Treadmill exercise training blunts suppression of splenic natural killer cell cytolysis after footshock

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Treadmill exercise training blunts suppression of splenic natural killer cell cytolysis after footshock. J Appl Physiol 88: 2176-2182, 2000.—This study extended to treadmill exercise training our prior report (Dishman RK, Warren JM, Youngstedt SD, Yao H, Bunnell BN, Mougey EH, Meyerhoff J L, J aso-Friedmann L, and Evans DL. J. Appl Physiol 78: 1547–1554, 1995) that activity wheel running abolished the suppression of footshock-induced natural killer (NK) cell cytosis. Twenty-four male Fischer 344 rats were assigned to one of three groups (n = 8, all groups): 1) a home-cage control group, 2) a sedentary treatment group, or 3) a treadmill-running group (0° incline, 25 m/min, 35 min/day, 6 days/wk). After 6 wk, the treadmill and sedentary groups received 2 days of footshock. Splenic NK cytotoxicity was determined by standard 4-h51Cr release assay. Percentages of lymphocytes were determined by flow cytometry. Plasma levels of ACTH, corticosterone, and prolactin concentration were measured by radioimmunoassay. After footshock, percentage of lysis relative to home-cage controls was 40% and 80% for sedentary and treadmill-trained animals, respectively (P < 0.05). Our results indicate that the protective effect of chronic exercise on innate cellular immunity in the Fischer 344 male rat is not restricted to activity wheel running, nor is it explained by elevations in basal NK activity, increased percentages of splenic NK and cytotoxic T cells, or increased plasma levels of ACTH, corticosterone, and prolactin.

lymphocytes; ACTH; corticosterone; prolactin

NATURAL KILLER (NK) cells are important in immunosurveillance against spontaneously arising tumors, bloodborne metastasizing tumor cells, acquired immune deficiency syndrome, and certain viral, bacterial, and protozoan infections (50). Because the lysis of many microorganisms, virus-infected cells, and tumor cells, without prior exposure to or recognition of the major histocompatibility complex, is fundamental for a host’s resistance to infection, it is important to establish whether chronic exercise can influence NK cell cytotoxicity, as reported in human blood-sampling studies (16, 31, 38, 55). Those studies are difficult to interpret because of the migratory flux of lymphocytes between blood and lymph tissues. Moreover, the studies were restricted to measures of basal NK activity. A biologically plausible explanation of how basal NK activity is altered after moderately intense chronic exercise, as used in past studies of humans, has not been elucidated (38, 55).

Studies of rodents permit the usage of procedures that cannot be ethically performed on healthy humans, including 1) sampling of NK activity in lymphatic tissues other than blood and 2) experimental manipulations of plausible mechanisms of innate cellular immunity. Several studies of NK activity in mice and rats after chronic activity wheel running or treadmill training have found increased in vivo NK cytosis in the lung (25, 26, 32, 33) and increased (4, 32, 33), reduced (3, 5), or unchanged (10, 17, 37) in vitro NK cytolysis in the spleen. However, those studies were limited to the analysis of basal NK activity. Another approach to the study of chronic exercise and NK activity involves the coincident study of NK suppression induced by nonexercise conditions (9, 10). This approach permits the generalization of increased NK activity after chronic exercise to be tested by exposing exercise-trained animals to a novel stressor that is known to suppress NK activity. Such an approach permits testing of the hypothesis that exercise training confers an immunoprotective effect as a result of a generalized cross-stressor adaptation in neural and/or neuroendocrine modulation of the innate immune system (45). Footshock reliably leads to a 25–50% acute suppression of in vitro splenic NK activity in rats that persists for 24 h (8–10, 41, 42, 52). Using this approach, we previously reported that 6 wk of circadian activity wheel running abolished the suppression of splenic NK activity induced by 2 days of repeated footshock without affecting basal NK cytotoxicity (10).

That study did not determine whether the results were explained by adaptations to chronic physical exertion rather than to a cage environment that was enriched compared with that for standard rat husbandry. Activity wheel running appears to be a motivated circadian behavior among rats (43); therefore, it might have a positive immunomodulatory effect through psychophysiological mechanisms (21, 22, 52) that is independent of physiological adaptations to physical activity. The use of treadmill running permits the
addition of a standard exercise stimulus beyond that of normal circadian physical activity, which, in our experience, does not increase physical fitness in the Fischer 344 strain, as determined by the oxidative capacity in locomotory skeletal muscle (10).

Thus the present study extended our test to treadmill exercise training. Although prior studies of mice have reported that immune responses to exercise training were similar after treadmill or activity wheel running (32, 33), those studies only examined basal NK activity. Determining whether exercise training results in a cross-stressor adaptation in innate immunity requires that responses to a nonexercise stressor be measured as well. Although treadmill exercise can confound exertion with emotional stress during novel or early sessions of exposure, rats adapt to treadmill training after 6 wk, as indicated by hypothalamic-pituitary-adrenal (HPA) cortical hormone responses after running (53).

The primary purpose of this study was to determine whether treadmill exercise training would attenuate the suppression of splenic NK cytotoxicity induced by repeated bouts of uncontrollable footshock. Because altered cytotoxicity after footshock could result from changed proportions of cytotoxic lymphocytes and NK cells (18, 28), we estimated splenic NK, B lymphocyte, and T lymphocyte cell populations using flow cytometry. Also, exercise training could affect basal NK cell percentage and activity in conditions without stress, thus confounding direct comparisons of treadmill-trained and sedentary animals after footshock. Therefore, a study replicating the treadmill exercise training and the sedentary groups without footshock was performed.

Mechanisms explaining the effects of exercise on NK activity in rats are not known. Although sympathetic nerve activity appears to modulate splenic NK activity (9, 12), evidence also suggests that splenic NK cell activity or number is modulated by HPA cortical responses to stress (1, 7, 27). Adrenal corticoids influence distribution of T lymphocytes so that the percentage of NK cells would be increased (1). Studies of humans report that glucocorticoids suppress (14, 19) or have no effect (39, 48) on blood NK activity, whereas prolactin mitigates corticosterone’s suppressive effects (2). It is plausible that the downregulation of the HPA system, a characteristic of adaptations to chronic exercise of moderate intensity (47), might lead to a cross-stressor attenuation of NK suppression. Although we previously reported that plasma levels of HPA hormones did not explain blunted NK suppression after footshock among chronic activity wheel runners (10), we reexamined those hormones in this study. We have found that the ACTH response to novel footshock is not affected after 6 wk of activity wheel running (10); however, it is augmented after 6 wk of treadmill exercise training (54).

METHODS

Subjects

Fischer 344 rats (n = 48, age ~ 45 days, mass ~ 165 g) were obtained from Charles River (Raleigh, NC) and allowed to adapt to the vivarium for 2 wk. The animals were housed in individual cages in a vivarium maintained at 22 ± 2°C with a 12-h light-dark cycle, starting at 7 am. Water and lab chow (Ralston Purina, St. Louis, MO) were available ad libitum. Animals were handled daily and weighed once a week throughout the course of the study. At an age of 60 days, animals were randomly assigned to either a sedentary group (n = 8) or a treadmill exercise training group (n = 8), each receiving uncontrollable footshock, or to a second sedentary group (n = 8) that did not receive footshock and that acted as a home-cage control. To determine the effects of treadmill exercise training on basal NK activity, another 14 rats were randomly assigned to treadmill exercise training (n = 7) or sedentary (n = 7) conditions without exposure to footshock.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205).

Experimental Procedures

A randomized factorial design (treadmill exercise vs. sedentary) was used for hypothesis testing. A randomly assigned third group was sedentary and did not receive footshock (home-cage control); it provided baseline values on the dependent variables for control comparisons. A one-way randomized design (treadmill exercise vs. sedentary) without footshock was used for the nonshock control study. The dependent measures included percent specific lysis of YAC-1 cells determined by a standard 4-h 51Cr release assay at five different effector-to-target ratios (E/T) (200, 100, 50, 25, and 12.5:1); percentages of OX6+ (B), OX8+ (Ts/c), W3/25+ (Thy-1.1) (a Pan T cell marker; Pan T), and SCG+ (NK) cells were determined by flow cytometry; and plasma concentrations of ACTH, corticosterone, and prolactin were determined by radioimmunoassay.

Treadmill exercise training. After 2 wk in the vivarium, treadmill training began with familiarizing the rats with a Stanhope 2000 (Davis, CA) motor-driven treadmill. Each animal was placed in 1 of 20 running compartments for 15–20 min on each of 2 days. The rats then ran at a low speed (5–10 m/min at 0° incline) for 5 min. The running times were gradually increased from 5–10 min during 1 wk. On each of the 2 days, running performance was rated on a scale of 1–5, with 5 being the best rating (53, 54). At the end of the trial period, the animals receiving a mean rating ≥3 were randomly assigned to the experimental groups. Poor runners (n = 10) that consistently scored <3 on the running performance scale were excluded from the study to minimize dropouts during training and to optimize the group exercise training effect. Animals assigned to the treadmill exercise training condition ran at 0° incline 6 days/wk for 2 wk, during which the running time was increased from 10 to 35 min/day and treadmill speed was gradually increased from 15 to 25 m/min. The animals maintained this regimen (35 min/day, speed = 25 m/min, 6 days/wk) for an additional 5 wk. Electric shock was not used to promote running performance. The protocol elicits a significant increase in the oxidative capacity of locomotory muscle, as measured by the activities of oxidative enzymes in slow- and fast-oxidative glycolytic muscle fibers (11). In the present study, we measured the activity of citrate synthase in soleus muscle. Animals ceased treadmill running 36 h before footshock and death.

Footshock protocol. After 6 wk, animals assigned to the treadmill training and sedentary footshock groups were matched, according to mass, into pairs. Two sessions of
footshock testing, separated by 24 h, occurred. Animals that
received footshock were transported from their home cages to
a testing room 10 m from the vivarium immediately before
footshock. They were immediately returned to their home
cages after the first session of footshock. Each animal was
unrestrained in an individual Skinner box (28 × 20 × 21 cm)
and received repeated 2 mA of scrambled footshock, for a total
of 6 min of shock delivered during a 20- to 50-min period. The
duration and frequency of shocks were selected to permit
comparison of results with our earlier report on footshock
after activity wheel running (10). The shock duration ranged
from 2–30 s, with a 35-s interval between shocks. Duration
equal for both members of each pair of treadmill-trained
and sedentary rats.

Tissue collection. After the second acute session of the
2-day footshock protocol, each animal was returned to its
home cage. The animal was transported to an adjacent room
30 min later and killed by decapitation using a guillotine.
Suppression of NK activity was previously reported to occur
1–4 h after novel footshock (42, 52). Animals that were not
footshocked were transported from their home cages immedi-
ately before decapitation. Heparinized trunk blood was chilled
on crushed ice for ~1 h before being centrifuged at 2,000 g.
The available plasma was pipetted into collection tubes
containing 50 µl of aprotinin and stored at −70°C. Spleens
were removed immediately after decapitation and were main-
tained in RPMI 1640 (Flow Laboratories, Rockville, MD)
containing 10% heat-inactivated fetal bovine serum (GIBCO
Laboratories, Grand Island, NY) within polypropylene vials
kept at room temperature until the cytotoxicity assay was
performed 1–2 h later. Soleus muscle was removed from the
left hindlimb, quick frozen in liquid nitrogen, and stored at
−70°C.

Cytotoxicity and Flow Cytometry

Reagents and media. Cell lines were maintained in RPMI
1640 containing 10% heat-inactivated fetal bovine serum at
37°C in 6% CO2 during the cytotoxicity assay. Na251CrO4 was
purchased from Amersham (Arlington Heights, IL), and the
fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG
and IgM were purchased from Sigma Immunocchemicals (St.
Louis, MO).

Monoclonal antibodies. Phenotyping of cells for deter-
ing subpopulations was done by flow cytometry using the
following anti-rat monoclonal antibodies (MAbs): W3/25 (CD4
equivalent for T helper cells), MRC OX-8 (CD8 for T cytotoxic/
suppressor cells), OX-6 (B cells), OX-7 (Thy-1.1/generic T cell
antigen), and MAb 5C6 (specific for rat NK cells) purchased
from Serotec Limited (Oxon, UK). In the control experiment,
phenotyping of cells was limited to percentage of 5C6+ cells
in nylon wool nonadherent (NWNA) cells.

Tissue preparation and cell purification. Preparation of
tissue for cytotoxicity experiments and cytometry were done
by surgical removal of the spleen followed by single cell
suspensions. Red blood cells were removed by gently mixing
the cells in Tris-HCl-ammonium chloride lysing buffer. After
white blood cell isolation, one-half of the cells were passed
through nylon wool columns and the nonadherent fraction
was used for the cytotoxicity assay. This NWNA fraction is
known to contain both T and NK cells and to have <1% of
contaminating B cells (as detected by Ig antibodies). Enrich-
ment by nylon wool extraction exceeded 70% purity of 5C6+
cells in both the experimental and the control studies.

Cytotoxicity assays. Spleen cells were tested for NK activity
with a51Cr release assay using YAC-1 cells (a mouse T cell
lymphoma cell line) as targets. YAC-1 cells were labeled with
100 µCi Na251CrO4 (Amersham) for 3 h at 37°C. After two
washes in 7 ml of RPMI 1640, the cells were resuspended in
RPMI containing 10% fetal bovine serum at 100,000 cells/ml,
and 100 µl was added to 96-well, round-bottomed, microtiter
plates (Costar, Cambridge, MA). Effector cells were washed
in RPMI 1640, resuspended at the desired concentrations,
and added to the targets at different E/T in a final volume of
200 µl. The cells were cocultured for 4–6 h at 37°C, the
plate was centrifuged at 100 g for 5 min, and 100 µl of
each supernatant was removed, without disturbing the pellet,
to determine radioactivity (Bio Gamma II, Beckman Instru-
mients, Irving, CA). The results were expressed as the percent-
age of specific release (%SR), i.e., percentage of cytotoxicity,
and were determined using the following formula: %SR =
[(test release — spontaneous release)/(total release — sponta-
neous release)] × 100. Spontaneous release averaged 10% of
total release.

Immunofluorescence and flow cytometry. In all cases, either
a normal conjugate control using anti-mouse IgM FITC or
irrelevant IgM MAb isotype control, 5G1, was used (23).
Analysis was done on an EPICS 541 cytometer (Couler
Electronics, EPICS Division, Hialeah, FL). A 5-W argon-ion
laser (Coherent, Palo Alto, CA) was tuned to 488-nm light.
Fluorescence was detected by using a 488-nm dichroic mirror,
a 488-5P laser blocking filter, and a 550-SP dichroic mirror.
Green fluorescence was detected with a 525-BP interference
filter. Data storage and analysis were accomplished using an
EASY II system (Couler Electronics).

Radioimmunoassay

A radioimmunoassay technique was used to determine the
plasma concentrations of the HPA cortical hormones. Radioim-
muunoassay for corticosterone was performed using an anti-
body produced in rabbits at Walter Reed Army Institute of
Research (35). The sensitivity of the assay was 2.0 µg/100 ml
plasma. The intra- and interassay coefficients of variation
were 5% and 10%, respectively. Materials for the assay of
prolactin were provided by the National Institutes of Health,
through the Rat Pituitary Hormone Distribution Program.
The sensitivity for the prolactin assay was ~0.8 ng/ml. The
intra- and interassay coefficients of variation were 6% and
12%, respectively.

The procedure for the ACTH assay with a commercial kit
(Instar, Stillwater, MI, no. 24310) was described previously
(36). Assay sensitivity was 5 pg/ml. The within-assay coeffi-
cient of variation was 8.2% at 33 pg/ml and 2.0% at 112 pg/ml.
The interassay coefficient of variation was 10.6% at 33 pg/ml
and 6.4% at 109 pg/ml.

Spectrophotometry

Citrate synthase activity was assayed to determine whether
the treadmill exercise training increased the oxidative capac-
ity of locomotory muscle. The soleus muscle was homogenized
in a 100 mM KPO4 and 10 mM glutathione buffer (pH 7.4).
Citrate synthase activity was measured spectrophotometri-
cally (Bausch and Lomb Spectronic model 1001) at 412 nm
and 30°C (46).

Statistical Analysis

We used SPSS Windows (SPSS, version 9.0, Chicago, IL)
for our statistical procedures. Percentage of specific lysis was
compared among groups across the five E/T, using a mixed-
model ANOVA with the E/T repeated. Sphericity adjustments
were made using Huynh–Feldt e. Effects for plasma hormones
and percentages of splenic lymphocytes were determined
using one-way ANOVA across groups for each dependent
variable. Duncan’s post hoc tests were conducted at P < 0.05.
Group comparisons of percentage of NK cells in the control study, body mass, and citrate synthase activity were made using a t-test for independent samples. Missing observations and data points exceeding the criterion of Grubb and Beck (15) for outliers were <4% of all observations and were replaced by the cell mean for each variable. Sample size was based on an expected effect size of 1.0 standard deviation (SD). Eight animals per cell provided a power of 0.80 at an \( \alpha \) of \( P < 0.05 \). Values are reported as means ± SE in the text and means ± SE in Figs. 1–3.

**RESULTS**

The suppression of NK cell cytotoxicity observed among sedentary animals after repeated footshock was blunted by treadmill exercise training. After footshock, NK activity was ~80% of control for the treadmill training group, but it was 40% of control for the sedentary group. The percentage of NK cells compared with total lymphocytes did not differ among groups. Plasma levels of ACTH, corticosterone, and prolactin were elevated above home-cage control levels after footshock but did not differ among the experimental groups. In the control study without footshock, treadmill-trained animals did not differ from sedentary controls on NK activity, percentage of NK cells compared with total lymphocytes, or plasma levels of ACTH, corticosterone, and prolactin. NK activity was ~95% of sedentary controls, indicating that 6 wk of treadmill exercise training did not elevate basal NK activity.

**Cytotoxicity.** Repeated-measures ANOVA of percentage of specific lysis across the E/T indicated an expected increase in NK activity with increasing E/T \[ F(4, 84) = 91.2, \( \epsilon = 0.44, P < 0.001 \) \], a group effect, \[ F(2, 21) = 6.82, P = 0.005 \] and a group × E/T effect, \[ F(8, 84) = 5.35, P < 0.001 \]. Post hoc tests indicated that the sedentary unshocked controls and the footshocked treadmill group had higher percentages of specific lysis at each E/T compared with the sedentary footshock group, and this difference was even greater at E/T of 100:1 and 200:1 (\( P < 0.05 \); See Fig. 1).

Fig. 1. Percentage of specific lysis of YAC-1 cells in male Fischer 344 rats tested under home-cage control conditions and without controllable footshock. Values are means ± SE. A group effect indicated that sedentary rats had suppressed natural killer (NK) cell activity after footshock compared with controls and rats that had been treadmill exercised for 6 wk (\( P = 0.005 \)). A group × effector-to-target ratio effect (\( P = 0.001 \)) was explained by larger differences at ratios of 100:1 and 200:1. Treadmill-trained and unshocked home-cage control groups did not differ (\( P > 0.10 \)). \( P = 0.05 \).

Fig. 2. Percentage of specific lysis of YAC-1 cells in male Fischer 344 rats tested under home-cage control conditions without footshock. Values are means ± SE. Rats that had been treadmill exercise trained for 6 wk (\( n = 7 \)) did not differ from rats that remained sedentary for 6 wk (\( n = 7, P > 0.10 \)).

A similar increase in the percentage of specific lysis across increasing E/T also occurred in the control study when the nonshocked treadmill-trained and sedentary groups were compared on basal cytotoxicity \[ F(4, 48) = 50.59, \( \epsilon = 0.83, P < 0.001 \); however, there was no group effect \[ F(1, 12) = 0.264, P = 0.772 \] (Fig. 2).

**Lymphocyte subsets.** Percentages of B, Pan T, Th, Thc, and NK cells did not differ among experimental groups (\( P > 0.23–0.68 \) (Table 1). Percentages of NK cells in NWNA cells also did not differ between the sedentary (75.29 ± 1.5%) and treadmill-trained (74.7 ± 3.5%) groups that did not receive footshock in the control study \( t(12) = 0.402, P = 0.695 \).

**Hormones.** Group effects were found for plasma levels of ACTH \[ F(2, 20) = 4.94, P = 0.018 \] and corticosterone \[ F(2, 20) = 6.81, P = 0.006 \]. Post hoc tests indicated that the levels in sedentary and treadmill groups, respectively, of ACTH (103.6 ± 15.5, 106.75 ± 13.3) and corticosterone (16.2 ± 2.0, 20.85 ± 4.3) were elevated (\( P < 0.05 \)) compared with levels of ACTH (60.25 ± 5.1) and corticosterone (4.0 ± 3.2) in the home-cage control group that was not shocked. Levels of prolactin in sedentary (8.3 ± 3.7) and treadmill-trained groups (9.2 ± 1.6) were not significantly higher compared with the home-cage control group (2.6 ± 0.76) \[ F(2, 20) = 2.59, P = 0.10 \]. Treadmill exercise training had no effect on plasma hormone levels after footshock (Fig. 3).

**Body mass and citrate synthase activity.** By week 6 of the experiment, sedentary animals were ~7% heavier on average than the treadmill-trained group. The treadmill-trained animals were 10.2% heavier than the home-cage control group. There were no group differences (\( P > 0.10 \)).

**Table 1.** Percentages of splenic lymphocyte subsets in 24 male Fischer 344 rats

<table>
<thead>
<tr>
<th>Lymphocyte Subsets</th>
<th>Home-cage</th>
<th>Sedentary</th>
<th>Treadmill-trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>15 ± 13</td>
<td>13 ± 11</td>
<td>18 ± 11</td>
</tr>
<tr>
<td>Pan T</td>
<td>11 ± 7</td>
<td>7 ± 4</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>T&lt;sub&gt;s&lt;/sub&gt;</td>
<td>1 ± 1</td>
<td>2 ± 2</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>T&lt;sub&gt;sc&lt;/sub&gt;</td>
<td>24 ± 5</td>
<td>24 ± 18</td>
<td>18 ± 13</td>
</tr>
<tr>
<td>NK</td>
<td>45 ± 7</td>
<td>54 ± 17</td>
<td>57 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 8 \) for each group. Sedentary and treadmill-trained values were taken 30 min after footshock. B, OX6+ cells; Pan T, Thy 1.1 cells; T<sub>s</sub>, W3/25+; T<sub>sc</sub>, OX+8 cells; NK, natural killer cells. There were no group differences (\( P > 0.10 \)).
than the treadmill group (225 ± 15 vs. 210 ± 13 g) [t(22) = 2.52, P = 0.02]. Citrate synthase activity (µmol·min⁻¹·g wet wt⁻¹) in the soleus muscle was higher in the treadmill-trained group (42 ± 3) compared with the sedentary group (38 ± 2) [t(22) = 3.9, P = 0.001].

**DISCUSSION**

Our results indicate that treadmill exercise training protects against the suppression of splenic NK activity induced by footshock. This finding extends prior reports (32, 33) of increased murine basal splenic NK cell numbers and activity after chronic activity wheel or treadmill running by demonstrating a protective cross-stressor attenuation of NK suppression that is independent of changes in basal NK activity. Rats that were treadmill exercise trained for ~6 wk, but did not receive footshock, had NK activity and percentages of NK cells that did not differ from sedentary control animals. We believe the attenuation of NK suppression after footshock observed in the treadmill training group is specific to single cell activity and not to a differential migration of lymphocytes, as percentages of the lymphocyte subsets did not differ between groups. An assay of single cell activity is needed to confirm this view.

The treadmill group had lower body mass than the sedentary animals by the end of the study, but we did not assess body composition or control food intake. Thus the role of energy balance on splenic NK activity cannot be determined from our study or from prior studies in this area (32, 33). Like us, Nasrullah and Mazzeo (37) reported that 15 wk of treadmill exercise training had no effect on basal NK cytotoxicity in 8-mo-old male Fischer 344 rats, despite their lower body mass and higher citrate synthase activity in soleus muscle when compared with sedentary rats. Other investigators have reported that male mice had increases in basal splenic NK activity that were similar after chronic activity wheel running and treadmill training, but only treadmill training led to increased peak oxygen uptake (33) or increased activity of citrate synthase in soleus muscle (32).

Footshock was administered during the early diurnal photoperiod, when basal cortisol levels were low, to optimize the cortisol response to stress. Although mitogenic lymphocyte proliferation after footshock is equivalent during the early diurnal and nocturnal periods of the day in Fischer 344 male rats (29), we are unaware of studies examining whether NK activity after footshock differs according to time of day. The treadmill exercise training also was diurnal. In our experience, fitness adaptations to the protocol we used do not differ according to time of day, but as far as we know, the interaction of the light-dark cycle with neuroendocrine and immune responses to treadmill training has not been previously reported.

The mechanisms underlying the effects of physical activity on NK cell activity are not clarified by our observation that the activity wheel and sedentary groups did not differ on plasma levels of ACTH, corticosterone, and prolactin. We reported similar results in a comparison between sedentary Fischer 344 males and those having 24-h access to activity wheels for 6 wk (10). The relation between neuroendocrine and immune responses after chronic exercise has not been well described. Our observations are consistent with past findings that intracerebral, but not intraperitoneal, injections of antibodies for corticotropin-releasing factor abolish the suppression of splenic NK activity after footshock in Wistar rats without affecting blood ACTH and corticosterone levels (22). The lack of association observed between hormone levels and NK activity in the present study is limited to one data point taken 30 min after footshock. Samples of hormonal responses taken during, immediately after, and 24 h after repeated footshock would more fully address the possible neuroendocrine effects on NK activity. For example, infusion of cortisol (5 µg/kg) for 1–5 h did not affect NK...
activity in lymphocytes taken from human blood (48). In another report (39), NK activity increased 4 h after, but decreased 24 h after, a 300-mg infusion of hydrocortisone. The NK activity paralleled the decrease and increase, respectively, of the percentage of T lymphocytes, suggesting an increase in percentage of NK cells. Glucocorticoid modulation of NK activity might differ according to lymph compartment and between humans and rats. Also, studies should examine whether activity wheel training affects regulatory responses by corticotropin-releasing factor on sympathetic responses to footshock or alters the sensitivity of NK cells to glucocorticoids and prolactin (50).

Alternative explanations for a cross-stressor attenuation in NK activity after treadmill exercise training might involve brain and splenic noradrenergic and opioidergic systems. In rats, brain opioids such as β-endorphin and metenkephalin can both inhibit and increase brain noradrenergic activity (13) and modulate splenic NK activity in response to conditioned electric shock (8). NK suppression after footshock is prevented by the opiate antagonists naloxone and naltrexone (41, 42), and it is mimicked by injection of morphine into either the peripheral circulation or the lateral ventricle of the brain, in the area of the central gray (51). Neuropeptide Y (NPY), which is colocalized with peripheral noradrenergic neurons, might also influence NK cell activity. In one report, NK activity was inversely related to plasma levels of NPY (20), which is coreleased with catecholamines during exercise in men (30) and with norepinephrine during footshock in rats (6, 56). Immobilization stress also increases splenic norepinephrine release and suppresses NK activity in rats (44). We have observed that treadmill exercise training leads to augmented ACTH responses to both footshock and immobilization in female rats (53, 54), so it will be interesting to determine whether treadmill exercise training has a protective effect against NK suppression after immobilization stress that is similar to our current observation after footshock.

The effects of neuropeptides and catecholamines on NK percentage and cytotoxicity may differ according to the lymph compartment and between humans and rats. Plasma catecholamines appear to increase migration of NK cells into the blood circulation (34); thus the increase in NK activity after infusion of epinephrine in humans might be explained by increased percentages of NK in circulating blood (48, 49). In contrast, chemical sympathectomy and β-adrenoreceptor blockade abolished and blunted, respectively, the suppression of rat splenic NK activity induced by intracerebroventricular injection of corticotropin releasing factor, which has an excitatory effect on the rat brain noradrenergic system (21).

We conclude that treadmill exercise training protects against the suppression of splenic NK activity induced by footshock in young male Fischer 344 rats. Similar to our earlier report on activity wheel running (10), this protective effect was not explained by elevations in basal NK activity or increased percentages of NK or cytotoxic T cells. Whereas activity wheel running might exert a protective effect by enriching the cage environment beyond that of standard rat husbandry, the blunting of NK suppression after footshock by progressive exercise training leading to an increase in fitness level indicates that physical activity has an independently protective cross-stressor immunomodulatory effect. The hypothesized influence of HPA responses on NK activity was not supported. We recommend that responses by splenic norepinephrine and NPY to treadmill exercise running and footshock be examined as possible explanations for our observations.

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