Role of brain IL-1β on fatigue after exercise-induced muscle damage

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STRENUOUS UNACCUSTOMED EXERCISE is known to result in micro-trauma to muscle, muscle soreness, and inflammation (34, 37), particularly exercise that includes a large eccentric component (lengthening of a muscle that is actively developing tension) like downhill running. The effect of eccentric-biased exercise on performance recovery has been fairly well studied but has for the most part been confined to experiments involving isolated single muscle groups. These studies have examined individual muscle contractile components (12), motor performance (10), maximal strength (19), and glycogen resynthesis (6). However, the effect of eccentric exercise on running performance has yet to be fully elucidated. We have shown that full recovery of both voluntary wheel running and treadmill running performance takes up to 72 h (4) after downhill running. The degree to which central nervous system (CNS) vs. muscle impairments contributes to these deficits has not been determined.

It has been suggested that an immune inflammatory response may be involved in the development of at least some of the negative effects associated with eccentric exercise such as pain, impaired muscle regeneration, and performance deficits (21, 27). Various markers of inflammation such as creatine kinase (CK) and C-reactive protein (CRP) have been shown to be elevated after this type of exercise (27). The exercise-induced inflammatory response is further evidenced by increased circulating lymphocytes and neutrophils (28), swelling within the exercise-damaged tissue (35), and elevations in inflammatory cytokines, including IL-1β, IL-6, tumor necrosis factor-α, and others (4, 11, 35). These cytokines can be produced and secreted by many immune cells along with a variety of other cell types in both muscle and brain. Peripherally, they regulate inflammatory and immunological processes involved in the repair of damaged tissue. Cytokines are also potent effectors of CNS function. Most importantly to this study, they are largely involved in groups of symptoms collectively referred to as sickness behaviors, of which fatigue is a major component (8). Both mechanical damage and corresponding inflammation are likely to affect both the skeletal muscle and the CNS that together may decrease exercise performance (8, 30).

The extent to which the delayed recovery of exercise performance after eccentric exercise is dependent on inflammatory cytokines within the brain is not known. However, increased brain concentrations of IL-1β whether injected directly in the brain or elevated in response to an inflammatory challenge like peripheral lipopolysaccharide (LPS) administration has been shown to induce several centrally mediated negative behavioral responses (1, 8), including fatigue (30, 33). In addition, we have shown increased brain IL-1β (cerebellum and cortex) after downhill running in conjunction with delayed recovery of running performance (4). Alternatively, the administration of its receptor antagonist (IL-1ra) has been shown to abrogate some of these responses (1). However, there’s been no systematic study of the effects of IL-1β on running performance after severe exercise. Therefore, the purpose of this experiment was to determine the role of brain IL-1β on running performance after prolonged uphill or downhill treadmill running in mice. Running performance was assessed by measuring both voluntary wheel running activity and treadmill running to fatigue.

We hypothesize that the administration of IL-1ra to mice after the more inflammatory downhill running bout will improve subsequent running performance, whereas administration of
IL-1β in the less-inflammatory uphill running bout would reduce subsequent running performance.

METHODS

Animals

Male C57BL/6 mice (n = 64), 8 wk of age, were purchased from Harlan Sprague-Dawley Laboratories and were acclimated to our facility for a period of at least 3 days before any experimental intervention. Mice were either housed four per cage (n = 32) or placed individually in cages that contained a running wheel (n = 32; Mini-Mitter, Bend, OR). All mice were cared for in the animal facility at the University of South Carolina Medical School. Mice were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% humidity and low noise) and given food (Purina Chow) and water ad libitum. All experiments were performed at the end of the active dark cycle (0700). All aspects of this experimental protocol were approved by the University’s Institutional Animal Care and Use Committee.

Exercise Protocol

Mice were randomly assigned to one of the following four groups: downhill IL-1ra (DWN-IL-1ra), downhill saline (DWN-SAL), uphill IL-1β (UP-IL-1), or uphill saline (UP-SAL). The initial up/downhill run consisted of a 150-min bout. For this session, all mice were placed on a stationary rodent treadmill for a few minutes to allow them to become familiar with the running environment before a progressive ramping of speed and grade during the first 20 min of exercise. This brief acclimation period was incorporated into the 150-min exercise bout to prevent any adaptation to the running protocol that is known to lessen the muscle-damaging effects. After the first 20 min, mice were running at 22 m/min at a grade of −14 or 14%. Mice were gently hand prodded occasionally to maintain running status.

Voluntary wheel running activity. Immediately after the 150-min up/downhill run, a subset of mice [DWN-IL-1ra (n = 8), DWN-SAL (n = 8), UP-IL-1 (n = 8), and UP-SAL (n = 8)] were individually placed back in activity-wheel cages to which they had become acclimated over the past 7 days. After the end of the initial up/downhill run (8 h), the downhill runners received intracerebroventricular injections of either IL-1ra (1.8 µg/mouse in 2 µl of saline) or saline (2 µl), whereas the uphill runners received intracerebroventricular injections of IL-1β (900 pg/mouse in 2 µl of saline) or saline (2 µl). This time point and these doses were chosen based on previously published research on the behavioral effects of intracerebroventricularly injected IL-1β and IL-1ra (1). After injections, mice were placed back in their activity wheel cages, and 24-h voluntary wheel running activity was measured automatically via computer using Vital View physiological and behavioral monitoring software (Mini-Mitter) for seven consecutive days. However, only data collected during the “active” dark period (1900–0700) were analyzed. Earlier experiments in our laboratory have shown that voluntary wheel running activity is minimal during the “inactive” light period (0700–1900). Voluntary activity was quantified for total distance, time on wheel (time), and peak speed (P-speed) as calculated using the following equations: distance = (no. of wheel rotations during a 2-min interval) × [circumference of the running wheel (0.7581 m)] × time; time = (no. of 2-min intervals where wheel rotations were >0) × 2; P-speed = [(95th percentile of rotations during a given time interval) × [circumference of wheel (0.7581 m/2)] (25) and compared with baseline (mean of 3 active cycles before the experimental up/downhill run) values.

Treadmill run to fatigue. A second subset of mice (n = 32) [DWN-IL-1ra (n = 8), DWN-SAL (n = 8), UP-IL-1 (n = 8), and UP-SAL (n = 8)] was given an identical intracerebroventricular injection scheme 22 h after the initial up/downhill run and was returned to their respective cages. Treadmill running performance was measured 2 h later. Mice were run to fatigue on a motorized rodent treadmill at 36 m/min and 8% grade. Fatigue was defined as the time when mice were no longer able or willing to keep up with the treadmill speed despite continued gentle hand prodding for a period of 2 min (9). In this protocol, mice typically fatigue in −150 min.

Intracerebroventricular Injections

Intracerebroventricular injections were performed according to a method described by Pelleymounter et al. (26). Briefly, mice were anesthetized with isoflurane for 30–40 s. The injection site was found by visualizing an equilateral triangle between the eyes and the back of the head, with the apex of the triangle as the injection site. Anesthetized mice were injected with a 10-µl Hamilton syringe fitted with a 30-gauge needle. The needle was shortened by a “sleeve” made from rubber tubing so that the actual injection depth was 4–4.5 mm. Injection accuracy was verified after completion of each study by a second freehand injection with India ink (5 µl). Success rate (as determined by dye injection) with this method has been >90% in our laboratory. Additionally, at the end of the experiment when mice were killed, needle marks were examined on the skull surface. Any animals with misplaced marks were not included in the experiment. DWN-IL-1ra mice each received 1.8 µg of recombinant mouse IL-1ra (rmIL-1ra; R&D Systems, Minneapolis, MN) in 2 µl of physiological aprotic saline (0.9%), whereas DWN-SAL mice received a similar volume of saline. UP-IL-1 mice each received 900 pg of rmIL-1β (R&D Systems) in 2 µl of aprotic saline, and UP-SAL mice received a similar volume of saline. These doses have previously been shown to be behaviorally effective. Both IL-1β and IL-1ra were reconstituted with PBS containing 0.1% BSA as specified by the manufacturer. IL-1ra was used in the downhill runners to block the expected increase in brain IL-1β shown previously (4), and IL-1β was used in the uphill runners who do not have increased brain IL-1β in this running protocol (4).

Statistical Analysis

Voluntary wheel running activity data were analyzed using two-way repeated-measures ANOVAs. Treadmill data were analyzed using a two-way ANOVA. All data were analyzed using commercial software (SigmaStat; SPSS, Chicago, IL). Statistical significance was set with an alpha value of P < 0.05. Post hoc analysis was done utilizing Student-Neuman-Keul’s tests. Data are presented as means ± SE.

RESULTS

Voluntary Wheel Running Activity

Downhill running resulted in a delay in the recovery of voluntary wheel running compared with uphill runners.

Distance. Total running distance for DWN-SAL in the first 12-h active cycle (1900–0700) after the downhill run (0700–0930) was ~20% of baseline (P < 0.01; Fig. 1A). This improved to 63% for the active cycle on day 2 (P < 0.05). By day 3, mice had fully recovered. In contrast, mice receiving IL-1ra (DWN-IL-1ra) performed much better on day 1, having running distances that were ~70% of baseline, which was not different from the running response in UP-SAL. UP-IL-1 had a similar response compared with DWN-SAL, running 6% (P < 0.001), 45% (P < 0.05), and 68% (P < 0.05) of baseline distances on days 1, 2, and 3, respectively, before full recovery. Mean baseline running distances (meters) were not different between groups (DWN-SAL = 3,379.1 ± 599.6, DWN-IL-1ra = 2,831.1 ± 686.1, UP-SAL = 3,679.4 ± 777.1, and UP-IL-1 = 2,793.4 ± 582.7).

Statistical significance was set with an alpha value of P < 0.05. Post hoc analysis was done utilizing Student-Neuman-Keul’s tests. Data are presented as means ± SE.
A downhill run compared with baseline values (Fig. 1 running during each of the first two active cycles after the baseline (*); Fig. 1. Effect of IL-1β/H9252R1346 EXERCISE, BRAIN IL-1β and IL-1ra on recovery of voluntary wheel running after a prolonged, novel bout of uphill and downhill running. Each point represents (mean ± SE) distance (A), time (B), and peak speed (C) vs. baseline run on wheel during each of 7 consecutive active cycles (900–0700). *P < 0.05, downhill-saline vs. baseline (θ) and uphill-IL-1 vs. baseline (*); n = 8 mice/group.

Time. DWN-SAL experienced shorter periods of wheel running during each of the first two active cycles after the downhill run compared with baseline values (Fig. 1B). Time spent on activity wheels for these mice during days 1 and 2 was 30% (P < 0.001) and 74% (P < 0.05) of baseline, respectively. Mice in this group were no different from baseline at day 3. This decrease in running time was partially blocked in DWN-IL-1ra. Time spent on the running wheels did not differ significantly from baseline values on days 1 and 2 or UP-SAL on days 1 and 2. In contrast, UP-IL-1 spent only 12% (P < 0.001) as much time on the wheels compared with baseline on day 1, improving slightly to 52% (P < 0.001) and then 67% (P < 0.05) on days 2 and 3 before full recovery on day 4.

Peak speed. DWN-SAL experienced a decrease of 38% (P < 0.05) in peak speed (Fig. 1C) compared with baseline on day 1 and was no different from baseline on day 2. Again, this reduction in peak speed was not apparent in DWN-IL-1ra, which responded similarly to UP-SAL. UP-IL-1 experienced the largest reduction in peak speed on day 1, attaining only 7% (P < 0.001) of that attained during baseline. By day 2, this improved to 75% (P < 0.05) of baseline levels. Recovery was complete by day 3.

Treadmill Running Performance

Treadmill run times to fatigue were also significantly reduced in DWN-SAL vs. UP-SAL at 24 h after the up/downhill run (37.3 ± 9.5 vs. 90.7 ± 10.5 min; P < 0.01; Fig. 2). This profound decrease in run time was no longer apparent in DWN-IL-1ra (91.3 ± 17.2 min; P < 0.01 vs. DWN-Sal).

DISCUSSION

The present investigation was designed to further determine the role of elevated brain IL-1β on recovery of both voluntary and forced running performance after a single, novel bout of muscle-damaging exercise. This was done by 1) blocking the expected increase in IL-1β activity after downhill running (4) via an intracerebroventricular injection of IL-1ra and 2) artificially increasing brain IL-1β in the uphill runners (previously shown not to have an increase in brain IL-1β) via intracerebroventricular injection of IL-1β. The administration of IL-1ra to the brain of downhill runners significantly improved run times to fatigue compared with their controls, whereas the administration of IL-1β to uphill runners reduced running performance. These are the first data to our knowledge that show a direct effect of brain IL-1β on recovery of running performance after exercise-induced muscle damage. It is also the first data to identify an important CNS component to performance recovery that would otherwise normally be ascribed only to recovery of muscle function.

It is well known that eccentric-biased exercise can result in microtrauma, fatigue, muscle soreness, and functional deficits in muscle lasting up to 96 h (23, 34). This has been indicated repeatedly by histology (17), increased damage, and inflammatory markers, such as CK and CRP (27), and proinflammatory cytokines, including IL-1β (3, 14), along with reduced muscle endurance/power and increased pain (19). Most investigators have attributed this delay in recovery of performance to muscle damage itself, and perhaps perceived pain, which could also be the case in this experiment. Indeed, we have previously found that plasma CK is elevated along with increased concentrations of inflammatory cytokines (IL-1β and IL-6) in skeletal muscle in downhill runners using a similar protocol (Davis, unpublished observation). However, to our knowledge, no studies have addressed extramuscular factors, especially those involving the CNS, in performance recovery after muscle-damaging exercise.

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![Fig. 1](http://ajpregu.physiology.org/)

![Fig. 2](http://ajpregu.physiology.org/)
Peripheral inflammatory challenges such as that caused by LPS injection have been shown to increase IL-1 in various brain regions, including the cerebellum, cortex, brain stem, diencephalon, and hippocampus (29) while triggering physiological and behavioral disturbances such as hyperalgesia (36) and reduced food-motivated behavior (2) and social exploration (1). In a previous experiment, we examined the effects of muscle-damaging downhill running on performance recovery and the inflammatory response in multiple regions of the mouse brain (brain stem, cerebellum, cortex, and midbrain) and found increased IL-1β only in the cerebellum and cortex. It is yet unclear what the precise interaction might be with respect to the cerebellum, cortex, and inflammation, but nuclei within these two brain regions are known to modulate behavioral responses to some inflammatory stimuli (16). The mechanism(s) by which these behavioral changes may occur is not fully understood but may include 1) LPS-activated brain endothelial cell production of IL-1β (31) or 2) vagal afferents (15).

The repertoire of nonspecific symptoms of sickness, which include decreased food and water intake, impaired learning and memory, hyperalgesia, as well as fatigue, is induced by both peripheral or central infusion of IL-1β (1, 33, 36) or by molecules that induce the synthesis of endogenous IL-1β (e.g., LPS). There is substantial evidence, however, which suggests that the effects of IL-1β within the brain are responsible for sickness behavior and not peripheral effects. This is supported by the abrogation of various components of sickness behavior by brain administration of IL-1ra and by the observation that much higher peripheral doses of IL-1β are needed to elicit the same magnitude of behavioral changes compared with central doses (1).

Fatigue is a common complaint in disease states such as Parkinson’s disease (PD), various cancers, autoimmune disorders, and viral infections (7, 18, 20, 32) and may be associated with increased levels of IL-1β. This cytokine has been shown to be elevated in the brains of subjects with PD (22), and IL-1β appears to be involved with cachexia associated with various cancers (13). Moreover, in a pilot study by Omdal and Gunnarsson, there was a rapid and persistent improvement in fatigue scores in patients suffering from rheumatoid arthritis who underwent IL-1ra therapy (24).

The results of this study support the hypothesis that increased brain IL-1β plays an important role on fatigue after exercise-induced muscle damage. That is not to say that it is the only factor involved. Clearly, factors in the periphery may be playing a role as well. It is well known for instance that muscle-damaging eccentric exercise can cause muscle soreness that may persist for up to 96 h after exercise cessation (23). Most of the studies that implicate peripheral factors in prolonged fatigue and muscle impairment after eccentric exercise employ widely differing methodologies than those used in this study. Most are confined to contrived laboratory experiments involving single muscle groups and have not investigated recovery of whole body performance and rarely address behavioral factors. Nonetheless, we acknowledge peripheral factors are likely to be involved, even though our data suggest a major role of brain IL-1β as indicated by the complete return to normal values in the IL-1ra-treated downhill runners.

In conclusion, our models of physical running performance recovery after muscle-damaging exercise confirm our previous results (4) from an earlier experiment by our laboratory. Downhill running delayed both voluntary wheel running and forced treadmill running compared with the more metabolically demanding uphill running. The new findings from the current experiment demonstrate that elevations in central IL-1β play an important role in these recovery models. Increased brain IL-1β may represent a possible mechanism of CNS fatigue after severe exercise.

REFERENCES
17. Hesselin MK, Kuipers H, Geurten P, and Van Straaten H. Structural muscle damage and muscle strength after incremental number of isometric


