Raised plasma G-CSF and IL-6 after exercise may play a role in neutrophil mobilization into the circulation

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Yamada, Mutsuo, Katsuhiko Suzuki, Satoru Kudo, Manabu Totsuka, Shigeyuki Nakaji, and Kazuo Sugawara. Raised plasma G-CSF and IL-6 after exercise may play a role in neutrophil mobilization into the circulation. J Appl Physiol 92: 1789–1794, 2002; 10.1152/japplphysiol.00629.2001.—We examined the hypothesis that the short, intense exercise-induced increase in circulating neutrophil counts is affected by the interaction between the endocrine and immune systems. Twelve male winter-sports athletes underwent a maximal exercise test on a treadmill. Blood samples were collected before, immediately after (Post), and 1 h (Post 1 h) and 2 h (Post 2 h) after the exercise. The neutrophil counts increased significantly at Post 1 h (P < 0.05) and remained significantly high even at Post 2 h (P < 0.05), showing a leftward shift. Plasma granulocyte colony-stimulating factor (G-CSF) increased at Post (P < 0.05), and interleukin-6 (IL-6) increased at Post 1 h (P < 0.05). Plasma G-CSF at Post significantly correlated with the numbers of both neutrophils and stab cells at Post 1 h (P < 0.05). Plasma IL-6 at Post 1 h levels also correlated significantly with the number of neutrophils at Post 2 h (P < 0.05). The increase in the levels of plasma G-CSF and IL-6 after intensive exercise may play a role in the mobilization of neutrophils into the circulation system.

maximal exercise; granulocyte colony-stimulating factor; interleukin-6; neutrophil counts

Several studies have shown that physical exercise affects the interactions between the endocrine and immune systems (20, 26, 31). Recent interest has focused on the influence of physical activity on immunity, which is particularly important for persons involved in sports at all levels. Moderate exercise (40–60% maximal $O_2$ uptake) appears to stimulate the immune system (25), reduce the risk of cardiovascular disease, promote health and fitness, and decrease susceptibility to infection (17, 28). In contrast, acute, intensive, and prolonged exercise has been associated with immune suppression and increases the risk of myocardial infarction, sudden death (17), and susceptibility to infection (11, 14).

Neutrophils play a critical role in the host defense mechanism against various bacterial infections. Such defense mechanisms are involved in the killing of invading microorganisms by the liberation of reactive oxygen species and lysosomal enzyme (3).

Circulating neutrophils increase in number after endurance or acute exercise (13, 15). Mobilization of neutrophils from the margined pools into the peripheral circulation is mediated by the exercise intensity-dependent secretion of stress hormones, such as catecholamines (1, 15, 30), cortisol (15, 24, 31, 32), and growth hormone (GH) (29, 31). Catecholamines wash out neutrophils from the margined pools into the circulation through shear force due to exercise-induced hemodynamics. Cortisol mobilizes neutrophils from the bone marrow reserves (29), which can be confirmed by the increase in stab cells (a leftward shift) (29, 31). However, there is a time lag between the increase in serum cortisol and neutrophilia (9), and an increase in serum cortisol concentration would not influence the blood neutrophil count, at least immediately after the exercise. Furthermore, there are some other possible mediators that aid in neutrophil mobilization. Therefore, the association between chemical mediators and neutrophil mobilization is important in understanding part of the interaction between the endocrine and immune systems after exercise.

Recent studies have shown that several cytokines also respond to strenuous exercise (10, 18). Levels of interleukin-6 (IL-6), suggested to be related to muscle damage (5, 21), have been found to increase a few hours after the cessation of exercise (18, 21). Granulocyte colony-stimulating factor (G-CSF) plays a main role in the proliferation, differentiation, prolonged survival, and marrow release of cells of neutrophil lineage (2, 8, 33). Moreover, G-CSF possesses essential neutrophil activating functions, such as the oxidative burst, degranulation, phagocytosis, and chemotaxis (19). Our laboratory's previous study demonstrated that plasma G-CSF concentration increased after long, intensive...
MATERIALS AND METHODS

Changes in leukocyte counts after the intensive exercise

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Post 1 h</th>
<th>Post 2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocytes</td>
<td>5.66 ± 1.26</td>
<td>10.28 ± 1.59†</td>
<td>6.85 ± 2.32</td>
<td>8.91 ± 2.30*</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.95 ± 0.34</td>
<td>4.98 ± 0.78†</td>
<td>1.28 ± 0.25</td>
<td>1.30 ± 0.20</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.29 ± 0.11</td>
<td>0.62 ± 0.17†</td>
<td>0.33 ± 0.18</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.20 ± 1.09</td>
<td>4.51 ± 1.41</td>
<td>5.10 ± 2.08‡</td>
<td>7.11 ± 2.14†</td>
</tr>
<tr>
<td>Stab cells</td>
<td>0.94 ± 0.48</td>
<td>1.35 ± 0.64</td>
<td>2.32 ± 0.98‡</td>
<td>2.73 ± 0.82†</td>
</tr>
</tbody>
</table>

Values are means ± SD in 10^6 cells/μl; n = 12 athletes. Groups are Pre, Post, Post 1 h, and Post 2 h; before, immediately after, and 1 and 2 h after exercise, respectively. Significant difference compared with Pre: *P < 0.05, †P < 0.01.

exercise (32). However, the effects of short, intensive exercise on G-CSF have not been examined.

The mobilization of neutrophils is complicated and remains unclear, including the possible importance of the interaction between the endocrine and immune systems. In this study, therefore, we measured the levels of various cytokines and stress hormones to investigate the relationship between the mobilization of neutrophils and chemical mediators after short, intensive exercise.

RESULTS

Exercise characteristics of skiers. The mean heart rate at the end of acute, intensive exercise was 201 ± 10 beats/min. The mean of the highest oxygen consumption of each subject was 3.8 ± 0.3 l/min, corresponding to 60.7 ± 4.8 ml·kg⁻¹·min⁻¹. The mean running time was 10.3 ± 2.3 min.

Leukocyte counts. As shown in Table 1, the total leukocyte counts at Post significantly increased compared with Pre values (+82%, P < 0.01). The total leukocyte counts tended to be elevated at Post 1 h, although not significantly (+16%), and then further increased significantly at Post 2 h (+57%, P < 0.05). The initial leukocytosis was caused by the increases of lymphocytes and monocytes (P < 0.05). After the exercise, neutrophil counts gradually increased, and the difference was significant at Post 1 h and Post 2 h compared with the Pre value (P < 0.05) (Table 1), suggesting that the delayed increase of leukocyte num-

Preparation of samples for cytokine assays. Blood samples were collected in EDTA and centrifuged at 1,000 g for 10 min, and plasma supernatant was stored at −80°C. Urine samples were also centrifuged at 1,000 g for 10 min and stored frozen at −80°C. Plasma IL-6 and G-CSF concentrations were determined by using commercially available ELISA kits (high sensitivity, R&D Systems, Minneapolis, MN) with a detection limit of 0.1 pg/ml for both IL-6 and G-CSF.

Measurement of stress hormones. Serum samples were separated from whole blood using Vacutainer blood-collection tubes (Becton Dickinson, Franklin Lakes, NJ) by centrifugation at 3,000 g for 10 min. The concentration of cortisol was measured by 125I radioimmunoassay (gamma coat cortisol, Incstar, Stiwiller, MN), and GH concentration was measured by immunoradiometric assay (GH kit, Daiichi Radiotope Institute, Tokyo, Japan).

Statistics. All data are presented as means ± SD. The differences were analyzed by using two-way ANOVA followed by post hoc tests using Fisher's protected least significant adjustment. Correlations between variables were assessed by Pearson's correlation coefficient (r). The level of significance was set at P < 0.05.

MATERIALS AND METHODS

Study subjects. Twelve healthy male cross-country skiers who were at the national junior competition level (aged 17.0 ± 1.2 yr, height 175.1 ± 4.4 cm, body weight 63.3 ± 5.3 kg) participated in this study during an off-season period (training period). None of the subjects was involved in any training for 2 days before the study. In addition, 12 sedentary (untrained) men (aged 19.3 ± 1.5 yr, height 170.8 ± 4.1 cm, body weight 61.8 ± 5.8 kg) served to examine the effect of the circadian rhythm on chemical mediators. There were no significant differences in height and body weight between the experimental and control groups. After being informed of the study design and the possible risks, all subjects gave their written consent to participate. This study protocol was approved by the Ethics Committee of Hirosaki University School of Medicine.

Exercise protocol. All skiers performed an incremental graded maximal exercise test on a treadmill until exhaustion. The treadmill speed started at 220 m/min for the first 2 min and 220 m/min at a 4% grade for the next 2 min. The speed was increased by 10 m/min each minute thereafter until exhaustion. None of the sedentary men engaged in this course of acute, intensive exercise.

Blood sampling and differential leukocyte count. Peripheral blood samples were collected via the median antecubital vein from all subjects preexercise (Pre), immediately after (Post), and 1 h (Post 1 h) and 2 h (Post 2 h) after the exercise. The total leukocyte count was calculated in each blood sample using a Micro Diff II (Beckman Coulter, Tokyo, Japan). May-Giemsa staining was performed on coverslipped whole blood smears, and differential counting of blood leukocytes (leukocytes, macrophages, and stab cells) was performed microscopically (>1,000) by examining at least 200 cells per slide, as described previously (30). The number of each cell type was calculated by the percentage of each differential count. Blood samples were also analyzed for hematocrit changes to determine plasma volume shift.

Urine sampling. Simultaneously, urine samples were collected from all subjects at Pre, Post, Post 1 h, and Post 2 h, and G-CSF and IL-6 concentrations were measured.

Fig. 1. Ratio of stab cells to neutrophils after intensive exercise. Values are mean relative ratios ± SD of 12 athletes. Groups are Pre, Post 1 h, and Post 2 h; before, immediately after, and 1 and 2 h after exercise, respectively. Significantly different compared with Pre: *P < 0.05, **P < 0.01.
Fig. 2. Levels of granulocyte colony-stimulating factor (G-CSF) after intensive exercise. A: levels of plasma G-CSF. B: levels of urine G-CSF. Values are means ± SD of 12 athletes. *Significantly different compared with Pre, \( P < 0.05 \).

Fig. 3. Levels of interleukin-6 (IL-6) after intensive exercise. A: levels of plasma IL-6. B: levels of urine IL-6. Values are means ± SD of 12 athletes. *Significantly different compared with Pre, \( P < 0.05 \).

Fig. 4. Levels of serum stress hormones after intensive exercise. A: levels of serum cortisol. B: levels of serum growth hormone. ●, The 12 athletes; □, the 12 sedentary controls. Values are means ± SD. *Significantly different compared with Pre, \( P < 0.05 \).
bers might be caused by neutrophilia. The stab cell counts gradually increased in the same manner as neutrophils (Table 1). In addition, the ratio of stab cell to neutrophil counts was significantly increased at Post 1 h and Post 2 h (P < 0.05) (Fig. 1). The hematocrit was not affected by the intensive exercise, suggesting that there were no marked shifts in plasma volume. On the other hand, these changes were not seen in the sedentary male control group.

**Cytokine concentration.** Plasma G-CSF levels increased significantly at Post compared with Pre (P < 0.05) (Fig. 2A). Urine G-CSF levels also increased significantly at Post 1 h compared with Pre (P < 0.05) (Fig. 2B). Plasma IL-6 levels increased significantly at Post 1 h compared with Pre (P < 0.05) (Fig. 3A). Urine IL-6 levels increased slightly after the exercise but not significantly, compared with Pre (Fig. 3B). On the other hand, the levels of cytokine concentration at rest did not change during the study period in the sedentary male control group.

**Stress hormone concentration.** The levels of serum cortisol in the athletes increased significantly at Post and Post 1 h compared with Pre (P < 0.05) (Fig. 4A). GH concentration also increased significantly at Post compared with Pre (P < 0.05) (Fig. 4B). Both of these values returned to their Pre levels at Post 2 h. On the other hand, the levels of both stress hormones at rest did not change during the study period in the sedentary male control group.

**Correlation between neutrophil counts and chemical mediators.** The level of plasma G-CSF at Post correlated positively, not only with the neutrophil counts (r = 0.673, P < 0.05) but also with the stab cell counts at Post 1 h (r = 0.583, P < 0.05) (Fig. 5, A and B). In addition, the level of plasma IL-6 at Post 1 h also correlated positively with the neutrophil counts at Post 2 h (r = 0.626, P < 0.05) (Fig. 5C). Positive correlations were also observed between the level of serum GH at Post and neutrophil counts at Post 1 h (r = 0.592, P < 0.05), and between the level of serum cortisol at Post 1 h and stab cell counts at Post 2 h (r = 0.607, P < 0.05) (Table 2).

**DISCUSSION**

The major finding of this study was that the levels of plasma and urine G-CSF and plasma IL-6, as well as the levels of serum cortisol and GH, increased in accordance with the increase in the number of neutrophils after the intensive exercise session.

**Table 2. Correlations between serum levels of stress hormones and neutrophil counts**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Correlation Coefficient</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Cortisol at Post vs. neutrophil count at Post 1 h</td>
<td>0.236</td>
<td>0.471</td>
</tr>
<tr>
<td>Cortisol at Post vs. stab cell count at Post 1 h</td>
<td>0.514</td>
<td>0.088</td>
</tr>
<tr>
<td>Cortisol at Post vs. neutrophil count at Post 2 h</td>
<td>0.137</td>
<td>0.678</td>
</tr>
<tr>
<td>Cortisol at Post vs. stab cell count at Post 2 h</td>
<td>0.447</td>
<td>0.149</td>
</tr>
<tr>
<td>Cortisol at Post 1 h vs. neutrophil count at Post 1 h</td>
<td>0.240</td>
<td>0.463</td>
</tr>
<tr>
<td>Cortisol at Post 1 h vs. stab cell count at Post 1 h</td>
<td>0.058</td>
<td>0.861</td>
</tr>
<tr>
<td>Cortisol at Post 1 h vs. neutrophil count at Post 2 h</td>
<td>0.212</td>
<td>0.519</td>
</tr>
<tr>
<td>Cortisol at Post 1 h vs. stab cell count at Post 2 h</td>
<td>0.607</td>
<td>0.035</td>
</tr>
<tr>
<td>GH at Post vs. neutrophil count at Post 1 h</td>
<td>0.592</td>
<td>0.041</td>
</tr>
<tr>
<td>GH at Post vs. stab cell count at Post 1 h</td>
<td>0.290</td>
<td>0.371</td>
</tr>
<tr>
<td>GH at Post vs. neutrophil count at Post 2 h</td>
<td>0.477</td>
<td>0.119</td>
</tr>
<tr>
<td>GH at Post vs. stab cell count at Post 2 h</td>
<td>-0.038</td>
<td>0.909</td>
</tr>
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GH, growth hormone.
G-CSF is produced by macrophages, lymphocytes (7), fibroblasts (4), and endothelial cells (12) in response to certain stimuli. In a previous study, endurance exercise increased the level of plasma G-CSF (32). In the present study, intensive exercise also stimulated the secretion of G-CSF, although the origin of G-CSF was not clear. We found that urine G-CSF levels increased at Post 1 h. G-CSF is metabolized and resolved by several organs but mostly excreted in urine (16). Therefore, this increase of urine G-CSF levels at Post 1 h might reflect the increased levels of G-CSF plasma at Post.

In the present study, plasma IL-6 concentration increased significantly at Post 1 h. IL-6, one of the proinflammatory cytokines, is associated with muscle damage (5, 21). IL-6 levels after exercise have been shown to be correlated with creatine kinase (MM3) and myoglobin (Mb), both indicative of muscle damage (5, 21, 31). A previous study demonstrated that Mb leaked easily from damaged muscles and disappeared immediately through renal excretion, suggesting that Mb is a more sensitive indicator than creatine kinase (31). In this study, Mb levels tended to increase after exercise and were significantly elevated at Post 1 h (unpublished observation). Moreover, we found a positive correlation between Mb at Post 1 h and the level of plasma IL-6 at Post 1 h. Therefore, it was possible that IL-6 levels increased as a result of muscle damage.

Acute exercise is known to mobilize the peripheral leukocytes, the magnitude of which reflects the intensity and duration of the exercise (11, 15). Several researchers have reported that short-term exercise resulted in a biphasic leukocyte response (11, 13, 15). The immediate transient response of leukocytes composed of lymphocytes, monocytes, and neutrophilia was followed by a delayed response mainly due to neutrophilia (11, 13, 15), as observed in our results.

Mobilization of neutrophils from the marginated pools is mediated by exercise intensity-dependent secretion of stress hormones, such as catecholamines (1, 15, 30), cortisol (15, 24, 31, 32), and GH (27, 31). Catecholamines wash out neutrophils from the marginated pools into the circulation. Furthermore, the levels of epinephrine after the exercise correlated positively with increased neutrophil counts (30). Although catecholamines were not measured, the levels of other stress hormones increased after the intensive exercise sessions in this study. Cortisol mobilizes neutrophils from the marginated bone marrow pools (9, 29), but there is a time lag between the increase in serum cortisol and neutrophilia (9, 15). Therefore, after intensive exercise, an increase in serum cortisol concentration would not influence the blood neutrophil count, at least immediately after the exercise. In this study, the levels of serum cortisol increased at Post and Post 1 h, and the levels at Post 1 h correlated positively with stab cell counts at Post 2 h. Cortisol thus mobilized neutrophils after Post 1 h, possibly leading to a gradual increase in neutrophil counts during the recovery period. Moreover, in the present study, there was a positive correlation between neutrophil counts at Post 1 h and the increased levels of serum GH at Post. Therefore, the levels of GH after the exercise might affect the delayed-onset neutrophilia.

On the other hand, we demonstrated a correlation between the increased levels of plasma G-CSF and both neutrophil and stab cell counts at Post 1 h. G-CSF plays a role in the marrow release of neutrophil lineage cells and in decreasing the transit time for neutrophils through the bone marrow into the peripheral blood (22). Moreover, several recent reports have shown that G-CSF is a faster mobilizing agent than corticosteroids for the collection of granulocytes for transfusion (6). Therefore, the increased levels of G-CSF might be associated with the mobilization of neutrophil with a leftward shift after exercise. Stress hormones and IL-6 possess pharmacological mobilization actions (23). Stress hormones might operate as a trigger of neutrophil mobilization and priming, and IL-6 may augment the inflammatory process. Therefore, a positive correlation between the level of IL-6 at Post 1 h and neutrophil counts at Post 2 h might suggest that IL-6 is involved in the later mobilization of neutrophil during the recovery period. Smith et al. (27) also showed that acute, eccentric exercises (bench-press and leg-curl exercise) elicited a significant increase in macrophage colony-stimulating factor (M-CSF) and IL-6. Although this is a different exercise protocol from that of the present study, it is a related study. However, there were two points of difference between the two studies. The present study showed that G-CSF (not M-CSF) and IL-6 might also regulate neutrophilia and that this regulation was for “delayed-onset” neutrophilia.

Although dramatic increases in plasma IL-6 and G-CSF were reported in the previous studies using marathon races (18, 32), the difference with the small increase in the present study should be attributed to the difference in exercise condition, that is, the duration of exercise in combination with muscle damage. In any case, it is newly demonstrated that cytokines are secreted into the circulation immediately, in response to short-time exercise.

In conclusion, G-CSF and IL-6 might also regulate delayed-onset neutrophilia with a leftward shift after intensive exercise. The mobilization of neutrophils after exercise may thus be regulated by both the stress hormones and G-CSF in addition to M-CSF and IL-6.

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