (EX-H: water temperature 36°C), exercise with a thermal clamp (EX-C: 24°C), passive heating (PA-H: 38.5°C), and control (CON: 35°C). EX-H and EX-C was comprised of 2 h of deep water running at 58% maximal oxygen uptake. Blood samples were collected at pre-, post-, and 1 h postimmersion. Core body temperature was unaltered on CON, clamped on EX-C (–0.02°C), and rose by 2.23°C and 2.31°C on EX-H and PA-H, respectively. Exercising with a thermal clamp did not blunt the neutrophilia postexercise (EX-C postexercise: 9.6 ± 1.1 and EX-H postexercise: 9.8 ± 1.0 x 10⁹/liter). Neutrophil degranulation decreased (P < 0.01) similarly immediately after PA-H (–21%), EX-C, and EX-H (–28%). EX-C blunted the circulating norepinephrine, cortisol, granulocyte-colony stimulating factor, and IL-6 response (P < 0.01) but not the plasma epinephrine and serum growth hormone response. These results show a similar neutrophilia and decrease in neutrophil degranulation after prolonged exercise with and without a thermal clamp. As such, the rise in core body temperature does not appear to mediate neutrophil trafficking and degranulation responses to prolonged exercise. In addition, these results suggest a limited role for cortisol, granulocyte-colony stimulating factor, and IL-6 in the observed neutrophil responses to prolonged exercise.

immediate; phagocyte; environment; heat; immersion

Although evidence supports a relationship between neuroendocrine and immune responses to exercise (16, 36), a clear role for stress hormones in the neutrophilia of prolonged exercise (lasting >1 h) has not been identified. Compared with thermoneutral conditions, greater circulating leukocyte counts, catecholamines, and cortisol have been observed following short fixed-duration exercise in hot conditions and passive heat exposure (6, 20, 42). Indeed, elevations in circulating leukocyte and neutrophil counts similar to exercising levels have been observed after infusion of epinephrine (21), cortisol (40), and growth hormone (GH) (19). Exercising in cold water (thermal clamp), albeit lasting only 40 min, substantially blunted the rise in circulating catecholamines, cortisol, and GH and was associated with smaller increases in circulating leukocytes compared with thermoneutral conditions (9, 29). More recent studies have also indicated a likely role for granulocyte-colony stimulating factor (G-CSF) in the mobilization of neutrophils into the circulation following sustained hyperthermia in rats (12) and brief incremental exercise to exhaustion in humans (44). In addition, interleukin-6 (IL-6) concentration 1 h after exercise correlated more strongly than cortisol with circulating neutrophil counts 2 h after exercise, which also indicated a possible role for IL-6 in the delayed neutrophilia of exercise (44). Unfortunately, these studies used short-duration exercise lasting ~10 min (44) or 40 min (9, 29) or were conducted in animals (12). Therefore, a role for raised circulating catecholamines, stress hormones, and more recently cytokines (G-CSF and IL-6) as potential mediators of the neutrophilia of prolonged exercise has not been studied in humans. In the present study, we adopted a thermal clamp model to delineate the influence of stress hormones and cytokines in the neutrophilia of prolonged exercise.

Little is known about the possible mechanism(s) responsible for suppressed in vitro neutrophil degranulation after prolonged exercise (3, 33). Elevated cortisol within the physiologic range increased in vitro neutrophil chemotaxis (34) and has been implicated in the decreased bacterial-stimulated neutrophil degranulation after prolonged exercise (33). However, a more recent study showed that blunting the circulating cortisol response to prolonged exercise with carbohydrate feeding did not significantly alter neutrophil degranulation responses during recovery, indicating a less likely role for cortisol in the decreased bacterial-stimulated neutrophil degranulation after prolonged exercise (3). Controversy also surrounds the temperature dependency of neutrophil function(s). Although some authors report enhanced neutrophil bactericidal capacity and migration at higher in vitro temperatures (38–39°C) (24, 31), others report inhibited neutrophil motility and unaltered oxidative burst activity as in vitro temperature is raised (27, 30). Running at 75% maximal oxygen uptake (V̇O₂max) for 1 h in hot [28°C: final rectal temperature (T₀) = 39.8°C] compared with thermoneutral (18°C: T₀ = 38.7°C) conditions led to larger increases in circulating norepinephrine, cortisol, GH, and neutrophils and lower unstimulated myeloperoxidase re-

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Subjects. Thirteen healthy, recreationally active men (means ± SE: age 21 ± 1 yr; height 180 ± 2 cm; body mass 74.9 ± 2.1 kg; \( V\dot{O}_{2\max} 58.3 ± 1.0 \text{ ml.kg}^{-1} \cdot \text{min}^{-1} \)) volunteered to participate in this study. Subjects gave written, informed consent before starting the study, which received local ethics committee approval. There were no reported symptoms of infection, and subjects did not take medication or nutritional supplements in the 6 wk before the study.

Experimental procedures. In a randomized order, subjects performed four 2-h water-immersion trials separated by 7 days. Trials were exercise-induced heating (EX-H: water temperature 36.3 ± 0.3°C), exercise with a thermal clamp (EX-C: 23.5 ± 0.9°C), passive heating (PA-H: 38.5 ± 0.2°C), and control (CON: 35.3 ± 0.2°C). The purpose of CON was to maintain core body temperature. Exercise trials (EX-H and EX-C) comprised deep water running at 58.1 ± 0.7 and 58.9 ± 0.5% \( V\dot{O}_{2\max} \) respectively, determined using expired gas collected in Douglas bags (Harvard Apparatus, Edenbridge, UK). \( V\dot{O}_{2}, V\dot{C}O_{2}, \) and \( V_{E} \) were determined using a combined paramagnetic oxygen and infrared carbon dioxide analyzer (Servomed 1420B, Crowborough, UK) and a dry gas meter (Harvard Apparatus, Edenbridge, UK). During the 24-h period before each experimental trial, subjects were required to refrain from exercise, caffeine, and alcohol and record their food intake in an effort to standardize their nutritional status.

At 0800 on the morning of each experimental trial, subjects consumed a standardized breakfast (816 kcal; 78% carbohydrate, 14% fat, and 8% protein) and drank only water until their arrival at the laboratory at 1130. The experimental trials were performed at 1200. On arrival at the laboratory, subjects were asked to empty their bladder and bowels, and nude body mass was obtained (Seca 705, Hamburg, Germany). To ensure that subjects arrived euhydrated, a urine osmolality measure was performed, and all recorded samples were below the 900 mosM threshold for euhydration (35). Subjects inserted a flexible thermistor 12 cm beyond the anal sphincter for monitoring \( T_e \) (YSI 4000A, Dayton, FL). Subjects were then seated for 15 min before a resting blood sample was collected from an antecubital vein by venepuncture. In all trials, subjects wore swimming shorts only. HR (Polar Electro, Kempele, Finland), \( R_e \), expired gas, and thermal sensation (McGinnis 1–13 rating of thermal comfort) (17) were recorded at 10-min intervals throughout exercise and seated immersion trials. Water consumption was permitted ad libitum. Immediately postimmersion, a venous blood sample was obtained before nude body mass was recorded. All subjects remained fasted until a further venous blood sample, and nude body mass was obtained 1 h postimmersion. For all blood samples, 19 ml of blood were collected with the subject in a seated position.

Analytical methods. Whole blood samples were collected into four separate vacutainer tubes (Becton Dickinson, Oxford, UK). Two 4-ml vacutainers containing K3EDTA (1.6 mg EDTA/ml blood), one 6-ml vacutainer containing lithium heparin (1.5 IU heparin/ml blood), and one 5-ml vacutainer containing silica clot activator for serum separation (SST II). Blood collected using the K3EDTA tube was stored at room temperature before hematological analysis within 6 h of collection. Hematological analyses including hemoglobin, hematocrit, and total and differential leukocyte counts were performed using an automated cell counter (Gen S, Beckman Coulter, Fullerton, CA). Plasma volume changes were estimated (11), and all blood parameters were corrected accordingly.

A 1.0-ml aliquot of fresh whole blood from the lithium heparin tube was immediately added to a snap-seal microcentrifuge tube containing 50 μl of bacterial stimulant solution (Stimulant, Sigma, Poole, UK). The blood and bacterial stimulant were gently mixed and incubated for 1 h at 37°C, with a gentle mix after 30 min as described previously (33). After incubation, the mixture was centrifuged for 2 min at 5,000 g, and the supernatant was immediately removed and stored at −80°C. The remaining heparinized blood was spun at 1,500 g for 10 min, and plasma was immediately stored at −80°C. Plasma elastase concentration was measured in unstimulated samples and after treatment of whole blood with bacterial stimulant using sandwich-type ELISA kits specific for elastase (Biovendor, Heidelberg, Germany). Aliquots of heparinized plasma were used for the determination of cortisol and G-CSF (DRG Diagnostics, Marlburg, Germany, and R & D Systems, Oxford, UK, respectively). Aliquots of EDTA plasma were used for the determination of epinephrine and norepinephrine using high-pressure liquid chromatography (Clinrep complete kit for catecholamines, Recipe Chemicals, Munich, Germany) as described previously (8). Due to limited sample volume, epinephrine and norepinephrine were analyzed on \( N = 9 \). Finally, aliquots of serum were used for the determination of IL-6 and GH using ELISA (R & D Systems and DRG Diagnostics, respectively). For each assay, all subject samples were analyzed on the same day and with standards on each plate. Intra-assay coefficient of variation was 6.3, 4.0, 4.0, 7.8, 11.0, 5.3, and 3.6% for elastase, cortisol, G-CSF, epinephrine, norepinephrine, IL-6, and GH, respectively.

Statistical analysis. Data in text, tables, and figures are presented as means ± SE. The data were examined using a four-way repeated-measures ANOVA design. Assumptions of homogeneity and sphericity in data were checked, and, where appropriate, adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Significant differences were analyzed using post hoc Tukey’s honestly significant difference test. Statistical significance was accepted at \( P < 0.05 \). The sample size was estimated to be \( N = 10 \) (http://www.dssresearch.com/toolkit/sscalc/size.asp) using previous data examining the effects of exercise on neutrophil degranulation responses (41). Alpha and power levels were set at 0.05 and 0.8, respectively, both of which are standard estimates. To allow for dropout, \( N = 13 \) subjects were recruited.
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RESULTS

T_{re}, physiological variables, and RPE. There was a significant trial × time interaction for T_{re} [F(4,44) = 80.0; P < 0.01]. Progressive increases in T_{re} occurred during the 2-h immersion on EX-H and PA-H (P < 0.01; Fig. 1). T_{re} increased by 2.23 ± 0.08°C and 2.31 ± 0.07°C on EX-H and PA-H, respectively, remained unaltered on CON, and was successfully clamped on EX-C (T_{re} peak at 20 min: 0.18 ± 0.08°C; P < 0.01).

There was a significant trial × time interaction for HR [F(36,432) = 28.2; P < 0.01; Table 1]. RPE [F(12,144) = 30.7; P < 0.01], ventilation rate [V_{E}; F(3,27) = 238; P < 0.01], and thermal sensation [F(5,56) = 13.4; P < 0.01], HR increased during 2-h immersion on EX-H, EX-C, and PA-H compared with preimmersion (P < 0.01; Table 1). In contrast, there were no significant changes from preimmersion observed for HR during CON. Exercising with a thermal clamp (EX-C) resulted in lower HR and RPE responses compared with exercise associated with a rise in T_{re} (EX-H; P < 0.01; Table 1). Reports of thermal sensation were significantly higher on EX-H and PA-H between 10 and 120 min compared with preimmersion and both EX-C and CON (10–20 min, P < 0.05; 30–120 min, P < 0.01). In addition, there were no differences in thermal sensation during CON and EX-C compared with preimmersion; however, exercising with a thermal clamp (EX-C) evoked significantly lower thermal sensation than CON for the entire duration of immersion (P < 0.05; Table 1).

Body mass losses were significantly greater on EX-H compared with CON (P < 0.05; Table 1). After correction for fluid intake, estimated total sweat loss was significantly greater on EX-H and PA-H compared with EX-C and CON (P < 0.01; Table 1). Plasma volume decreased on all trials compared with preimmersion (CON, P < 0.05; EX-H, PA-H, and EX-C, P < 0.01; Table 1). The decrease in plasma volume postimmersion was significantly greater on EX-C and PA-H compared with CON (P < 0.01; Table 1).

Neutrophil responses. There was a significant trial × time interaction observed for circulating neutrophil count [F(6,72) = 13.2; P < 0.01; Fig. 2A], plasma elastase concentration [percentage change: F(2,19) = 3.6; P < 0.05; Fig. 2B], and bacterial stimulated elastase release per neutrophil [percentage change: F(6,54) = 2.4; P < 0.05; Fig. 2C]. The magnitude of the neutrophilia was similar when exercising with (EX-C) and without (EX-H) a thermal clamp and was greater after exercise (EX-C and EX-H) than passive heating (PA-H) at post and 1-h post (P < 0.01; Fig. 2A). Unstimulated plasma elastase concentration increased following exercise (EX-C and EX-H) compared with preimmersion and was greater than PA-H and CON at this time (Fig. 2B). Unstimulated plasma elastase concentration remained elevated on EX-H above baseline, CON, and PA-H at 1-h postimmersion (P < 0.05; Fig. 2B). Bacterial stimulated elastase release per neutrophil decreased significantly after exercise (EX-C and EX-H) and heat stress alone (PA-H; Fig. 2C). Heat stress alone and exercising with (EX-C) and without (EX-H) a thermal clamp evoked a 21, 28, and 28% reduction in neutrophil degranulation at postimmersion (P < 0.01). Bacterial stimulated elastase release per neutrophil remained 21–29% below preimmersion values 1-h postimmersion on the EX-C, EX-H, and PA-H trials and was significantly lower than CON at this point (P < 0.01; Fig. 2C).

Circulating hormone and cytokine responses. There was a significant trial × time interaction observed for plasma epinephrine [F(6,48) = 4.4; P < 0.01; Fig. 3A], plasma norepinephrine [F(6,48) = 8.2; P < 0.01; Fig. 3B], plasma cortisol concentration [F(4,42) = 24.9; P < 0.01; Fig. 4A], serum GH concentration [F(2,24) = 9.3; P < 0.01; Fig. 4B], plasma G-CSF concentration [F(2,24) = 8.8; P < 0.01; Fig. 4C], and IL-6 concentration [F(2,26) = 7.4; P < 0.01; Fig. 4D].

Table 1. The effects of 2 h of exercise at 58% \( V_{O_{2\max}} \)

<table>
<thead>
<tr>
<th>Trial</th>
<th>HR, beats/min&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RPE&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ve, l/min&lt;sup&gt;b&lt;/sup&gt;</th>
<th>TS&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Sweat loss, liters&lt;sup&gt;c&lt;/sup&gt;</th>
<th>PVC, %&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Interaction</th>
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<tr>
<td>EX-H</td>
<td>164 ± 3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18 ± 1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>68.6 ± 2.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.0 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.3 ± 0.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>−6.7 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>EX-H &gt; EX-C &gt; PA-H &gt; CON</td>
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<tr>
<td>EX-C</td>
<td>134 ± 2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.0 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>69.2 ± 2.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.0 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>−9.7 ± 1.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>EX-H &gt; EX-C</td>
</tr>
<tr>
<td>PA-H</td>
<td>120 ± 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.0 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>69.2 ± 2.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.0 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>−9.0 ± 1.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>EX-H &gt; PA-H &gt; CON</td>
</tr>
<tr>
<td>CON</td>
<td>73 ± 3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.0 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>69.2 ± 2.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.0 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>−4.2 ± 1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>EX-C &gt; PA-H &gt; CON</td>
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Values are means ± SE. Shown are the effects of 2 h of exercise at 58% \( V_{O_{2\max}} \) in thermoneutral water (exercise-induced heating; EX-H) and cold water (EX-C) and of 2 h of passive immersion in thermoneutral water (CON) and hot water (PA-H) on heart rate (HR), ratings of perceived exertion (RPE) (6-20 scale), ventilation (Ve), thermal sensation (TS; 1-13 scale), body mass loss (BML), sweat loss (estimated), and plasma volume change (PVC). *Immediately postimmersion compared with preimmersion. <sup>a</sup>Mean for trial and <sup>b</sup>after correction for fluid intake. <sup>c</sup>Significant difference from preimmersion (P < 0.05). <sup>d</sup>Significant difference from preimmersion (P < 0.01). Interaction: > > P < 0.05 and > P < 0.01.

Fig. 1. The effects of 2 h of exercise at 58% \( V_{O_{2\max}} \) immersed in thermoneutral water (exercise-induced heating; EX-H; ●) and cold water (EX-C; ■) and 2 h of passive immersion in thermoneutral water (CON; ○) and hot water (PA-H; □) on rectal temperature (T_{re}). Values are means ± SE. Significantly greater than preimmersion (**P < 0.01): a, EX-H > PA-H + EX-C > CON (P < 0.05); b, EX-H > PA-H > EX-C + CON (P < 0.01); c, EX-H + PA-H > EX-C + CON (P < 0.01).
These results indicate that exercising with a thermal clamp (EX-C) blunts the circulating norepinephrine, cortisol, G-CSF, and IL-6 response (*P < 0.01; Figs. 3A and B) compared with preimmersion. Postimmersion increases in circulating cortisol, G-CSF, and IL-6 tended to occur only when Tre increased (EX-H and PA-H), with the exception being plasma G-CSF, which also increased at postimmersion on CON (*P < 0.01). Circulating cortisol, G-CSF, and IL-6 remained elevated 1 h postimmersion on EX-H and PA-H (*P < 0.01; Figs. 3A and 4, A, C, and D). Serum GH increased postimmersion on EX-H, PA-H, and EX-C (*P < 0.01; Fig. 3B) and returned to within preimmersion levels by 1 h postimmersion.

**DISCUSSION**

The purpose of this study was to investigate thermal effects and associated hormone and cytokine involvement in neutrophil responses after prolonged exercise. By using a water-immersion technique, we were able to clamp the rise in core body temperature during prolonged exercise (Tre = -0.02°C on EX-C and 2.23°C on EX-H vs. preimmersion). The addition of the PA-H trial was a particular strength of the study design because this enabled us to identify the effect of a rise in core body temperature alone on neutrophil responses (Tre = 2.31°C vs. preimmersion). In direct contrast to our hypothesis, these results show a similar neutrophilia and decrease in bacterial-norepinephrine were significantly elevated postimmersion on EX-H, PA-H, and EX-C (*P < 0.01; Figs. 3A and B) compared with preimmersion. Postimmersion increases in circulating cortisol, G-CSF, and IL-6 tended to occur only when Treh increased (EX-H and PA-H), with the exception being plasma G-CSF, which also increased at postimmersion on CON (*P < 0.01). Circulating cortisol, G-CSF, and IL-6 remained elevated 1 h postimmersion on EX-H and PA-H (*P < 0.01; Figs. 3A and 4, A, C, and D). Serum GH increased postimmersion on EX-H, PA-H, and EX-C (*P < 0.01; Fig. 3B) and returned to within preimmersion levels by 1 h postimmersion.

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stimulated neutrophil degranulation after prolonged exercise with and without a thermal clamp. As such, the rise in core body temperature does not appear to mediate the observed neutrophil responses to prolonged exercise.

In partial support of our hypothesis, EX-C blunted increases in circulating norepinephrine, cortisol, G-CSF, and IL-6 but not epinephrine and GH. Given the similar neutrophilia on EX-C and EX-H, these results suggest a more limited role for cortisol, G-CSF, and IL-6 in the neutrophilia of prolonged exercise than previously indicated after short-term exercise (9, 29, 44). Similar increases in circulating catecholamines and GH immediately after PA-H and EX-H with no significant neutrophilia on PA-H at this time indicate a less likely role for catecholamines and GH in the neutrophilia immediately after prolonged exercise. A less likely role for epinephrine is in accordance with the finding that β-blockade (propranolol) did not alter the neutrophilia of submaximal exercise (lasting ≤25 min) (14). Propranolol lowered exercising HR (~14%) but did not alter cardiac output. Consequently, the authors proposed a mechanical effect of raised cardiac output to account for the neutrophilia immediately after exercise via demargination from the pulmonary circulation (14). Since we recorded HR and not cardiac output (HR was lower during EX-C vs. EX-H), measurement of cardiac output using the CO2 rebreathing technique might support a pivotal role for raised cardiac output in the neutrophilia immediately after more prolonged exercise. Similar increases in circulating catecholamines and GH immediately after PA-H and EX-H supports early work indicating a role for ventilatory patterns in neutrophil recruitment into the circulation (2). However, more recent work has discounted an involvement of raised V˙E in the neutrophilia of short-term exercise (13).

Approximately one-half of all neutrophils in the blood are marginated to the vascular endothelium, and one-half are freely circulating (1). Consequently, demargination of neutrophils (e.g., via the increase in cardiac output) might account for an approximate doubling of circulating neutrophil number immediately after exercise. It might then be reasonable to assume that a small component of the three-fold increase in circulating neutrophils on EX-C and EX-H at postimmersion, and possibly a larger component at 1 h postimmersion, is accounted for by increased bone marrow release and/or increased half-life of neutrophils in circulation. Typically, bone marrow neutrophil release is indicated by an increase in circulating band neutrophils (26). However, large variation exists in the percentage of band neutrophils reported in resting healthy males: large variation even exists between papers from the same research group, e.g., 10% (38) and 29% (44). Exercise of similar duration and intensity to the present study did not alter the percentage of band neutrophils (22). In addition, since EX-C abolished increases in circulating levels of known mediators of bone marrow neutrophil release [e.g., cortisol, G-CSF, and IL-6 (37, 44)], the contribution of bone marrow release to the observed neutrophilia at postimmersion and 1 h postimmersion might be considered small. A more objective and quantitative method of determining bone marrow contribution to the neutrophilia of prolonged exercise may be to determine neutrophil expression of CD10^/CD16^{low} using flow cytometry (26). The present findings indicate that the mediators of the neutrophilia immediately after and 1 h after prolonged exercise may be different from those identified for short-term exercise and require further clarification.

Fig. 4. The effects of 2 h of exercise at 58% V˙O_{max} immersed in thermoneutral water (EX-H; ●) and cold water (EX-C; ▲) and 2 h of passive immersion in thermoneutral water (CON; □) and hot water (PA-H; △) on plasma cortisol (A), serum GH (N = 12; B), plasma G-CSF (C), and serum IL-6 concentration (D). Values are means ± SE. **Significantly different from preimmersion P < 0.01. *Significantly greater than EX-C (P < 0.01). *Significantly greater than CON (P < 0.01).
In direct contrast to our hypothesis, EX-C evoked a similar reduction in bacterial-stimulated neutrophil degranulation as with EX-H and PA-H. Since EX-C blunted increases in circulating cortisol, G-CSF, and IL-6, it is unlikely that these are important mediators of neutrophil degranulation as suggested previously (10, 18, 33). Raised circulating epinephrine and GH and decreased neutrophil degranulation immediately after EX-C, EX-H, and PA-H indicate a more likely role for these hormones in the decrease in neutrophil degranulation. In cardiopulmonary bypass patients, cardiac-selective β-blockade (esmolol) prevented the increase in circulating elastase 5 h after surgery, indicating a depressive effect of epinephrine on neutrophil degranulation (4). Epinephrine (albeit ≥10 nM) has been shown to downregulate in vitro bacterial-stimulated neutrophil elastase release via cAMP-mediated enhancement of the clearance of cytosolic Ca²⁺ (39). Since propranolol attenuated this effect, a role for β₂-adrenoreceptors in the anti-inflammatory interaction of epinephrine and neutrophils was suggested. Further support comes from studies showing that beta-agonists have favorable outcomes for recovery from acute lung injury where suppressing the harmful effects of activated neutrophils is beneficial (43). Research examining a role for epinephrine in the decrease in neutrophil degranulation after prolonged exercise is limited. One recent study showed that carbohydrate ingestion blunted the plasma epinephrine response (postexercise 0.6 nM vs. 1.4 nM on placebo) but did not alter the neutrophil degranulation response to the second of two 90-min bouts of cycling at 70% VO₂max (23). Previous research has not identified a role for GH in the decrease in neutrophil degranulation after prolonged exercise. Repeating PA-H with propranolol (β-blockade) and somatostatin (GH-blocker) may verify whether these hormones are involved in the decrease in neutrophil degranulation immediately after and also possibly 1 h after immersion. There is some evidence that neutrophils remain in a refractory state for up to 2 h following activation (15). A role for complement C5a in secretory desensitization in human neutrophils has been shown (15), and circulating C5a concentration increased sixfold after a marathon race (7). A potential role for raised circulating C5a in the decrease in bacterial-stimulated neutrophil degranulation after prolonged exercise also warrants investigation. Exposing neutrophils collected at preimmersion with plasma collected at postimmersion may unravel if circulating factors in the blood are responsible for the decrease in neutrophil degranulation after prolonged exercise (and passive heating).

In line with a recent study (22), these findings indicate a limited effect of additional heat stress (EX-H +2.2°C Tₑₑ vs. EX-C), at least within the range of Tₑₑ reported here, on the neutrophil degranulation response to prolonged exercise. A limitation of the previous study was that Tₑₑ was only 0.6°C greater immediately after exercise in hot compared with thermoneutral conditions (22). We recognize that, due to ethical committee restrictions, the absolute final Tₑₑ (EX-H 38.4°C) represents relatively modest heat stress compared with responses reported in individuals undertaking vigorous exercise in hot conditions (32). We also recognize that more frequent blood sampling during immersion and for a longer time period during recovery may provide further insight into the potential mediators of neutrophil responses. For example, neutrophil degranulation had not returned to preimmersion levels at 1 h postimmersion (EX-C, EX-H, and PA-H). Indeed, the time course of recovery for neutrophil degranulation after prolonged exercise remains a topic of debate. Neutrophil degranulation recovered to within preexercise levels by 2 h postexercise after cycling for 120 min at 60% VO₂max (22) but remained depressed for 24 h after cycling to fatigue (lasting on average 164 min) at 55% VO₂max (33).

Conclusion. These results show a similar neutrophilia and decrease in neutrophil degranulation after prolonged exercise with and without a rise in core body temperature. As such, the rise in core body temperature does not appear to mediate neutrophil trafficking and degranulation responses to prolonged exercise. In addition, these results suggest a limited role for cortisol, G-CSF, and IL-6 in the observed neutrophil trafficking and degranulation responses to prolonged exercise.

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