Effects of different durations of exercise on macrophage functions in mice

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Received 28 January 2000; accepted in final form 15 September 2000

Sugiura, Haruo, Hiroko Sugiura, Hiroyuki Nishida, Ryoichi Inaba, S. M. Mirbod, and Hirotoshi Iwata. Effects of different durations of exercise on macrophage functions in mice. J Appl Physiol 90: 789–794, 2001.—The effects of differing durations of daily exercise on macrophage functions in mice were studied. Male ICR mice aged 4 wk were divided into five groups: a nonexercise group (control) and four exercise groups with differing daily exercise durations of 15–120 min (Exr groups). The exercise applied was 5 days/wk treadmill running at 13 m/min for 12 wk. The potentiation of the phagocytosis function of the reticuloendothelial system and the glucose consumption of peritoneal macrophages in the Exr 30, 60, and 120 groups were significantly higher than those in the control group. Superoxide anion production of peritoneal macrophages in both the absence and the presence of phorbol 12-myristate 13-acetate in the Exr 60 and 120 groups was significantly higher than that in the control group. The acid phosphatase and β-glucuronidase activities of peritoneal macrophages in the Exr 30, 60, and 120 groups were significantly increased. These results suggest that treadmill running exercise for at least 30 min/day (30–120 min) effectively enhances macrophage functions in mice. These data provide preliminary evidence indicating that chronic exercise-induced increases in phagocytic activity exhibit a dose-dependent relationship with exercise duration.

exercise duration; reticuloendothelial system; phagocytic activity

It is well known that exercise training has a number of beneficial physiological effects, e.g., increased insulin sensitivity, improved lipid profile, increased maximal oxygen uptake, maintenance of muscle strength, and increased bone mass (3). Comitantly, it is documented that physical exercise modulates functions of the immune system (10, 19, 29). Macrophages are, for many reasons, the most appropriate cell subset in which to study the influence of physical exercise on the status of the immune system. In addition to the non-specific host defense mechanism in the efferent pathway of the immune system (phagocytosis and elimination of microorganisms), macrophages play a central role in the afferent pathway of the immune system (11). Many reports have been made on the effects of exercise training on immune functions, such as macrophage and lymphocyte functions (32). Recent studies indicate that regular and moderate exercise stimulates some aspects of the immune response (16), whereas intense exercise training may lead to immunosuppression and elevated risk for infectious diseases (23). Furthermore, it is known that phagocytes such as macrophages play an important role in the defense mechanisms of such infectious diseases.

Macrophages constitute an important part of a first line of defense against microbial invaders and malignancies by nature of their phagocytic, cytotoxic, and intracellular killing capacities. In addition, macrophages are important as antigen-presenting cells (1). With regards to the influence of exercise training on macrophage functions, several investigators have reported exercise-induced increases in macrophage chemotaxis, adherence, respiratory burst, and phagocytic activities after a single bout of exercise (8, 10, 25). The effects of continued exercise training on macrophage function have been the subject of only a few studies. Chronic treadmill running exercise for 16 wk in young mice increased macrophage cytolytic activity (19). Fernandez and De la Fuente (13) reported that stimulation of phagocytic functions (chemotaxis capacity, phagocytosis capacity, and superoxide anion production) was higher for peritoneal macrophages in mice after swimming exercise for 90 min/day over a period of 20 days, compared with sedentary control mice. In connection with T cell proliferative activity, an indicator for cellular immunity, endurance exercise training has been reported to enhance T cell mitogenesis with mitogen stimulation (7, 28, 29, 30). In contrast, it has been reported that heavy exercise induces immunosuppression (6, 20, 22). The effects of treadmill running and voluntary exercise on macrophage and lymphocyte functions in mice were reported in our previous studies (28, 29). We have previously shown that treadmill exercise...
running exercise and voluntary running exercise in mice for 8–12 wk enhance phagocytic and elimination activities in peritoneal macrophages and augment lymphocyte proliferative responses to concanavalin A. Thus not only positive (9, 13, 18, 19, 28, 29) but also negative (15, 21, 22) effects of exercise training on immune functions have been reported. No documentation has been made regarding the possible impact of exercise training on immune function. Moreover, the varying results on the effects of exercise on immune functions reported in these studies may be influenced by the quality and/or quantity of exercise applied in the studies. In fact, little attention has been paid to the effects of differing conditions of exercise on immune functions (14, 30).

The purpose of this study was to evaluate the effects of differing durations of daily exercise on immune functions, particularly on macrophage function, in mice. We hypothesized that a dose-dependent relationship exists between exercise duration and exercise training-induced increase in macrophage function. Macrophage function was evaluated by measuring phagocytosis activity of the reticuloendothelial system, glucose consumption, superoxide anion production, and lysosomal enzyme activity by peritoneal macrophages as an indicator.

METHODS

Animals. The experimental protocol, animal care, and treatment were approved by the committee for animal studies at Gifu Pharmaceutical University. A total of 61 male ICR strain mice, 3 wk old, weighing 10–12 g, obtained from Japan SLC (Hamamatsu City, Japan), was used for this experiment. They were housed, five to six per cage, with a pelleted basal diet, CE-2 (CLEA Japan, Tokyo, Japan), and water ad libitum, in an animal room under a 12:12-h light-dark cycle at a temperature of 22 ± 1°C and humidity of 60 ± 5%. After a week of acclimation, they were used for the experiment.

Exercise program. The 61 mice were divided into five groups: four exercise groups with differing daily exercise duration regimens of 15 (Exr 15 group, n = 12), 30 (Exr 30 group, n = 12), 60 (Exr 60 group, n = 12), and 120 min (Exr 120 group, n = 12) and the treadmill control group (control, n = 13). The exercise applied was treadmill running (without electrical shock treatment) at 12.12±0.5 m/min for 12 h-light-dark cycle at a temperature of 22 ± 1°C and humidity of 60 ± 5%. After a week of acclimation, they were used for the experiment.

The period of exercise was 5 days/wk for 12 wk, and the time the treadmill running exercise was conducted was 6–8 PM. To control for any noneffect of treadmill running such as handling, novel environment, noise, and vibration, animals of the control group were kept in the same room and placed on a slow-moving treadmill (5 m/min, 5 min/day, 5 days/wk). To remove the acute effects of the treadmill running exercise, the animals were killed by bleeding under ether anesthesia 72 h after the last treadmill running exercise; 30 mice were used for carbon clearance assay and 31 for isolation of peritoneal macrophages.

Before the main experiments, the liver, spleen, and anterior tibialis muscle in all groups were weighed.

Assay of carbon clearance activity. The phagocytic activity of the reticuloendothelial system was determined from measuring clearance of colloidal carbon from the peripheral blood, as described previously (27). Briefly, the carbon suspension was injected intravenously at 0.1 ml/10 g body wt under ether anesthesia. Blood samples were collected into heparinized capillary tubes from the retro-orbital plexus of mice anesthetized with ether at 5 and 10 min after the injection. Immediately after collection, the blood was mixed with 0.1% sodium carbonate solution. Optical density of the mixture was measured at 675 nm with a spectrophotometer (Hitachi U-2000A, Tokyo, Japan). Each group consisted of six mice.

The phagocytic index was calculated according to the following equation

\[
\text{Phagocytic index} = \left(\log \text{OD}_{t5} - \log \text{OD}_{t10}\right)/\left(t10 - t5\right)
\]

where \(t5 = 5 \text{ min}, t10 = 10 \text{ min}, \text{OD}_{t5} = \text{optical density at 5 min after the injection of carbon solution}, \text{and OD}_{t10} = \text{optical density at 10 min after the injection of carbon solution}.

Isolation of macrophages. All procedures were conducted under aseptic conditions. Six to seven mice were selected from each group. Mice were killed by bleeding under ether anesthesia, and peritoneal cells (PEC) were obtained by intraperitoneal injection of Hanks’ solution (Nissui Seiyaku, Tokyo, Japan). The PEC were pooled in each group. Macrophages in the PEC suspension were isolated by the cell adhesion method. The PEC were suspended in RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) containing 10% heat-inactivated FCS (GIBCO Laboratories Life Technologies, Grand Island, NY) and incubated in a culture plate (Corning Laboratory Sciences, Corning, NY) for 2 h at 37°C in a 5% CO₂ incubator. After nonadherent cells were removed by the plate being washed with Hanks’ solution, the adherent cells were harvested from the bottom by using a rubber policeman and were resuspended in 10% FCS-RPMI 1640 medium. The cells were used for experiments as resident peritoneal macrophages at a concentration of 2 × 10⁶ cells/ml. Cell viabilities checked by the Trypan blue dye exclusion test were >95%.

Assay of glucose consumption in peritoneal macrophages. Glucose remaining in the peritoneal macrophages culture supernatant was determined by use of a commercial kit, Glucose B-test Wako (Wako, Osaka, Japan). Briefly, the supernatants (20 μl) obtained from the macrophage culture sample (2 × 10⁶ cells/well) for 24 h at 37°C were incubated with 3.0 ml of color reagent for 20 min at 37°C. The optical density of the solution at 505 nm was measured, and the remaining glucose was determined from a calibration curve with standard glucose solution. The results were expressed as percent glucose consumption, calculated from the following formula

\[
1 - \left(\frac{\text{glucose content in culture medium cultured with macrophages/glucose content in culture medium without macrophages}}{100}\right)
\]
Assay of enzyme activities. Enzyme activities in peritoneal macrophages were determined by the method reported previously (27). The intracellular activities of acid phosphatase (APh) and β-glucuronidase (Glu) of the solution were measured by the p-nitrophenyl phosphate methods (Wako) and Glu kit (Sigma Chemical), respectively. The APh activity was expressed as international unit (IU) per 2 × 10⁵ cells, and Glu activity was expressed as IU per 4 × 10⁵ cells.

Statistical analysis. Results are presented as means ± SE. Data were analyzed statistically by using one-way ANOVA and post hoc Scheffé’s test for multiple comparisons. Significance levels were set at \( P < 0.05 \).

RESULTS

General observation. The mean body weight and relative liver, spleen, and muscle weights in each group are indicated in Table 1. No significant effects of treadmill running exercise on body weight or relative liver and spleen weights were observed. The muscle weight increased with increased duration of running. The weight of the tibialis anterior muscle in the Exr 30, Exr 60, and Exr 120 groups, but not in the Exr 15 group, after a 24-h culture was significantly higher (50–59%) than that in the control group (\( P < 0.05 \) or \( P < 0.01 \)).

Glucose consumption capacity. The results of glucose consumption capacity in peritoneal macrophages are illustrated in Fig. 2. Glucose consumption of peritoneal macrophages in the Exr 30, Exr 60, and Exr 120 groups, but not in the Exr 15 group, after a 24-h culture was significantly higher than that in the control group (\( P < 0.01 \)).

Superoxide anion production capacity. As shown in Fig. 3, superoxide anion production of peritoneal macrophages in the absence of PMA was significantly enhanced (from 93 to 96%) in the Exr 60 and Exr 120 groups, but not in the Exr 15 and Exr 30 groups, compared with that in the control group (\( P < 0.01 \)). In the presence of PMA, superoxide anion production in peritoneal macrophages in the Exr 60 and Exr 120 groups, but not in the Exr 15 and Exr 30 groups, was significantly higher (from 74 to 86%) than that in the control group (\( P < 0.01 \)). The highest value of superoxide anion production of peritoneal macrophages in the presence of PMA was observed in the Exr 120 group.

APh and Glu activities. The data of intracellular activities of APh and Glu of peritoneal macrophages are summarized in Table 2. APh activity of peritoneal macrophages in the Exr 30, Exr 60, and Exr 120 groups, but not in the Exr 15 group, was significantly higher (50–59%) than that in the control group (\( P < 0.05 \) or \( P < 0.01 \)). Glu activity of peritoneal macrophages in the Exr 30, Exr 60, and Exr 120 groups, but not in the Exr 15 group, was significantly higher than that in the control group (\( P < 0.01 \)). The highest value of Glu activity in peritoneal macrophages was observed in the Exr 60 group.

Table 1. Body weight gain and relative liver, spleen, and muscle weights in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>4 wk</th>
<th>16 wk</th>
<th>Liver (mg/g per body wt)</th>
<th>Spleen (mg/g per body wt)</th>
<th>Anterior Tibialis (mg/g per body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>22.0 ± 0.2</td>
<td>45.8 ± 1.1</td>
<td>55.9 ± 0.9</td>
<td>4.26 ± 0.16</td>
<td>3.20 ± 0.17</td>
</tr>
<tr>
<td>Exr 15</td>
<td>12</td>
<td>22.1 ± 0.3</td>
<td>45.8 ± 0.7</td>
<td>54.3 ± 1.8</td>
<td>3.90 ± 0.18</td>
<td>3.63 ± 0.14</td>
</tr>
<tr>
<td>Exr 30</td>
<td>12</td>
<td>22.7 ± 0.2</td>
<td>44.3 ± 1.0</td>
<td>55.2 ± 1.4</td>
<td>4.10 ± 0.28</td>
<td>4.18 ± 0.15**</td>
</tr>
<tr>
<td>Exr 60</td>
<td>12</td>
<td>22.5 ± 0.2</td>
<td>46.2 ± 0.4</td>
<td>55.0 ± 1.1</td>
<td>4.06 ± 0.14</td>
<td>3.98 ± 0.12†</td>
</tr>
<tr>
<td>Exr 120</td>
<td>12</td>
<td>22.5 ± 0.5</td>
<td>46.7 ± 1.0</td>
<td>57.2 ± 1.7</td>
<td>5.98 ± 0.63</td>
<td>4.19 ± 0.23**</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), number of mice examined; Control, nonexercise group; 4 exercise groups with differing daily exercise durations of 15 (Exr 15), 30 (Exr 30), 60 (Exr 60), and 120 min (Exr 120). ANOVA revealed a significant difference among the exercise groups: \( F(4, 56) = 4.1, P < 0.01 \). *Significant difference from the control group. †\( P < 0.01 \), ‡\( P < 0.05 \) (post hoc Scheffé’s test).
DISCUSSION

The effects of physical exercise on immune functions in experimental animals have been reported by many investigators (see, for example, Refs. 13, 18, 19, 28, 29); however, the effects of differing exercise durations on immune functions, especially macrophage functions, have not yet been reported. In this study, male ICR strain mice aged 4 wk were used to examine the effects of different durations of exercise on the phagocytic activity of the reticuloendothelial system in vivo; glucose consumption, superoxide anion production, and lysosomal enzyme by peritoneal macrophages were used as indicators of macrophage functions in vitro.

Japel and co-workers (17) reported that treadmill running exercise increases phagocytosis activities of peritoneal and splenic macrophages in mice. They could not observe any particular differences between peritoneal and splenic macrophages in response to exercise. This was the reason for only peritoneal macrophages being examined in this study. The results in this study indicate that treadmill running exercise effectively enhanced macrophage function in mice and that exercise-induced increases in macrophage function, especially phagocytosis and elimination activities, exhibit a dose-dependent relationship with exercise duration. To our knowledge, this is the first study to investigate the relationship between exercise duration and macrophage function in mice.

Initially, in the present study, the effects of differing durations of exercise on the phagocytic activity of the

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**Table 2. Effects of different durations of exercise on intracellular activities of APh and Glu in peritoneal macrophages in mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>APh/2 × 10^5 cells (× 10^4 IU)</th>
<th>Glu/4 × 10^5 cells (× 10^1 IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.54 ± 0.09</td>
<td>3.07 ± 0.03</td>
</tr>
<tr>
<td>Exr 15</td>
<td>4.00 ± 0.12</td>
<td>3.42 ± 0.13</td>
</tr>
<tr>
<td>Exr 30</td>
<td>4.28 ± 0.02*ab**</td>
<td>4.77 ± 0.08*ab**</td>
</tr>
<tr>
<td>Exr 60</td>
<td>4.60 ± 0.13*abc</td>
<td>5.27 ± 0.01*abcde</td>
</tr>
<tr>
<td>Exr 120</td>
<td>4.55 ± 0.11*abc**</td>
<td>4.29 ± 0.14*abc**</td>
</tr>
</tbody>
</table>

Values are means ± SE. APh, acid phosphatase; Glu, β-glucuronidase. For isolation of macrophages, see Fig. 2. ANOVA procedure revealed a significant difference among the exercise groups: APh, F(4, 15) = 14.7, P < 0.0001; Glu, F(4, 15) = 50.7, P < 0.0001. *Significantly different from the control group. **Significantly different from the Exr 15 group. "Significantly different from the Exr 30 group. †Significantly different from the Exr 120 group. **P < 0.01, †P < 0.05 (post hoc Scheffe’s test).
Macrophages play a critical role in the primary host defense against infection (5). Macrophage glucose consumption and superoxide anion production increase as a result of macrophage activation (31, 33). In the present study, the glucose consumption capacity of peritoneal macrophages after 24 h incubation was significantly higher in all Exr groups except for the Exr 15 group, compared with the control group. Also, superoxide anion production of peritoneal macrophages in both the absence and the presence of PMA was significantly enhanced in the Exr 60 and Exr 120 groups, but not in the Exr 15 group, compared with the control. In the Exr 120 group, the production of superoxide anion in the peritoneal macrophages was most markedly increased. Ferrandez and De la Fuente (13) in their recent study reported that phagocytosis activity and NBT reduction of murine peritoneal macrophages were enhanced after swimming exercise (90 min/day for 20 days). Although the style of exercise is different, these results suggest that exercise training activates peritoneal macrophages. Because glucose consumption and superoxide anion production by macrophages are related to the pentose phosphate pathway of glycolysis (33), it is likely that treadmill running exercise for at least 30 min/day activates the pentose phosphate pathway in peritoneal macrophages. Moreover, we found that the superoxide anion production capacity of peritoneal macrophages in the absence of PMA was enhanced after treadmill running exercise. It can be concluded that oxidative metabolism is increased with increased exercise duration, as also shown by other researchers (9, 13).

It is well known that activities of lysosomal enzymes in the peritoneal macrophages increase after macrophage activation (2). In this study, we evaluated the function of the elimination stage of the phagocytic process by measuring the lysosomal enzymes APh and Glu in peritoneal macrophages from exercising mice. APh and Glu activities of peritoneal macrophages in the Exr 30, Exr 60, and Exr 120 groups, but not in the Exr 15 group, were significantly higher than that in the control group, although in the present study, Glu activity of peritoneal macrophages was slightly lower in the Exr 120 group than in the Exr 60 group. The results described here suggest that treadmill running exercise for at least 30 min/day activates the macrophages through modulation of lysosomal enzyme activities of APh and Glu in peritoneal macrophages. Also, running exercise partially affects the ability of lysosomal enzymes in peritoneal macrophages to respond appropriately to foreign agents. It has previously been discussed that heavy exertion can cause negative changes in immune functions (22), and the present results suggest that long-term treadmill running exercise of ~120 min or more possibly causes adverse effects on macrophage functions. To elucidate this speculation, further surveys with various exercise durations are required. Thus, on the basis of the data presented in this study and materials discussed here, we infer that, when the exercise is limited to 15 min/day, its effect on the phagocytosis activities would not be noticeable. To improve the macrophage functions, we assume that exercise training of >30 min/day should be applied.

It is known that strenuous physical exercise leads to an augmented generation of reactive oxygen species and reactive nitrogen species, resulting in immunosuppression. On the other hand, chronic exercise seems to reduce the capacity of leukocytes for oxidant release and leads to an adaptation of antioxidative mechanisms, which may contribute to a limitation of exercise-induced oxidative stress (24). Smith (26) reported in a review article that moderate exercise increased reactive oxygen production capacity in neutrophils and that it enhanced infectious defense capacity. Hence, in the present study, it would have been desirable to examine reactive oxygen and nitrogen species, as well.

In conclusion, our results suggest that treadmill running exercise for at least 30 min (30–120 min) per day stimulates the phagocytic functions of peritoneal macrophages in mice. These data provide preliminary evidence indicating that chronic exercise-induced increases in phagocytic activity exhibit a dose-dependent relationship with exercise duration.

Finally, the results reported here should be evaluated with caution, as the measure of immune function employed in this study may encounter some limitations such as lack of antigen specificity, cell selection, and so on. Future studies free of the mentioned limitations would further our understanding on the clear effect of chronic exercise on immune functions.

This study was supported in part by Grants-in-Aid for Scientific Research (nos. 10780032 and 12680045) from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES