

ANIMAL MODEL OF PERFORMANCE ENHANCEMENT BY NUTRITIONAL SUPPLEMENTS WITH ANTI-INFLAMMATORY ACTIVITY

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1. INTRODUCTION

It is well known that intense exercise can induce muscle damage and inflammation depending on exercise mode, intensity, and duration (Schwane et al., 1983; Willoughby et al., 2003). Exercise with a large eccentric component (lengthening of a muscle that is actively developing tension) produces the greatest muscle fiber damage, inflammation, delayed-onset muscle soreness (DOMS) and various functional deficits. It is now thought that many of these responses to muscle-damaging exercise may be triggered by a large increase in inflammatory cytokines in the working muscle, plasma and perhaps even the brain (Dantzer, 2004; Schwane et al., 1983; Sheng et al., 2001; Willoughby et al., 2003).

Exercise-induced increases in inflammatory cytokines such as IL-1 β , TNF- α , and IL-6 were originally thought to be expressed only in immune cells, but now are known to be expressed to varying degrees in many other tissues. They are regulated by a variety of stimulators and suppressors within the inflammatory pathways. The cyclooxygenase-2 (COX-2) prostaglandin cascade and NF-KappaB-mediated cytokine pathways are the most studied pathways (Chun and Surh, 2004). Muscle damage with the production of free radicals in response to unaccustomed exercise can trigger both pathways that lead to increased inflammatory cytokine production, pain, and performance deficits (Reddy and Rao, 2000; Baldwin, 2003).

Recent evidence suggests that various herbal extracts including curcumin (extract of the Indian spice, turmeric) have potent anti-inflammatory activity in a variety of inflammation models. Curcumin has been shown to inhibit both COX-2 and NF-KappaB mediated inflammation pathways (Chun and Surh, 2004; Chun et al., 2003; Han et al., 2002). In fact, evidence suggests that in some experimental conditions curcumin can have similar anti-inflammatory activity as some of the common non-steroidal anti-inflammatory drugs (NSAIDs) like Indomethacin, Vioxx, Celebrex, and Ibuprofen, but without many of the side effects such as gastrointestinal distress and cardiovascular complications (Graumlich, 2001; Mukherjee et al., 2001). However, there are no reports of the effects of curcumin on exercise-induced muscle damage and subsequent recovery of performance.

The downhill treadmill running model of exercise-induced muscle damage, inflammation, and functional deficits is well characterized in rats (Armstrong et al., 1983; Brown and Donnelly, 1999; Komulainen et al., 1999; Kyparos et al., 2001; Schwane et al., 1983; Smith et al., 1991), but evidence in mice is limited. Furthermore, there is no information in either rats or mice about the time course of performance recovery following exercise-induced muscle damage and to what extent this is associated with inflammatory cytokine expression in skeletal muscle. The purpose of this series of studies was to 1) develop an animal model of performance recovery following muscle-damaging exercise and 2) to evaluate the potential benefits of curcumin supplementation in this model. We hypothesized that the anti-inflammatory activity of curcumin would hasten recovery due to an attenuation of the primary and especially secondary inflammation that is associated with delayed regeneration of muscle fibers, soreness, and central nervous systems symptoms of fatigue. The beauty of this model is that the "control" condition is uphill running that requires more energy and is physically more demanding but produces little or no muscle damage and inflammation.

2. METHODS

2.1 Animals

Male CD-1 mice (8 weeks of age, Charles River Labs) were housed in the animal facility, and maintained on a 12:12 light-dark cycle in a low-stress environment (20C, 50% relative humidity, low noise). Water and food (Purina Chow) were available ad libitum.

2.2 Experimental Groups

Mice were randomly divided into the following groups: uphill treadmill running (UP); uphill treadmill running with curcumin (UP+C); downhill treadmill running with curcumin (DW); downhill treadmill running with curcumin (DW+C); and resting cage control (CON).

2.3 Exercise Protocol

Recovery of endurance capacity was assessed as treadmill run times to fatigue (36m/min, 8% grade) at 24,

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48, 72 & 96 hr after an intense 2.5 h uphill (concentric-biased exercise) or downhill (eccentric-biased exercise) run on a motorized treadmill in male mice. Downhill running consisted of 150 min starting at 16m/min, -5% grade with increments every 5 min to 24m/min, -14% grade). Uphill running was done at the same speed, but grade was increased from 5% to 14% at the same times as grade was decreased in the downhill condition. At the appropriate time point, the animals were placed once again on the treadmill and encouraged to run using mild hand prodding until fatigue, which was defined as a refusal to run after one minute of continued encouragement. We have previously shown that mice will readily run for up to 3 hours prior to fatigue with this type of protocol (Davis et al., 1998).

2.4 Tissue Collection

Separate groups of mice were sacrificed at 24, 48 and 96 hr after the uphill and downhill runs for blood and muscle collection. At the appropriate time point, mice were sacrificed by halothane overdose and the soleus muscle from both legs was immediately removed, cleaned of residual blood, and flash frozen in liquid nitrogen. They were then stored at -80°C for analysis of cytokine protein concentration. Blood was also collected from the inferior vena cava using a heparinized syringe, and plasma was stored at -80°C for analysis of creatine kinase (CK, marker of skeletal muscle damage).

2.5 Analytical Method

Creatine kinase was measured in plasma according to manufacturer's instructions (Diagnostic Chemicals Limited, Oxford, CT). Muscle inflammatory cytokine concentrations (IL-1 β , TNF- α , IL-6) were measured in muscle homogenates (in Iscove's modified Dulbecco's medium) via ELISA according to manufacturer's instructions (R&D Systems, Minneapolis, MN). The cytokine concentration was expressed as pg/mL of homogenate.

2.6 Curcumin Feedings

Curcumin was evaluated in the second set of experiments, where mice were given curcumin (10 mg) once daily in a highly palatable food pellet (0.41g; bacon flavor) on three days prior to the downhill or uphill run. No supplements were given during the recovery period. Control animals received only the bacon-flavored pellet. The experimental protocol was otherwise identical to the original experiment except that performance was only measured at 48 & 72 hr.

2.7 Statistical Analysis

Data was analyzed with commercial software (SigmaStat, Chicago, IL). Statistical analysis consisted of two- and three-way ANOVA with Newman Keuls post-hoc tests to examine individual group differences. Significance was set at $p < 0.05$. Data are presented as means $\pm$ SEM.

3. RESULTS

Treadmill run time to fatigue was suppressed by over 50% for up to 72 hr after the initial bout of intense exercise in the downhill runners as compared to the uphill runners (Figure 1). Recovery of performance (endurance capacity) was not complete until 96 hr.

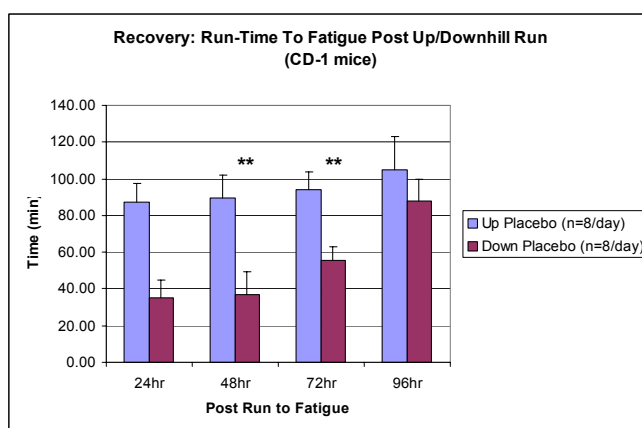


Figure 1

An elevation in plasma CK has been used as an indirect marker of skeletal muscle damage (Armstrong et al., 1983). Figure 2 shows that eccentrically-biased downhill running (DOWN) elicited approximately a 5-fold increase above CON at 24 hr, with no change in UP. It remained elevated in DOWN at 48 hr before dropping to CON levels at 96 hr.

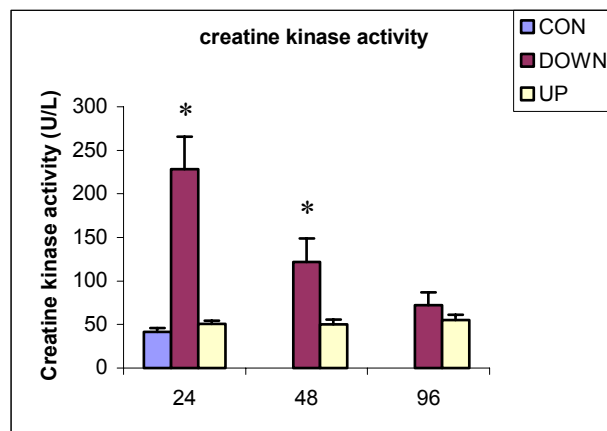


Figure 2

Muscle inflammatory cytokine concentration can be associated with an increase in invading immune cells, inflammation, and functional deficits (Bruunsgaard et al., 1997). In this study, DOWN was associated with increases in muscle IL-1beta and IL-6 (Figures 3, 4), but not TNF-alpha (data not shown) at 24 hours as compared to CON and UP. No differences were found for any cytokine at 48 and 96 hours following the downhill or uphill run.

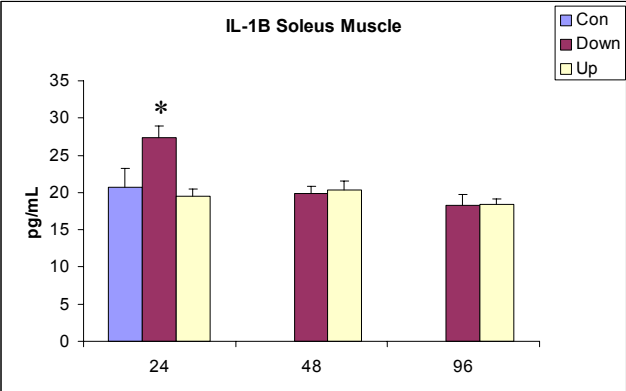


Figure 3

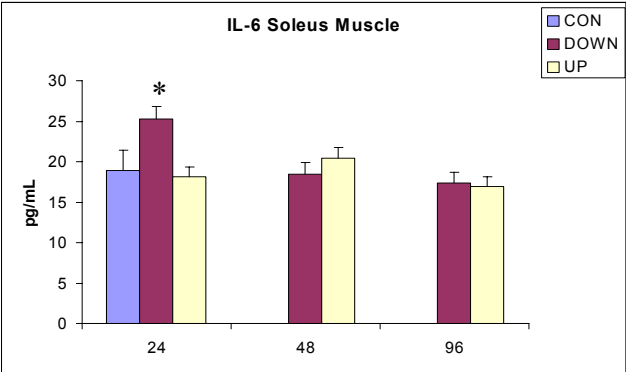


Figure 4

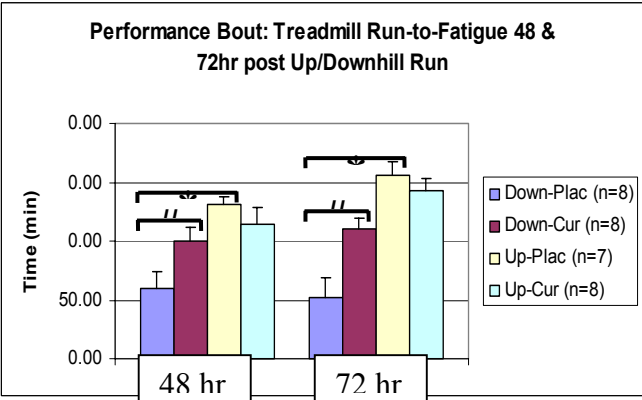


Figure 5

Figure 5 shows the effects of curcumin supplementation on recovery of performance following downhill running. As expected from previous experiments using this model, treadmill-running performance was impaired at 24 and 48 hr in the downhill running groups versus the uphill running mice. However, this effect was blunted (i.e., run time to fatigue was increased) in the downhill running groups that received curcumin supplements. Curcumin had no effect in the uphill runners.

4. DISCUSSION AND CONCLUSIONS

The induction of mechanical damage and inflammatory cytokines in skeletal muscle following a novel bout of eccentrically-biased exercise (downhill running) can be associated with a reduction in muscle performance during the normal progression of tissue regeneration. Although the morphological and biochemical responses that result from downhill treadmill running have been well characterized in rats, there is still very limited evidence in mice (Armstrong et al., 1983; Kanulainen et al., 1999; Kyparos et al., 2001). The several studies involving mice have focused primarily on twitch tension and the morphological changes associated with degeneration and regeneration; there has been no reports of cytokine analysis at either the message or protein level (Armand et al., 2003; Carter et al., 1994) or the time course of performance recovery following the damage. To our knowledge, this is the first study to report the time course of performance recovery and its association with muscle inflammatory cytokines following exercise-induced muscle damage. It is also the first study to examine the effect of the anti-inflammatory nutraceutical curcumin on recovery of endurance performance following downhill running. The data show that endurance performance does not recover fully for up to 96 hr after a novel bout of downhill running, which is associated with increases in plasma CK and muscle inflammatory cytokines (IL-1beta and IL-6). In addition, and perhaps more important from a military perspective, the data shows faster recovery of endurance performance following ingestion of curcumin for 3 days prior to the muscle-damaging exercise. Whether or not continued feedings of curcumin during the recovery period would further enhance this effect remains to be determined.

Both concentric and eccentric muscle contractions can induce damage and functional deficits, albeit to different degrees, but the time course of recovery is still unclear. Eccentric contraction is defined as a muscle that is lengthening while tension is still developing, and it has been shown to be the most damaging form of muscle contraction (Armstrong et al., 1983). Downhill running was first evaluated as a possible eccentric injury model because humans experience much more intense delayed

onset muscle soreness (DOMS) 1-3 days after an eccentrically-biased exercise than concentrically-biased exercise (Armstrong et al., 1983).

The current study found a large increase in plasma CK activity in downhill runners compared to resting control animals at 24 hr post-run, with return to resting levels by 96 hours. This was associated with a decrease in treadmill run time to fatigue in animals running downhill compared to the uphill runners at 24 and 48 hours. The increase in circulating CK resembles the second peak in the biphasic response that Armstrong and colleagues reported after using several different running protocols involving level, uphill and downhill running in rats. He found that there was a biphasic response of CK to downhill running, but not with level or uphill running (Armstrong et al., 1983). There are a limited number of papers that have investigated the CK response in mice with downhill running and none have measured the response at 24-48 hours (Cheung and Tidball, 2003). The decrease in performance recovery may be associated with the histological, morphological, and ionic changes that occur within the skeletal muscle fiber following eccentric exercise (Armstrong et al., 1983; Smith et al., 1997). However, the mechanical damage and corresponding inflammation, due mainly to infiltrating cells such as neutrophils and macrophages, are likely to affect both the skeletal muscle and the central nervous system (fatigue, perceived discomfort, impaired mood, and perhaps other cognitive deficits) that together result in decreased endurance performance (Dantzer, 2004; Schwane et al., 1983; Sheng et al., 2001; Willoughby et al., 2003).

Scientific investigation into the development of new anti-inflammatory drugs has increased dramatically in recent years. The outcome has been a wide variety of non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin, Vioxx, Celebrex, and Ibuprofen that are often used to reduce muscle and joint pain (Mukherjee et al., 2001; Reddy and Rao, 2000; Wargovich et al., 2001). However, a major drawback to chronic ingestion of many of the NSAIDs, such as practiced by some athletes and elite Warfighters, is the degradation of the mucosa lining of the stomach, leading to gastrointestinal ulcers and unexpected and potentially deadly bleeding, as well as increased risk of other cardiovascular events (Graumlich, 2001; Mukherjee et al., 2001; Reddy and Rao, 2000; Wargovich et al., 2001). Another drawback is the high cost of these drugs. Therefore, the uses of natural anti-inflammatory agents, such as curcumin, that presumably do not have these disadvantages are generating much interest. Our results suggest that curcumin can enhance recovery following muscle-damaging exercise, which is of great potential benefit to athletes and military personnel who are often exposed to unusually stressful exercise conditions on a recurrent basis. This positive effect is presumable working via curcumin's well-

characterized anti-inflammatory activity that leads to a reduction in inflammatory cytokine expression by partial inhibition of the COX-2 and/or NF-kappaB pathways (Chun and Surh, 2004; Chun et al., 2003; Han et al., 2002), but future experiments are required to confirm this hypothesis. Curcumin also has the advantage of being a natural food/food additive since it is the major constituent (curcuminoid) in the Indian spice turmeric and therefore can easily be incorporated into combat rations, energy bars, or other food choices for ingestion by athletes and military personnel.

In summary, this study describes the development of a mouse model that can be used to rapidly screen novel anti-inflammatory supplements for potential benefit in recovery of performance decrements following intense muscle-damaging exercise. It can also be used to better understand the biomedical mechanisms (at the level of both muscle and brain) that cause the effect that are essential for development of safe and effective novel nutritional strategies for use in athletes and military personnel. This is the first report of a beneficial effect of curcumin feedings on the time course of recovery of endurance running performance following a bout of eccentrically biased exercise. Specifically the evidence suggests that 1) increases in muscle inflammatory cytokine concentrations (IL-1beta, IL-6) occur in conjunction with mechanical damage following intense eccentric-biased exercise (downhill running), 2) that recovery of endurance performance following such exercise can take up to 96 hours, and 3) that ingestion of the anti-inflammatory nutraceutical curcumin can hasten recovery of endurance performance in this model.

We anticipate that future experiments with this model will lead to better nutritional strategies to offset the detrimental effects of intense physical exercise and training on physical performance when adequate recovery rest periods are not possible. Of particular additional importance would be experiments with this model to determine the safety of such strategies, especially in comparison to a variety of NSAIDs currently on the market and widely used by individuals taking part in intense physical training.

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