Changes in neutrophil surface receptor expression, degranulation, and respiratory burst activity after moderate- and high-intensity exercise

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Neutrophils are a class of white blood cells that form the first line of defense against infection and play an important role in the immune response to tissue injury (16). Neutrophils respond to infection and tissue injury by recognizing and binding immunoglobulin G (IgG) molecules and complement proteins that coat the surface of foreign pathogens and host tissue fragments (1). This recognition-binding process is mediated by neutrophil surface receptors, including the complement 1 receptor CD35, the complement 3 receptor CD11b, and the low-affinity IgG receptor CD16. The engagement of these surface receptors initiates a cascade of intracellular events leading to the release of enzymes (degranulation) and reactive oxygen species (respiratory burst activity) from neutrophils (6, 9). Together, these enzymes and reactive oxygen species assist in the destruction and degradation of foreign pathogens and damaged tissue fragments (32).

A few studies have examined alterations in neutrophil receptor expression after exercise (11, 14, 26, 31). Several of these studies showed an increase in CD11b expression after moderate-intensity cycling (26) and high-intensity running (11, 14, 31). Smith et al. (26) found no change in CD16 expression, whereas Gray et al. (11) observed a trend toward reduced CD16 expression. It is unknown whether the expression of other neutrophil surface receptors such as CD35 is affected by exercise. Only one study has compared changes in CD11b expression across different exercise intensities (14). However, because the exercise trials in that study were not of a fixed duration, it was not possible to separate the effect of exercise intensity from the effect of exercise duration.

The systemic factors affecting neutrophil receptor expression after exercise are not well understood. Elevated serum levels of interleukin (IL)-6 and IL-8 have been correlated with enhanced neutrophil expression of CD11b and CD35 in sepsis patients (15, 20). In contrast, the expression of CD35 and CD16 expression is reduced after in vivo infusion of granulocyte-colony stimulating factor (G-CSF) (13, 24). Intense exercise stimulates the systemic release of IL-6, IL-8, and G-CSF (27, 29, 33). It is therefore possible that increases in the release of these cytokines after intense exercise may alter neutrophil receptor expression.

There are also few data available regarding the possible relationship(s) between altered neutrophil receptor expression and markers of exercise-induced muscle damage or changes in neutrophil degranulation and respiratory burst activity. Pizza et al. (18) found upregulation of CD11b after eccentric forearm exercise, together with an increase in plasma creatine kinase (CK) activity and myoglobin (Mb) concentration. However, these parameters were not correlated with each other. The results of a study by Smith et al. (26) suggested that upregulation of CD11b expression may be associated with neutrophil degranulation. Data from studies of pathological conditions suggest that neutrophil receptor expression is altered in response to inflammatory stimuli and tissue injury. For example,

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the expression of CD11b and CD35 receptors is enhanced in patients suffering sepsis (20). In contrast, the expression of CD11b, CD35, and CD16 is reduced in patients who have experienced acute trauma and burns (2, 25) and in individuals suffering from arthritis (10, 12, 23). These alterations may represent adaptive responses to control the level of neutrophil and complement activation during these pathological conditions (7, 10, 23). Alterations in neutrophil receptor expression could also affect neutrophil functional activity. For example, a reduction in the neutrophil expression of CD11b and CD16 in patients with thermal injuries has been associated with defects in respiratory burst activity, and this has resulted in an increased susceptibility to infection (2). Therefore, exercise-induced alterations in neutrophil receptor expression could have implications for the clearance of damaged muscle tissue, neutrophil function, and resistance to infection after exercise.

The aim of this study was to compare the effects of moderate- (MI) vs. high-intensity exercise (HI) of a fixed duration on changes in the expression of CD11b, CD16, and CD35 neutrophil surface receptors, degranulation (as measured by plasma and intracellular myeloperoxidase concentrations), and respiratory burst activity. We hypothesized that, relative to MI, HI would cause larger changes in neutrophil counts, receptor expression, plasma myeloperoxidase concentration, and respiratory burst activity. We also hypothesized that these alterations would be correlated to intensity-dependent increases in the systemic release of cytokines and stress hormones.

**METHODS**

Subjects. Subjects were 10 well-trained male runners and triathletes. Well-trained athletes were chosen because they needed to be able to complete 60 min of exercise at a high intensity [85% maximal oxygen consumption ($V_{O2\, max}$)]. The athlete characteristics were (means ± SD) age 28 ± 3 yr, weight 75 ± 6 kg, height 178 ± 5 cm, $V_{O2\, max}$ 61 ± 3 ml·kg⁻¹·min⁻¹, and heart rate maximum 186 ± 8 beats/min. All participants were informed about the purpose and risks of the study before written, informed consent was obtained. The experimental protocol was approved by the Medical Research Ethics Committee at The University of Queensland.

$V_{O2\, max}$ testing. The runners were tested on a treadmill (Austredex AC190, Melbourne, Australia) to determine their $V_{O2\, max}$. Each athlete was tested at least twice to ensure that the $V_{O2\, max}$ values were reliable. The protocol used to determine $V_{O2\, max}$ started at a treadmill velocity of 10 km/h and 0% gradient; treadmill speed was increased 2 km/h every 2 min up to 16 km/h, followed by 1 km/h increments per minute up to 18 km/h. The gradient was then increased by 2% each minute until $V_{O2\, max}$ was reached. Expired air was analyzed for expired oxygen fraction and expired CO₂ fraction every 30 s during exercise (Ametek gas analyzers SOV S-3A11 and COV CD3A, Pittsburgh, PA). Minute ventilation was recorded every 30 s with a turbine ventilometer (model 096, Morgan, Kent, UK). Gas analyzers were calibrated immediately before and validated after each test by a certified beta gas mixture (Commonwealth Industrial Gas, Brisbane, Australia); the ventilometer was calibrated before each test with a 1-liter syringe according to the manufacturer’s instructions. Oxygen consumption was calculated with an online data-acquisition and -analysis program (South Australian Sports Institute, Adelaide, Australia). Heart rate was measured every 30 s during this test (Polar Vantage, Polar, Finland). Exercised protcols. Once $V_{O2\, max}$ was determined, the athletes completed two separate 60-min exercise trials at either 60% (MI) or 85% (HI) $V_{O2\, max}$. The order of the two trials was counterbalanced among the athletes, and each trial was performed at least 2 wk apart. During each trial, oxygen consumption was measured, as described above, for 5 min at the beginning and for two 3-min periods after 20 and 40 min of the trial to ensure that each athlete was exercising at the correct relative intensity. Heart rate was measured every 5 min throughout each trial. All trials were conducted between 11:00 AM and 12:00 PM to minimize the effect of diurnal variation in cortisol release.

Blood sampling. A 20-ml blood sample was drawn from a forearm vein by venipuncture immediately pre- (Pre) and postexercise (Post) and after 1 h of recovery. To determine whether there was any diurnal variation in plasma cortisol concentration and neutrophil parameters, blood was sampled at rest from each athlete on a separate occasion at the same time of day as the exercise test. Blood (15 ml) was collected in a sterile tube containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ), and a further 5 ml were collected in a sterile tube containing EDTA. Immediately after collection, the blood was centrifuged for 10 min at 1,000 g to separate plasma. The lithium heparin plasma was separated into two 1-ml aliquots for the measurement of catecholamines, Mb, and CK and into two 500-μl aliquots for the measurement of growth hormone and cortisol. The EDTA plasma was separated into 500-μl aliquots for the measurement of cytokines and myeloperoxidase. All plasma samples were stored at −80°C until the day of assay.

Hematologic profile. A full blood cell count and hemoglobin and hematocrit values were obtained by an automated cell counter (Sysmex K-2000, Kobe, Japan). Cell counts and the plasma concentrations of stress hormones, cytokines, myeloperoxidase, and Mb were adjusted to account for changes in plasma volume according to the methods of Dill and Costill (8).

Neutrophil separation. Neutrophils were separated by dextran sedimentation (Amersham Pharmacia Biotech, Uppsala, Sweden), followed by layering on Histopaque-1077 (Sigma Chemical, St. Louis, MO) and hypotonic lysis. The cells were finally resuspended in HBSS containing 5.5 mM glucose and 0.9 mM CaCl₂ (Sigma Chemical). The neutrophils were ≥99% pure and viable, as determined by Trypan blue exclusion. One hour was normally required to separate the neutrophils, and the cells were kept at room temperature for more than 2 h before their activity was measured.

Neutrophil respiratory burst activity. Two separate assays were used to measure the respiratory burst activity of neutrophils in response to in vitro stimulation.

The first assay measured the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. Approximately 3 × 10⁴ isolated neutrophils were added to each of three cuvettes containing HBSS and either MTT only, MTT with PMA (100 nmol/l), or MTT with opsonized zymosan (OZ; 10 mg/ml). MTT (Sigma Chemical) was present in all three reactions at a final concentration of 1.2 mM. The first of these reactions was included to measure the “spontaneous” or basal activity of neutrophils without stimulation (28). All three cuvettes were then incubated at 37°C before the formazan crystals were dissolved in isopropyl alcohol. Last, the absorbance at 570 nm was read on a spectrophotometer (model 5625, ATI Unicam, Cambridge, UK). The absorbance from the basal reaction was subtracted from each of the stimulated reactions to obtain the response to stimulation. The interassay variation for this assay was <2% for all three reactions.

The second assay measured the oxidation of the chemiluminescent probe luminol. Approximately 3 × 10⁵ isolated neutrophils were added to each of two reaction cuvettes containing luminol (0.5 mM; Sigma Chemical), HBSS, and either 100 nM PMA or 10 mg/ml OZ (Sigma Chemical). These reagents were prepared as described previously (28). The oxidation of luminol after stimulation of neutrophils with PMA and OZ was then measured for 15 min at 37°C on a fluorometer (Turner model 110; Barnstead-Thermolyne) and logged on a chart recorder. Data were recorded as the peak height of chemiluminescence activity. The interassay variation for this assay was 3.8% for PMA and 6.1% for OZ.
Neutrophil surface receptor expression. Whole blood (50 μl) was added to polypropylene tubes and incubated for 20 min at room temperature with mouse anti-human monoclonal antibodies for the following receptors: phycoerythrin-conjugated IgG1 (Becton Dickinson) and FITC-conjugated IgG1 (Becton Dickinson) (both used as a negative control); phycoerythrin-conjugated CD11b antibody (2LPM19c clone) for the C3bi receptor (Dako, Glostrup, Denmark); FITC-conjugated CD16 antibody (3G8 clone) for the FcyRII receptor (Becton Dickinson), and the phycoerythrin-conjugated CD35 antibody (E1 clone) for the C3b receptor (Becton Dickinson). After this initial incubation, 1 ml of FACSlyse solution (Becton Dickinson) was added, and the samples were vortexed and incubated for another 9 min. Finally, the tubes were centrifuged for 3 min at 1,500 g before the supernatant was removed, and the cell pellet was resuspended in 250 μl of 1% formalin. Receptor expression was then read by flow cytometry. The neutrophil population was gated according to the forward- and side-scatter light profile. Fluorescence was measured with a FACSscan flow cytometer and CellQuest software (Becton Dickinson). Measurements were made on the FL1 (green) and FL2 (red) channel, and the gates were adjusted to the negative control quadrant. A total of 15,000 events was collected, and the data were recorded as mean fluorescence channel number. The interassay variation for each antibody assay was as follows: 12.7% for CD11b, 7.8% for CD16, and 11% for CD35.

Intracellular myeloperoxidase concentration. To measure neutrophil degranulation, the myeloperoxidase content of neutrophils was measured by flow cytometry. Fifty microliters of whole blood were fixed by adding 100 μl of Fix and Perm reagent A (Becton Dickinson), vortexed, and incubated for 15 min at room temperature. After 15 min, the blood was washed with 4 ml of PBS and centrifuged for 3 min at 1,500 g, and the supernatant was removed. The cell pellet was resuspended, and 2 μl of mouse anti-human FITC-conjugated MPO antibody (MPO-7 clone) (Dako) and 100 μl of Fix and Perm reagent B (Becton Dickinson) were added. The sample was then vortexed and incubated for another 15 min at room temperature. Finally, the sample was washed once more with 4 ml of PBS and centrifuged for 3 min at 1,500 g before the supernatant was removed and the cell pellet was resuspended with 250 μl of 1% formalin. The samples were then read by flow cytometry. The neutrophil population was gated according to forward- and side-scatter light properties. Fluorescence was measured on the FL1 (green) channel, and the gates were adjusted to the negative control quadrant. A total of 15,000 events was collected, and the data were recorded as mean fluorescence channel number. The interassay variation was 9.0%.

Plasma myeloperoxidase concentration. EDTA plasma was added undiluted to an ELISA kit (Bioxytech MPO-EIA, OxisResearch, Portland, OR). The absorbance was read spectrophotometrically on a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA) at 405 nm, and the concentration was calculated by using a standard curve. All measurements were made in duplicate. The interassay variation was 13.2%.

Plasma stress hormones. The concentrations of cortisol, growth hormone, and the catecholamines epinephrine, norepinephrine, and dopamine were measured in lithium heparin plasma. Cortisol and growth hormone were measured by ELISA (Neogen, Lexington, KY, and Bioclon, NSW, Australia, respectively). Samples were diluted 1:100 with extraction buffer for cortisol and, if required, up to 1:8 with zero standard for growth hormone. The absorbance was read spectrophotometrically on a microplate reader (Titertek Multiskan MCC450, Flow Laboratories, Helsinki, Finland) at 650 nm for cortisol and 490 nm for growth hormone. The concentration was calculated using a standard curve. All assays were performed in duplicate. The interassay variation was 3.0% for cortisol and 12.0% for growth hormone. The catecholamines were measured by radioenzymatic assay (Biotrak, Amersham Pharmacia Biotech). The radioactivity of the samples was measured with a liquid scintillation counter (Aloka Systems, Liquid Scintillation, Tokyo, Japan). All assays were performed in duplicate. The interassay variation was 7.5% for epinephrine, 7.6% for norepinephrine, and 5.9% for dopamine.

Plasma cytokines. IL-6 and G-CSF were measured in EDTA plasma by ELISA (R&D Systems, Quantikine HS, Minneapolis, MN, and Immuno-Biological Laboratories, Gunma, Japan, respectively). IL-8 was measured in EDTA plasma with an enzyme-amplified sensitivity immunooassay kit (Biosource, Nivelles, Belgium). This kit is similar to an ELISA, except that the wells of the microplate are coated with several monoclonal antibodies against distinct epitopes of the target molecule, thereby avoiding hyperspecificity and increasing sensitivity (27). The absorbance was read spectrophotometrically on a microplate reader (VERSAmax, Molecular Devices) at 490 nm for IL-6 and IL-8, and 450 nm for G-CSF. The concentration of each cytokine was calculated using a standard curve. IL-6 and IL-8 were measured in duplicate, whereas single measurements of G-CSF were made. The interassay variation was 5.4% for IL-6 and 16.6% for IL-8.

Plasma CK and Mb. Plasma CK activity was determined spectrophotometrically (VP-Super, Dinabott, Tokyo, Japan) with a commercially available kit (Dinabott). Plasma Mb concentration was measured on a biochemical analyzer (model TBA-30A, Toshiba, Tokyo, Japan) with a commercially available kit (Denka-Seiken, Tokyo, Japan). The interassay variation was 3.2% for CK and 4.4% for Mb.

Statistical analysis. Statistical significance was set at P < 0.05. Data are expressed as means ± SD. Before performing the statistical analysis, we tested the residuals for normality using a histogram and normality plots. If the residuals were not normally distributed, a log transformation was applied to stabilize the variance. The statistical significance of exercise-induced changes was assessed using a 2 (MI and HI) × 3 (sampling points) repeated-measures ANOVA to obtain main effects of time and time × trial interaction. The changes from preexercise to postexercise, and from preexercise to 1 h postexercise were compared between trials using Student’s paired t-tests. For these two comparisons, a Bonferroni adjustment was made, with statistical significance set at P < 0.025. Because some data sets were not normally distributed, Spearman’s rank correlation (r) was used to identify any significant relationships. Statistical analysis was performed using SPSS version 10.0 for Windows (SPSS, Chicago, IL). Effect size (ES) was also calculated as the difference between two sample means, divided by the average standard deviation of the two samples. ES was interpreted according to the suggestion of Thomas et al. (30) as follows: ~0.2 = small, ~0.5 = moderate, and ~0.8 = large.

RESULTS

Diurnal variation. When blood was sampled under resting conditions from each athlete on a separate occasion at the same time as the exercise trials, there were no significant changes in plasma cortisol concentrations or neutrophil parameters (data not shown).

Neutrophil and total leukocyte counts. Neutrophil count was significantly higher than preexercise values immediately after HI (62%; ES = 2.1; P < 0.01) and 1 h after both MI (100%; ES = 2.2; P < 0.01) and HI (320%; ES = 4.4; P < 0.01) (Fig. 1). The increase in neutrophil count from preexercise to 1 h postexercise was greater for HI than for MI (ES = 1.7; P < 0.01). Total leukocyte count followed a similar trend, with significant differences between MI and HI (Fig. 1).

Neutrophil surface receptor expression. CD11b expression was diminished 1 h after MI (~33%; ES = −0.8; P < 0.01) and HI (~36%; ES = −1.3; P < 0.025) (Table 1). CD16 expression decreased below preexercise values immediately after HI (−21%; ES = −0.6; P < 0.01) and 1 h after both MI (−25%; ES = −0.7; P < 0.01) and HI (−45%; ES = −1.4; P < 0.01). The reduction in CD16 expression from preexercise...
to 1 h postexercise was greater for HI than for MI (ES = 0.7; P < 0.01). CD35 expression decreased 1 h after MI (−16%; ES = −0.8; P < 0.025).

**Neutrophil respiratory burst activity.** Immediately after HI, there was a moderate, nonsignificant increase in MTT reduction at the basal level (22%; ES = 0.8; P = 0.19) (Table 2). The increase in MTT reduction in response to PMA stimulation immediately after HI was significant (+19%; ES = 0.9; P < 0.01), and there was a trend toward enhanced MTT reduction in response to OZ stimulation (20%; ES = 0.4; P = 0.06). The change in PMA-stimulated chemiluminescence activity was not statistically significant (P = 0.06). There were no significant changes in neutrophil respiratory burst activity after MI.

**Intracellular and plasma myeloperoxidase concentrations.** After HI, intracellular myeloperoxidase levels were lower immediately postexercise compared with preexercise (−12%; ES = −0.3; P < 0.01) and 1 h postexercise (−18%; ES = −0.4; P < 0.01), whereas no significant change was evident after MI (Fig. 2). The decrease in intracellular myeloperoxidase concentration was accompanied by a substantial rise in the plasma concentration of myeloperoxidase. Immediately after HI, plasma myeloperoxidase concentration was greater than preexercise (250%; ES = 1.6; P < 0.01). In contrast, there was no significant change in plasma myeloperoxidase concentration after MI. The increase in plasma myeloperoxidase concentration from pre- to postexercise was significantly greater for HI than for MI (ES = 1.0; P < 0.01).

**Plasma stress hormones.** The plasma concentrations of growth hormone, epinephrine, and norepinephrine were all elevated immediately after both trials (Figs. 3 and 4). In contrast, cortisol (290%; ES = 2.3; P < 0.001) and dopamine (127%; ES = 1.3; P < 0.025) increased only after HI (Figs. 3 and 4). The increase from pre- to postexercise was greater for HI than for MI for cortisol (ES = 2.9; P < 0.01), growth hormone (ES = 0.8; P < 0.025), and norepinephrine (ES = 1.5; P < 0.01). Cortisol levels (ES = 1.9; P < 0.01) also remained significantly higher 1 h after HI compared with MI.

**Plasma cytokines.** Plasma IL-6 concentration increased substantially above preexercise values immediately postexercise and 1 h postexercise after both trials (Table 3). The increase from pre- to postexercise was significantly larger for HI than for MI (ES = 1.0; P < 0.025). The increase in plasma IL-8 concentration was not statistically significant (P = 0.09). There was no significant change in plasma G-CSF concentration after either exercise trial.

**Plasma CK and Mb.** Exercise caused a large increase in plasma Mb concentration (Table 4). Plasma Mb concentration increased up to 1 h after MI (110%; ES = 1.5; P < 0.01) and HI (450%; ES = 3.1; P < 0.01). There was also a small increase in plasma CK activity immediately after MI (17%; ES = 0.3; P < 0.01) and HI (41%; ES = 1.0; P < 0.01). These changes were greater for HI than for MI for both Mb concentration (ES = 2.4; P < 0.01) and CK activity (ES = 2.7; P < 0.01).

**Correlations.** Significant ρ were obtained between CD16 expression at 1 h postexercise and postexercise plasma concentrations of IL-6 (ρ = −0.56, P < 0.05) and IL-8 (ρ = −0.53, P < 0.05), 1 h postexercise neutrophil counts and postexercise plasma concentrations of cortisol (ρ = 0.80, P < 0.01), growth hormone (ρ = 0.62, P < 0.01), epinephrine (ρ = 0.54, P < 0.05), and IL-6 (ρ = 0.66, P < 0.01) and 1 h postexercise plasma concentration of IL-8 (ρ = 0.70, P < 0.01).

**DISCUSSION.** The aim of this study was to examine the effects of exercise intensity on alterations in neutrophil surface receptor expression, degradation, and respiratory burst activity. Although both MI and HI reduced neutrophil receptor expression, the reduction in CD16 expression was greater after HI. The reduction in neutrophil receptor expression did not impair neutrophil degradation or respiratory burst activity. Decreased neutrophil receptor expression after exercise may be mediated by systemic cytokine release and could occur to control neutrophil activation.

The downregulation in the expression of CD11b, CD16, and CD35 after exercise could be part of a mild inflammatory response to exercise-induced muscle damage. Downregulation of CD11b and CD16 expression has been reported in individ-
Elevated levels of soluble CD35 have also been observed in synovial fluid obtained from individuals with arthritis, suggesting that CD16 is shed from neutrophils when they are mobilized from bone marrow. Therefore, the decrease in CD16 expression after exercise may reflect the presence of a new population of circulating immature neutrophils.

An alternative explanation for the observed decrease in CD16 expression after exercise is that this response reflected a shift toward the release of immature neutrophils newly recruited from bone marrow. This concept seems plausible for the following reasons. First, previous studies have found an increase in the number of immature neutrophils several hours after exercise (27, 29). Second, Huizinga et al. (13) reported that an increase in the release of neutrophils from bone marrow was associated with an increase in the level of soluble CD16, suggesting that CD16 is shed from neutrophils when they are mobilized from bone marrow. Therefore, the decrease in CD16 expression after exercise may reflect the presence of a new population of circulating immature neutrophils.

The present data indicate that the decrease in CD11b and CD35 expression was not related to exercise intensity. One possibility is that the downregulation of CD11b and CD35 expression was the result of the cleavage of receptors from the neutrophil cell membrane by proteolytic enzymes. Incubation of neutrophils in vitro with serine or metalloproteinase enzymes similar to those enzymes released during neutrophil degranulation causes the proteolytic degradation of the plasma membrane, which might explain the downregulation of CD11b and CD35 expression.

### Table 2. Neutrophil respiratory burst activity Pre, Post, and Post-1 h after 60 min of running at MI and HI

<table>
<thead>
<tr>
<th>Parameter, arbitrary units</th>
<th>Pre</th>
<th>Post</th>
<th>Post-1 h</th>
<th>Effect × Time Interaction</th>
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<tr>
<td>Basal MTT reduction</td>
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<td></td>
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<tr>
<td>MI</td>
<td>198±42</td>
<td>186±47</td>
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<tr>
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<td>PMA-stimulated MTT reduction*</td>
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<td></td>
<td></td>
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<tr>
<td>MI</td>
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<td>870±212</td>
<td>919±183</td>
<td>0.153</td>
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<tr>
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<td>937±166</td>
<td>861±221</td>
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<tr>
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<tr>
<td>PMA-stimulated chemiluminescence</td>
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<tr>
<td>MI</td>
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<td>OZ-stimulated chemiluminescence</td>
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<tr>
<td>MI</td>
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</table>

Values are means ± SD; n = 10 subjects. MTT, tetrazolium salt; PMA, phorbol myristate acetate; OZ, opsonized zymosan; *Absorbance for the stimulated reaction minus absorbance for the basal reaction. †Significantly different from Pre, P < 0.01.

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**Fig. 2.** Intracellular (A) and plasma (B) myeloperoxidase (MPO) concentrations Pre, Post, and Post-1 h after MI and HI. Data are means ± SD. MFI, mean fluorescence intensity. **Significantly different from Pre, P < 0.01. †Change from Pre significantly greater for HI than for MI, P < 0.01.

**Fig. 3.** Plasma concentrations of growth hormone (A) and cortisol (B) Pre, Post, and Post-1 h after MI and HI. Data are means ± SD. **Significantly different from Pre, P < 0.01. †Change from Pre significantly greater for HI than for MI, P < 0.01. §Change from Pre significantly greater for HI than for MI, P < 0.025.
membrane portion of the CD11b, CD16, and CD35 receptors (3, 7, 22). The proteolytic cleavage of neutrophil surface receptors could possibly be part of a regulatory control loop to prevent excessive neutrophil infiltration and activation within tissues. The large increase in plasma myeloperoxidase concentration that occurred after exercise in the present study is evidence that exercise induced neutrophil degranulation. Therefore, other granular enzymes released from neutrophils during exercise might have been responsible for the observed reduction in receptor expression after exercise.

The serum levels of IL-6 and IL-8 have been positively correlated with enhanced expression of CD11b in sepsis patients (15, 20). In contrast, we found an inverse correlation between CD16 expression and the plasma concentrations of these two cytokines. The biological significance of this observation is unclear. The increased plasma levels of IL-6 and IL-8, together with the reduction in CD16 expression, might reflect a mild inflammatory response to exercise. Infusion of G-CSF in vivo reduces neutrophil CD16 expression (13, 24). However, because there was no significant increase in plasma G-CSF concentration in our study, it is unlikely that G-CSF reduced CD16 expression after exercise.

Data from clinical studies of patients with sepsis and thermal injuries suggest that defective expression of CD11b and CD16 receptors on the surface of neutrophils is associated with impairment of respiratory burst activity and increased risk of infection (2, 21). However, in our study, the reduction in neutrophil receptor expression did not appear to affect the release of myeloperoxidase or respiratory burst activity. We can only speculate whether the decreased expression of these two receptors observed in our study has any implications for resistance to infection after exercise. If the expression of CD11b and CD16 remains low beyond 1 h after exercise, then it is possible that this could enhance the risk of infection.

This study is the first to demonstrate that, independent of any effect of exercise duration (4), changes in plasma myeloperoxidase concentration depend on exercise intensity. Although several other studies have demonstrated increases in the plasma concentration of myeloperoxidase (4, 5, 27), our study is also the first to report a concomitant decrease in intracellular myeloperoxidase concentration. Van Eeden et al. (31) reported no change in this parameter after an incremental exercise test to exhaustion lasting ~10 min. The duration of exercise in their study may not have been sufficient to stimulate the release of myeloperoxidase from neutrophils. The observation that intracellular myeloperoxidase concentration remained low 1 h post-exercise, when plasma myeloperoxidase concentration had returned to normal, could have resulted from an increase in the rate of clearance of myeloperoxidase from the circulation after exercise (27).

The intensity-dependent increase in neutrophil counts after exercise and correlations with stress hormones and cytokines support the work of others (19, 27, 29). The increase in neutrophil respiratory burst activity after HI is consistent with previous findings (28). This increase is likely due to the priming effects of growth hormone and IL-6 on the reduced NADP oxidase enzyme (26, 29) that is responsible for generating reactive oxygen species in neutrophils (16).
In summary, exercise decreased the expression of CD11b, CD16, and CD35 receptors on the surface of neutrophils. However, only CD16 expression was influenced by exercise intensity. The reduction in receptor expression did not appear to influence the release of myeloperoxidase from neutrophils or respiratory burst activity. Together, these alterations may represent a subclinical inflammatory response similar to that observed during sepsis (20). The shedding of receptors may serve as an adaptive mechanism for inhibiting excessive inflammatory reactions. Alternatively, if receptors shed from the cell surface after exercise are not replaced shortly thereafter, this could have deleterious consequences for host protection against infection (2, 21). In theory, the reduction in expression of cell surface receptors may render individuals more susceptible to infection during the purported open window after exercise. Future exercise studies could examine these concepts by measuring the balance between surface expression and the soluble levels of these receptors, as well as tracking changes in neutrophil receptor expression beyond 1 h after exercise.

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GRANTS

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