Voluntary Exercise Has Only Limited Effects on Activity of Antioxidant Enzymes and Does Not Cause Oxidative Damage in a Small Mammal

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EXPANDED ABSTRACT

KEY WORDS: • voluntary exercise • reactive oxygen species • antioxidant • enzymes • oxidative DNA damage • comet assay • vole

Regular physical exercise has a number of beneficial effects, including the reduction in the risk of cancer, osteoporosis, obesity and cardiovascular disease (1,2). Voluntary exercise through wheel running has also been shown to increase average life expectancy in rats by nearly 10% (3). However, physical exercise also considerably increases total oxygen consumption relative to resting levels, which may, in turn, increase the generation of reactive oxygen species (ROS4). If ROS generation exceeds the antioxidant protection and repair mechanisms, oxidative stress will occur and this process is thought to be intimately involved in the aging process (4) and mechanisms, oxidative stress parameters [e.g., Leeuwenburgh et al. (18)]. In the following study, we used a small (15–30 g) mammalian model, the short-tailed field vole (Microtus agrestis) to examine whether short-term (i.e., 1- or 7-d) voluntary wheel running, with or without an 8-h recovery period, had any effect on the activities of the antioxidant enzymes catalase (Cat), glutathione peroxidase (Gpx) and total superoxide dismutase (total-SOD) or DNA oxidation. We measured oxidative DNA damage in lymphocytes and hepatocytes, employing the comet assay and lesion-specific enzymes endonuclease III (endo III) and formamidopyrimidine DNA glycosylase (FPG) (19–21). Antioxidant enzyme activities were measured in skeletal muscle species, subject age and assay technique employed (11–13).

The majority of studies examining the relationship between exercise, ROS production and oxidative stress have exposed subjects to bouts of acute and/or exhaustive exercise, or examined the effects of endurance training (10), with fewer studies investigating the effects of voluntary exercise on oxidative stress parameters [e.g., Leeuwenburgh et al. (18)]. In the following study, we used a small (15–30 g) mammalian model, the short-tailed field vole (Microtus agrestis) to examine whether short-term (i.e., 1- or 7-d) voluntary wheel running, with or without an 8-h recovery period, had any effect on the activities of the antioxidant enzymes catalase (Cat), glutathione peroxidase (Gpx) and total superoxide dismutase (total-SOD) or DNA oxidation. We measured oxidative DNA damage in lymphocytes and hepatocytes, employing the comet assay and lesion-specific enzymes endonuclease III (endo III) and formamidopyrimidine DNA glycosylase (FPG) (19–21). Antioxidant enzyme activities were measured in skeletal muscle species, subject age and assay technique employed (11–13).

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VOLUNTARY EXERCISE AND OXIDATIVE STRESS

TABLE 1

<table>
<thead>
<tr>
<th>Exercise group</th>
<th>0500 h GMT</th>
<th>1300 h GMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.1 ± 1.03</td>
<td>17.7 ± 0.64</td>
</tr>
<tr>
<td>1-d runner</td>
<td>19.2 ± 0.76</td>
<td>19.1 ± 0.80</td>
</tr>
<tr>
<td>7-d runner</td>
<td>18.7 ± 1.28</td>
<td>20.4 ± 0.31</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10 in each group. None of the differences observed was statistically significant (one-way ANOVA, P = 0.480).

MATERIALS AND METHODS

Animals

Short-tailed field voles Microtus agrestis, derived from a captive breeding population at Aberdeen, UK, were maintained at 22 ± 3°C. Individuals (6 wk old) were exposed to a 16:8 light regime (lights on 0500 h GMT) and, when given running wheels, chose to run primarily during darkness. No individual had access to running wheels before the experiment started. Voles were weaned at 1 d, individually housed in shoebox cages containing sawdust and provided, ad libitum, with water and a pelleted rodent diet [rat and mouse breeder and grower diet; Special Diets Services, BP Nutrition, UK], which included vitamin E (103.2 mg/kg), α-tocopherol (93.8 mg/kg), β-carotene (0.9 mg/kg) and vitamin C (8.0 mg/kg). All voles were killed by cervical dislocation, complying with a local ethical committee and the U.K. Home Office.

Wheel-running apparatus

The wheel-running apparatus was designed by Dr. Phil Bagley (University of Aberdeen, UK) and consisted of 40 individual cages with attached running wheels. Each wheel was connected to a nonconcentric disc, which operated a microswitch on each complete rotation. Data collected on the microswitch and activity were transmitted to a PC-based acquisition system by an optically isolated logic level converter. The number of revolutions run by each individual was recorded during each 10-min period, over 24 h per day, and downloaded directly onto a spreadsheet package (Microsoft Excel).

Assays

The activities of the antioxidant enzyme catalase (Cat), selenium-dependent glutathione peroxidase (Gpx) and total superoxide dismutase (total-SOD) were measured in six experimental groups: control (no running wheel), 1-d (access to wheel) and 7-d (access to wheel), killed at 0500 h (no rest period after running) or 1300 h (8-h rest period after running). Sixty individuals (30 male, 30 female: 10 per group) were used to determine antioxidant enzyme activity in skeletal muscle (hind- and forelimb) and in the heart. The protocols employed are described fully elsewhere (25).

Oxidative DNA damage was determined in lymphocytes and hepatocytes in three groups, all killed at 1300 h after 8-h rest: control (no wheel), 1 d (access to wheel) and 7 d (access to wheel).

The comet assay, with the modification of an extra step after lysis in which DNA is digested with lesion-specific enzymes, has been described previously (26). Cells are embedded in agarose on a microscope slide and lysed in detergent and high salt to form nucleotides. For forced, endurance and exhaustive exercise, cells are treated with an extra step after lysis to form nucleotides. In the case of hepatocytes, the lesion-specific enzymes did not reveal any oxidized bases over and above these strand breaks, in any group.

DISCUSSION

This study investigated whether short-term voluntary exercise (1- or 7-d access to a running wheel, with or without an 8-h rest period) had any effect on the antioxidant enzyme status or on lymphocyte or hepatocyte oxidative DNA damage in short-tailed field voles Microtus agrestis. Wheel running has been shown to elevate daily energy expenditure by over 40% higher compared to that of nonrunning controls (24). However, despite this large increase in oxygen consumption, short-term voluntary exercise did not significantly alter the activities of the antioxidant enzymes, with the exception of heart total-SOD. The levels of oxidative DNA damage also did not differ between control animals and runners in either lymphocytes or hepatocytes.

Forced, endurance and exhaustive exercise generally induce an increase in both enzymatic and nonenzymatic antioxidants in heart and skeletal muscle (11,12,22,27,28). Rats with access, over 20 mo, to voluntary running wheels exhibited elevated activities in skeletal muscle of mitochondrial SOD and cytosolic Gpx, but not Cat or cytosolic SOD compared with

Statistical analyses

All values reported are means ± SEM, except where indicated. Data were analyzed employing SPSS (Version 9) statistical software and one-way analysis of variance. Significance was indicated by values of P < 0.05.

RESULTS

Body mass did not differ between the experimental groups (Table 1). The mean ± SEM distance run per day was 7.8 ± 1.2 km, with voles running almost exclusively during the 8 h of darkness (lights on 0500 h GMT). No differences were observed between the experimental groups in the activities of Cat, Gpx or total-SOD in either hind- or forelimb skeletal muscle. The activities of heart Cat and Gpx between groups were also not significantly different, although heart total-SOD was, with the lowest levels observed in 1- and 7-d runners killed immediately after exercise (0500 h).

Oxidative DNA damage in lymphocytes did not differ among the three groups measured (Fig. 1), when using either endo III or FPG. We also observed no significant differences between the experimental groups in hepatocyte DNA breaks (Fig. 2). In the case of hepatocytes, the lesion-specific enzymes did not reveal any oxidized bases over and above these strand breaks, in any group.

FIGURE 1 Oxidative DNA damage (arbitrary units) using the comet assay and lesion-specific enzymes endo III and FPG in lymphocytes of nonrunning control, 1- and 7-d runners killed at 1300 h. Values are means ± SEM, n = 6 in each group. No significant differences were observed between groups using either endo III (P = 0.712) or FPG (P = 0.783).
that of sedentary controls (18). It has been proposed that the basal levels of antioxidant enzymes, particularly skeletal muscle SOD, are sufficient during moderate oxidative stress (13), although by measuring total-SOD activity we cannot be certain that the activities of the different isoenzymes of SOD did not alter during this exercise protocol.

Moderate-intensity exercise has been shown to increase heart SOD activity in some (27) but not all studies (13). In our study, a significant difference was observed between groups in heart total-SOD activity, although there was no clear pattern of difference between the sedentary and running groups, with the lowest levels of SOD actually observed in the nonrunning controls, 1- and 7-d runners killed at 1300 h. Values are means ± SEM, n = 6 in each group. No significant differences were observed between groups in strand breaks (FPG), base addition, or strand breaks using the lesion-specific enzymes endo III and FPG.

In conclusion, it would appear that, although voluntary wheel running increased energy expenditure significantly compared to that of sedentary controls (24), this did not appear to induce oxidative stress responses. The fact that, despite the absence of a response, there was no evidence of oxidative DNA damage may be because during voluntary exercise the antioxidant protection and repair mechanisms are sufficient to cope with any accompanying increase in ROS production. The exercise regime employed in this study may also have been insufficient in intensity and/or duration to cause a significant increase in oxidative damage (29) or, perhaps, the antioxidant levels of the rodent diet used were fortified with respect to requirements in voles.

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LITERATURE CITED