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Long-Term Exercise Training Selectively Alters Serum Cytokines Involved in Fever

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Long-term exercise training selectively alters serum cytokines involved in fever. Chronic exercise training has a number of effects on the immune system that may mimic the physiological response to fever. Female rats that voluntarily exercise on running wheels develop an elevated daytime core temperature after several weeks of training. It remains to be seen whether the elevation in daytime temperature involves inflammatory patterns characteristic of an infectious fever. We assessed whether chronic exercise training in the rat would alter levels of cytokines involved in fever. Female Sprague Dawley rats at 45 days of age weighing 90–110 g were divided into two groups (exercise and sedentary) and housed at an ambient temperature of 22°C. Interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-α), iron, and zinc levels were analyzed. Rats underwent 8 weeks of exercise on running wheels. Exercise led to altered levels of some key cytokines that are involved in fever. Exercise animals had significantly higher IL-1β levels and lower IL-10 levels compared to sedentary animals. Although IL-6 levels were slightly lower in the exercise animals, these levels were not significantly affected by training. TNF-α activity was similar in the two groups. Training also led to a slight increase in serum zinc and decrease in serum unsaturated iron binding capacity (UIBC). The data suggest that chronic exercise training evokes immune responses that mimic some, but not all, aspects of fever. This may explain why exercise leads to elevated daytime core temperature.

Keywords: cytokines; exercise; fever

An exercise-induced rodent model of thermoregulation is an important model for nursing research because chronic exercise training induces nonspecific immune changes similar to endotoxin-induced nonspecific immune changes during illness. Many of the biological actions attributed to the release of endogenous cytokines have been found in people and animals during and immediately after exercise. Exercise produces lymphocytosis, elevation in core body temperature, and an increase in acute-phase proteins (Pedersen, 2000; Pedersen & Toft, 2000; Rowsey, Metzger, & Gordon, 2001), but the functional significance of these changes remains unclear.

Voluntary exercise on a running wheel is an effective method for altering thermoregulatory responses in rodents. Chronic exercise training on running wheels results in a large rise in core body temperature during the nighttime, when rats are exercising, as well as a significant increase in daytime temperature, when rats are inactive (Rowsey, Borer, & Kluger, 1993a, 1993b; Rowsey et al., 2001; Satinoff, Kent, & Hurd, 1991). The factors responsible for the daytime elevation in temperature are not known.

One would suspect, however, that circulating levels of cytokines may be involved. There is evidence that exercise produces a pyrogen-like substance that could be responsible for this daytime elevation in core temperature (Cannon & Kluger, 1983). In one study, 14 volunteers exercised on a bicycle ergometer...
for 1 hr at 60% of their aerobic capacity. Venous samples were taken before exercise, immediately after exercise, and 3 hr after exercise. Human plasma collected immediately (1 hr) after exercise was injected intraperitoneally into rats, causing a significant elevation of core temperature in those animals (Cannon & Kluger, 1983). Taylor et al. (1987) also sampled volunteers from a triathlon event involving canoeing, cycling, and running. Data showed exercise training was associated with induction of acute-phase reaction as evidenced by a decrease in serum iron concentration and an increase in C-reactive protein. Even more convincing support for the idea that exercise can result in a change in thermoregulatory set point comes from behavioral studies with the desert iguana (Bernheim & Kluger, 1976). After treadmill exercise, these animals selected a warmer thermal environment. However, administration of sodium salicylate immediately before exercise eliminated the increase in temperature preference for at least 3 hr after exercise. Results from these studies indicate that exercise induces the release of a circulating factor that exhibits pyrogen-like activity capable of causing changes in thermoregulatory set point.

Chronic exercise training in the rat may initiate a series of neuron-immune responses that alter a variety of physiological processes, including thermoregulation. Cytokines involved in fever may also increase with exercise. However, there is little known about circulating levels of these cytokines in the exercise-trained rat. The purpose of this study was to assess the effects of chronic exercise training on serum cytokine levels and to identify which cytokine may be responsible for the chronic activation of the immune system in the rat.

Material and Method

This study was approved by the IACUC committee at the Environmental Protection Agency, Research Triangle Park, NC. Female young adult rats provide excellent models to study the physiological effects of exercise because they voluntarily run more than their male counterparts (Rowsey, Metzger, Carlson, & Gordon, 2003). For this study, 16 pathogen-free, young adult female Sprague Dawley rats were obtained from Charles River Laboratories (Raleigh, NC) at 45 days of age weighing 90–110 g. The rats were housed individually, in the same room, in acrylic cages lined with wood shavings and maintained at an ambient temperature ($T_a$) of 22°C and a 12:12 light/dark photoperiod (lights on at 0600). Animals were allowed at least 2 weeks to acclimate to the animal facility and were then evenly divided by weight into exercise (provided running wheels) and sedentary (no wheels) groups. Rats were fed standard rodent chow and given water ad libitum.

Protocol

Animals in the exercise group were housed in a specifically designed cage with a running wheel (Nalge designed for Minimitter Company, Sun River, OR) that allows physiological monitoring of animals. While rats are at rest and running, this system allows easy, convenient simultaneous measurement of the animals’ physical activity and core temperature in response to chemical or environmental stimuli. Dimensions of fully assembled cages with the wheels are (length × width × height) 50 × 26.8 × 36.4 cm³. Previous studies (Rowsey et al., 1993a, 1993b, 2001; Rowsey & Kluger, 1994) have demonstrated that female Sprague Dawley rats voluntarily run with sufficient duration and intensity to result in an elevation of their day- and nighttime core temperature. Estrous measurements were not taken in this study because work by Yanase, Tanaka, & Nakayama (1989) indicated exercise-induced increase in core body temperature in the rat is a regulated response dependent on work intensity and not influenced by the estrous cycle. From previous work (Rowsey et al., 2003), we knew that after 8 weeks of running on wheels, animals maintain a consistent elevated daytime temperature indicating a type of chronic activation of the immune system. Consequently, in the current study, we used an 8-week time period of voluntary exercise, after which we took cytokine samples and harvested organs.

Sample collection and storage. At the end of 8 weeks of running and before sample collection, animals were removed from the animal room, brought to the laboratory, and allowed to acclimate overnight. Sample collections began at 10 a.m. the next morning. Each rat was euthanized by CO₂ asphyxiation and a blood sample was taken via cardiac puncture with a nonheparinized syringe and placed in tubes without ethylenediaminetetraacetic acid (EDTA) or heparin. All blood samples were allowed to clot for 2 hr at room temperature. Serum was separated (2,000 rpm × 20 min at 4°C),
aliquoted into 1.5 ml microcentrifuge vials, and stored at –20°C for cytokine (enzyme-linked immunosorbent assay [ELISA]) and serum chemistry (cholinesterase, iron, and zinc) determinations. Organs (heart, liver, and kidneys) from each animal were also harvested and weighed.

Cytokine determination. ELISA kits (R&D Systems, Minneapolis MN) were purchased for cytokine quantification in triplicate of tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) from rat serum. All four ELISA kits were rat specific and used an immobilized (capture) antibody that was specific to the measured cytokine. Standards and serum were pipetted into the wells, and the immobilized antibody was bound to any cytokine present. After washing away any unbound substances, an enzyme-linked polyclonal antibody, specific for the cytokine, was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate was added to the wells, and the intensity of developed color was proportional to the amount of cytokine in the first step. The detection signal was the color change produced by the interaction of the horseradish peroxidase and the appropriate substrate tetramethylbenzidine. The color reaction was then stopped by the addition of 100 μl of diluted HCl to each well, which changes the blue color to yellow. The absorbance of each well was read at 450 nm using end point analyses on a μQuant microplate reader attached to an IBM PC for data manipulation. A standard curve was constructed (Excel-Microsoft & GraphPad-Prism) for each time point. Samples were run in triplicate and then averaged to obtain an individual control value. The background absorbance for 0 pg/ml was then subtracted from each value.

Statistical Analysis
The effects of chronic exercise conditioning on serum cytokine levels were analyzed using unpaired t tests (SAS Institute, Cary, NC). Group means were compared to check the sensitivity of the results of the assay. Groups were compared using an exact test for differences in the ranked values of the assay (IL-1β, IL-6, IL-10, TNF-α). For each cytokine, differences in the level between exercised and sedentary animals were tested by a technique appropriate to its observed distribution. For cytokines with a markedly non-normal distribution or where the variances were not equivalent between the exercised and sedentary rats, an exact test of the ranks was conducted.

Results
Although we did not measure running activity of the exercise animals, previous work (Rowsey et al., 2001, 2003) has shown that animals that voluntarily run on running wheels average a total running distance of 7.6 km per 24 hr with little running activity during the daytime. After 8 weeks of access or no access to running wheels, body weights of the sedentary and exercise groups were 312.6 ± 6.9 g and 302.2 ± 9.3 g, respectively. At the time of euthanasia, rats were approximately 16 weeks old.

Exercise animals had significantly higher IL-1β activity levels (189.3 ± 24.9 pg/ml) and lower IL-10 activity (17.83 ± 5.10 pg/ml) compared to their sedentary counterparts (17.25 ± 8.64 pg/ml and 57.70 ± 11.26 pg/ml, respectively; Figure 1). Although not a statistically significant finding, exercise also caused a slight suppression of IL-6 (exercise, 288 ± 47.9 pg/ml; sedentary, 413.7 ± 90.8 pg/ml). TNF-α activity was similar in the exercise (29.32 ± 9.76 pg/ml) and sedentary (31.11 ± 9.22 pg/ml) animals.

Total serum iron levels were almost identical in exercise and sedentary rats, as shown in Table 1. However, unsaturated iron binding capacity (UIBC) was significantly lower in the exercised rats when compared to sedentary rats. Serum zinc levels were slightly higher in the exercised rats. Heart, kidney, and liver weights (Figure 2) were normalized to body weights. Both the heart and liver weights of exercise animals were significantly higher than those of sedentary animals (Figure 2). The hearts and livers of exercised rats were 28% and 20% larger than those of sedentary rats, respectively. Kidney weights of the two groups were almost identical.

Discussion
Fever is the predominant thermoregulatory response of humans acutely exposed to environmental toxins or bacterial infection (Gordon & Leon, 2005). Exercise often induces a pattern of hormonal and immunological responses that is similar to that of fever. In the current study, chronic exercise training altered serum levels of key cytokines involved in the
inflammatory process in rats. Following exposure to 8 weeks of running on wheels, animals in the exercise group displayed significant elevations in circulating levels of IL-1β, while IL-10 levels were markedly inhibited by exercise. IL-6 levels were slightly suppressed but not significantly affected by exercise, while TNF-α concentration was similar in the two groups. In this study, cytokines were measured at a single time point. Although this may be considered a limitation of the study, we chose to use a single time point because previous studies have shown that rats who exercise on running wheels maintain an elevated

| Table 1. Serum Chemistry Levels for Rats in Exercise and Sedentary Groups (Mean ± SE) |
|--------------------------------------|------------------|------------------|
| Serum Chemistry (μg/dl)              | Exercise         | Sedentary        |
| Serum iron                          | 330.7 ± 15.65    | 322.9 ± 18.90    |
| Serum UIBC                          | 331.9 ± 18.91    | 365.1 ± 23.54    |
| Serum zinc                          | 188.5 ± 5.16     | 172.8 ± 9.047    |

NOTES: UIBC = unsaturated iron binding capacity.

a. Significant difference between exercise and sedentary groups.
daytime temperature (Rowsey, Metzger, Carlson, & Gordon, 2006).

The neuroimmunological pattern of this cytokine response is similar to some facets of an infectious fever and may provide insight into the mechanism of the elevation in daytime core temperature of the exercised rats. IL-1β is released into the circulation during the onset of fever (Miller, Hopkins, & Luheshi, 1997; Turrin et al., 2001). Peripheral or central injection of IL-1β elicits a febrile response in rats and other species (Turrin et al., 2001). IL-6 is also involved in the onset of fever (Harden, du Plessis, Poole, & Laburn, 2006), but this cytokine was not significantly affected by exercise training. However, studies involving IL-10 suggest that this cytokine is involved in the recovery from fever (Leon, Kozak, Rudolph, & Kluger, 1999), as administration of IL-10 induced a hypothermic response or blocked the rise in temperature following a fever (Leon et al., 1999). In the current study, exercise training led to a significant reduction in circulating levels of IL-10. Finally, many studies have suggested that TNF-α is needed to limit the rise in core temperature during fever and/or to initiate the recovery from fever (Kozak, Conn, Kler, Wong, & Kluger, 1995; Leon, Kozak, Peschon, & Kluger, 1997). In the current study, TNF-α was unaffected by training.

The cytokine data of the current study, therefore, present an interesting pattern characterized by the marked increase in circulating levels of a cytokine that is associated with fever (IL-1β) and a reduction in levels of a cytokine involved in recovery from fever (IL-10). These patterns were apparently stimulated by 8 weeks of exercise training. Such changes are conducive for an elevation in the daytime core temperature.

Exercise training also led to a slight, though non-significant, increase in serum zinc and a significant decrease in serum U1BC. These findings are consistent with evidence that continuous strenuous exercise leads to a progressive fall in serum iron concentrations (Taylor et al., 1987) and an increase in total serum zinc (Cordova & Nava, 1998). Both findings resemble known actions of IL-1β. Fever, from endotoxin, bacteria, or virus, is normally associated with reductions in both zinc and iron.

Past research (Simon, 1984) has shown that IL-1β, IL-6, and TNF-α all contribute significantly to the temperature increases associated with exercise. However, the current data show that the levels of IL-1β, and not the other cytokines, increase significantly when rats are exposed to chronic exercise training. The most potent cell source of IL-1 is the antigen presenting cell, a macrophage that processes an antigen in specific immune responses. Exercise stimulates the production of IL-1 in the absence of a specific immune driver, which provides some evidence that an individual may benefit from exercise as a natural booster of the immune response.

The 28% elevation in heart mass in the current study provides ample evidence of a training effect in exercised rats. Rodents allowed to exercise for several weeks showed an expected increase in heart mass. The increase in heart weight is a result of increased work by the heart via exercise (Evangelista, Brum, & Krieger, 2003; Natali et al., 2002). Although the kidney of the exercise animals showed some hypertrophy, the overall weight of the kidney was not significantly different in exercise and sedentary animals. The liver, however, was significantly larger in the exercise animals. It would be interesting to consider how the changes in circulating levels of cytokines affect organ hypertrophy in chronically exercising animals. One could speculate that exercise produces a number of growth factors (e.g., fibroblast growth factor-2, hepatic growth factor) that work in conjunction with each other to cause cardiac and hepatic hypertrophy.

In summary, this study suggests that exercise evokes immune responses that mimic some aspects of fever. Exercise training induced a significant increase in the proinflammatory cytokine IL-1β, a significant decrease in the anti-inflammatory cytokine IL-10, and no significant changes in IL-6 and TNF-α levels. It is possible that anti-inflammatory and proinflammatory cytokines work together to limit the magnitude and duration of the inflammatory response associated with exercise.

Chronic disease is the major cause of death throughout the world. Cardiovascular diseases and cancer followed by diabetes mellitus account for the majority of deaths seen in the United States. It is well documented that exercise offers some protection against these major chronic diseases (Pedersen & Toft, 2000). Haight and Keatinge (1973) found male participants to have an elevated core body temperature after prolonged exercise that lasted for more than 11 hr post exercise. Participants maintained that elevated core body temperature while they rested in both warm and cool environments. In a more recent study, Bradford, Cotter, Thorburn, Walker, and Gerrard (2007) also found “fever-like” elevation in body temperature in male participants exposed to nonexhaustive exercise. Our model, in rats, shows a similar response. Rats that exercise voluntarily at night on running wheels increase their temperature...
during the day, which is their inactive period, by almost 0.5°C when compared to sedentary rats with no access to running wheels. It is unclear whether this elevated core body temperature post exercise contributes to the benefits associated with exercise. Further studies are needed to assess the benefits associated with this change in the daytime thermoregulatory milieu and whether it has some beneficial effects in lessening pathogen growth. Findings of such studies could provide evidence for prescribing exercise as therapy in chronic disease. Additional studies are also needed to assess whether cytokines are responsible for the exercise-induced organ hypertrophy we found in the current study and to determine its effects in health and disease.

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