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White blood cell response to uphill walking and downhill jogging at similar metabolic loads

L. L. Smith[1], M. McCammon[1], S. Smith[2], M. Chamness[2], R. G. Israel[1], and K. F. O'Brien[3]

[1] Human Performance Laboratory, East Carolina University, Greenville, NC 27858, USA
[2] Department of Clinical Laboratory Science
[3] Department of Biostatistics

Summary. The object of this study was to determine whether leukocytosis would occur in response to eccentric exercise, to concentric exercise, and/or to possible increases in serum corticol levels. Eight men performed 2 bouts of exercise at 46% $\dot{V}_{O_{2max}}$ for 40 min. Subjects initially walked up a 10% grade (UW); 2 weeks later they jogged down a 10% grade (DJ), a form of eccentric exercise known to induce delayed onset muscle soreness (DOMS). Venous blood samples were drawn before and after each exercise bout (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 h). Total and differential WBCc and serum cortisol levels were assessed. Results were analyzed using repeated measures ANOVA (2 × 11). Subjects experienced severe DOMS after DJ. There was a significant difference in TWBCc ($p < 0.0001$) between UW and DJ. Post-hoc testing revealed no significant increase over baseline values for UW; after DJ there was a 46% increase over baseline values ($p < 0.05$) initially seen at 1.0 h. These increases in TWBCc were predominantly a reflection of increases in neutrophils which were significant ($p < 0.0001$) when compared to baseline values at 1.0, 1.5 and 2.0 h (~60%). No significant neutrophil increases were seen after UW. Cortisol levels were similar for both groups pre-exercise (UW = 367.1 ± 38.6, DJ = 320.2 ± 44.16 nmol·L^{-1} $\bar{x}$ ± SE) and decreased similarly for both groups after exercise, and thus were not related to the post-exercise neutrophilia. In conclusion, the neutrophilia seen after the DJ appeared to be a response to differences in the exercise, not plasma cortisol levels. Thus a bout of eccentric exercise appears to induce a significant post-exercise increase in neutrophils seen between 1.0 and 2.0 h after the termination of exercise.

Key words: Leukocytosis — Cortisol — Delayed onset muscle soreness — Neutrophils — Eccentric contractions

Offprint requests to: L. L. Smith, Human Performance Laboratory, Minges Coliseum, East Carolina University, Greenville, NC 27858-4353, USA

Introduction

The white blood cell (WBC) response to a bout of exercise has previously been investigated in an attempt to clarify the biochemical events underlying delayed onset muscle soreness (DOMS) (Schwane et al. 1983; Bobbert et al. 1986). It is now well established that eccentric lengthening contractions induce DOMS (Armstrong 1984); it is also well documented that an elevated WBC count is associated with inflammation (Ryan and Majno 1977; Boggs et al. 1968). Therefore to determine whether there was any association between DOMS and inflammation, Schwane et al. (1983) and Bobbert et al. (1986) assessed the WBC count in human subjects after a bout of eccentric exercise. No significant elevations were seen immediately after or at 24, 48, or 72 h later which led these researchers to conclude that DOMS was not associated with inflammatory pain.

Recently Smith et al. (1986) found a significant increase in neutrophils at 2 h after a 45 min bout of bench stepping; but it was unclear whether the elevated WBC count was a response to the eccentric or concentric component of the exercise since bench stepping involves equivalent amounts of both types of contractions. Additionally, neutrophilia may have been induced by elevated levels of plasma corticol (Claman 1983).

Thus, the purpose of this study was to determine whether a significant increase in neutrophils would be seen shortly after eccentric exercise (downhill running) as opposed to an equivalent bout of exercise consisting of predominantly concentric contractions (uphill walking). Additionally, plasma cortisol levels were measured to determine whether any increases seen in WBC count could be attributed to increased levels of this hormone.

Methods

Subjects. Eight men served as volunteer subjects (Table 1). All were healthy, but none trained in any activity on a regular basis or engaged in activities involving extensive amounts of eccentric contractions such as weight training. Signed informed consent was obtained from all subjects and the project was approved by the University's Policy and Review Committee for Human Research. All subjects were exercised between 1300 and 1600 hours.

Experimental protocol

$\dot{V}_{O_{2max}}$ test. Subjects were initially screened to determine their maximum aerobic capacity. This was done using the standard Bruce Protocol (Bruce et al. 1973) on a motor-driven treadmill. Gas analysis was performed continuously (Beckman Horizon System II) and the highest minute value achieved was considered $\dot{V}_{O_{2max}}$. Heart rate was recorded during the last 10 s of each minute.

Uphill walking (UW). On the first exercise day all subjects were required to walk on a motor-driven treadmill, up a 10% grade. Metabolic equations, as derived by the American College of Sports Medicine (1986), were used to determine the speed which would elicit approximately 50% of the subjects' maximum aerobic capacity. Speeds varied between 63.3 and 85.0 $m \cdot s^{-1}$, with a mean speed of 75.0 ± 2.3 $m \cdot s^{-1}$. Oxygen uptake was monitored continuously during the first 10 min of exercise.

Downhill jogging (DJ). Two weeks after uphill walking all subjects were required to jog for 40 min on a motor driven treadmill down a 10% grade. Subjects jogged at speeds which ranged from 120.0 to 160 ($\bar{x} = 138.3 \pm 6.7$ $m \cdot s^{-1}$). The initial speed was selected to elicit an oxygen uptake similar to the steady state level seen during minutes six through ten of uphill walking (50% of $\dot{V}_{O_{2max}}$).

Blood sampling. Subjects were catheterized and blood samples were obtained from a radial vein 30 min before the exercise bout, with the second sample taken 5 min before; these two values were then averaged and considered the pre-exercise measure. Ten additional samples were taken at the following times: immediately after exercise (time 0) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0 h after exercise. Samples were collected in EDTA anticoagulant, mixed, and refrigerated. During the 5 h post-exercise period, subjects remained in the vicinity of the Human Performance Laboratory and performed no exercise.

Table 1. Physical characteristics of subjects ($N = 8$)

Characteristcs	$\bar{x} \pm SE$
Age	18.8 ± 0.5 years
Height	1.76 ± 0.02 m
Weight	68.39 ± 2.04 kg
Percent body fat	11.6 ± 1.9%
$\dot{V}_{O_{2max}}$	53.2 ± 1.4 $ml \cdot kg \cdot min^{-1}$

Blood analysis. Cell counts and blood smears were performed within 4 h of collection with the samples brought to room temperature and mixed gently by rotation on an automatic mixer. Duplicate 1:250 dilutions were performed from each sample using a Sequoia-Turner HD 251 Hematology Diluter. Red cells were lysed, samples were mixed and white cell counts were performed using a Sequoia-Turner Cell-Dyn 400 Hematology Analyzer. Duplicate blood smears were also stained with Weight-Giemsa stain (Ames Hema-tek slide stainer). One-hundred-cell white cell differentials were performed on each smear.

Cortisol was determined on EDTA plasma samples using a solid phase RIA kit (Clinical Assays). The plasma was separated from cells within two hours of collection and maintained at −40 degrees Centigrade until assay. All samples and standards were run in duplicate.

Delayed onset muscle soreness (DOMS). Subjects were instructed to assess DOMS at 24, 48 and 72 h after both exercise bouts. All were given diagrams of the front and rear view of the lower body and were asked to circle the involved area and then rate the intensity of soreness experienced in that area using a zero to ten scale (0 = no soreness, 10 = unbearable soreness). These cumulative values were then used to represent subjects' daily DOMS scores.

Statistical analysis. To evaluate the changes in the WBC count (total and differential) as well as changes in plasma cortisol levels, a two-way ANOVA with repeated measures across time was used. When significance was found for main effects ($p < 0.05$), a Tukey's post-hoc analysis was performed. Values reported are mean ± standard error.

Results

Oxygen uptake and heart rate

Oxygen uptake measured during minute six through ten of both exercise bouts was $45.8\% \pm 0.9\%$ and $47.2\% \pm 1.8\%$ $\dot{V}_{O_{2max}}$ for the UW and DJ respectively. Mean heart rate was significantly higher during the DJ (149.7 ± 5.4 $beats \cdot min^{-1}$) compared to the UW (129.5 ± 2.1 $beats \cdot min^{-1}$) ($p < 0.0001$).

Total and differential WBC

The total WBC response between exercise bouts (uphill/downhill) was significantly different ($p < 0.0001$). This was due to the elevated counts,

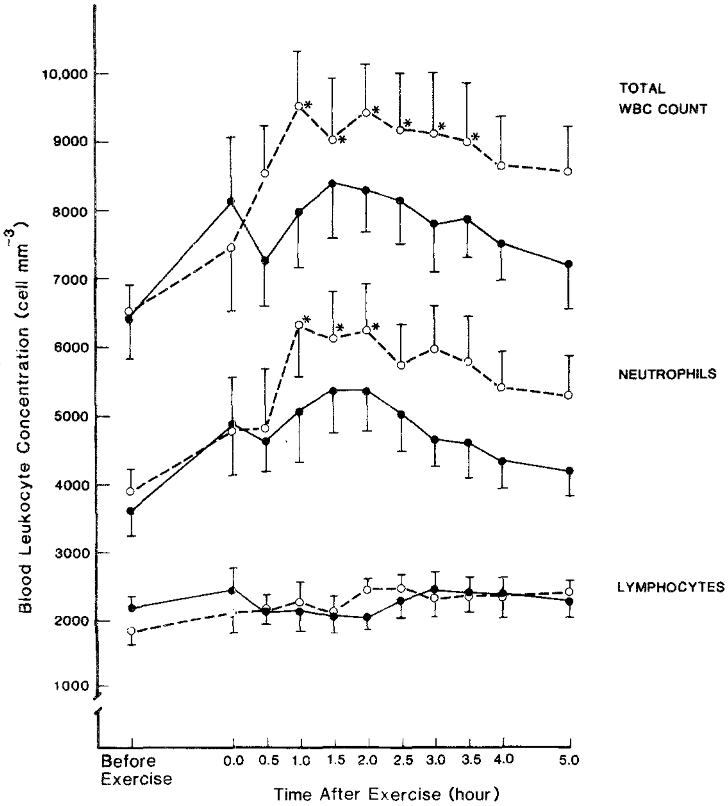

Fig. 1. Changes in leukocyte count before and after uphill walking ($N=8$, ● — ●) and downhill jogging ($N=8$, ○---○). Values represent means and standard error of means (* $p<0.05$ compared to before exercise)

at 1.0 through 3.5 h after DJ as compared to UW (Fig. 1). The neutrophil count between exercise bouts was also significantly different ($p<0.0001$) due to a significant ($p<0.05$) increase in neutrophils at 1.0 through 2.0 h after the DJ. There were no significant exercise effects seen between any of

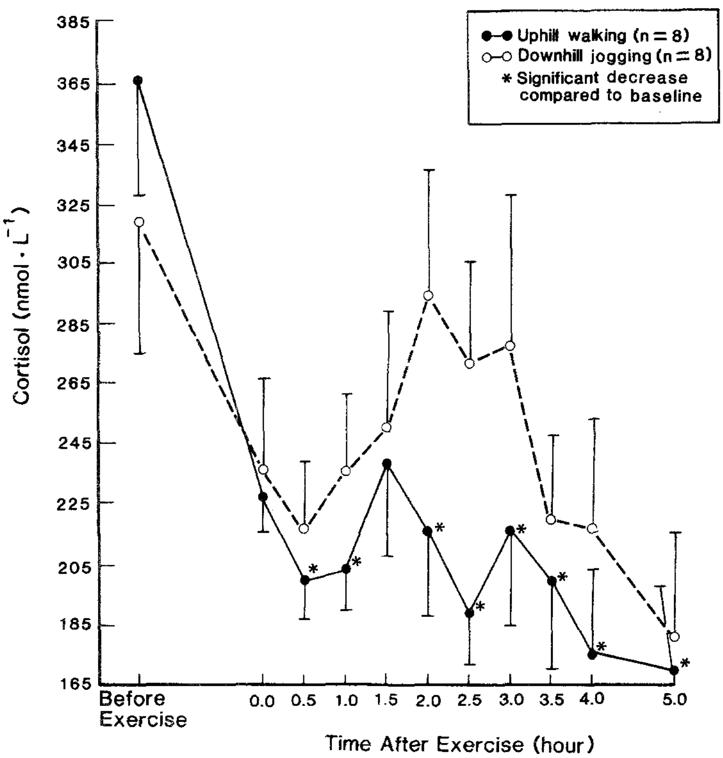

Fig. 2. Changes in plasma cortisol levels ($nmol \cdot L^{-1}$) before and after uphill walking ($N=8$, ●---●) and downhill jogging ($N=8$, ○---○). Values represent means and standard error of means (* $p<0.05$ compared to before exercise)

the other cell types (lymphocytes, monocytes and eosinophils) and none of these cells were at any time significantly elevated or depressed when compared with the pre-exercise baseline levels.

Plasma cortisol levels

Initial plasma cortisol levels were 367.1±38.6 $nmol \cdot L^{-1}$ before UW and 320.2±44.16 $nmol \cdot L^{-1}$ before DJ (Fig. 2). After both bouts of exercise cortisol decreased similarly ($p<0.0001$) to 229.1±11.0 and 265.0±30.4 $nmol \cdot L^{-1}$ for the UW and DJ respectively. A significant main effect ($p<0.02$) was also seen between exercise groups; this was due to cortisol decreasing significantly ($p<0.05$) for UW from pre-exercise values, during 0.5 to 5.0 h.

Intensity of DOMS

Minimal amounts of muscle soreness were reported after the uphill walk. However, as anticipated, subjects reported significant amounts of DOMS after downhill jogging ($p<0.0001$). Post-hoc testing revealed that these subjects experienced significant ($p<0.05$) amounts of soreness at 24 h (66.4±12.8), at 48 h (59.6±15.1), and 72 h (32.9±10.0).

Discussion

This study was designed to observe the effects of eccentrically (DJ) and concentrically (UW) biased exercise, at a similar metabolic cost, on changes in neutrophil count, over a five hour post-exercise period. The results indicated that there was a significant difference in neutrophil count between the two bouts of exercise with neutrophilia observed between one and two hours after DJ (Fig. 1). This is in agreement with Smith et al. (1986) and Franklin and Franklin (1988). Smith et al. (1988) reported a 77% increase ($p<0.01$) in neutrophils 2 h after a 45 min bout of bench stepping. Franklin and Franklin (1988) using resistive exercise, found a significant increase in neutrophils ($p<0.05$) 12 h post-exercise. Others (Schwane et al. 1983; Bobbert et al. 1986) who assessed counts immediately post and then not until 24 h after exercise reported that negative work did not induce neutrophilia. The results from the present study suggest that neutrophil levels should be assessed within 5 hours post-exercise.

In the present study low intensity exercise (<50% of max) was specifically chosen as to avoid increased circulating levels of cortisol (Galbo 1983). It is interesting to note that Schwane et al. (1983) found a significant increase in WBC count after a 45 min bout of level running. Schwane's subjects ran at a mean $\dot{V}_{O_2}$ which represented approximately 78% of their max; increases in cortisol are usually seen at about 60% of $\dot{V}_{O_{2max}}$ (Galbo 1983). It is possible that increased leukocyte levels seen after level running were in response to this stimulus. The possible impact of this stress hormone was reduced by our experimental design. Thus, the increases in neutrophil count which we observed can be attributed mainly to the eccentric exercise since no increases in cortisol were observed, yet neutrophil count increased.

Other factors associated with the downhill run could have had an impact on the neutrophil results. Increased epinephrine levels have been shown to induce neutrophilia (Ryan and Majno 1977). Although circulating levels of epinephrine were not measured in this study it is possible that levels were elevated in response to the downhill run. This may have contributed to the higher heart rate seen during this exercise bout. However, epinephrine would not appear to account for the neutrophilia seen one or 2 h later, since physiological and biochemical responses to epinephrine are immediate (Lehninger 1982) and the clearance rate is rapid.

One reason why the WBC response to negatively biased exercise has been of interest is that an elevated count may be indicative of acute inflammation (Ryan and Majno 1977). It is well established that tissue disruption occurs in response to negatively biased exercise and that this disruption is associated with subsequent delayed onset muscle soreness (Friden et al. 1981). Tullson and Armstrong (1978) suggested that acute inflammation might be the underlying mechanism in DOMS. This hypothesis was subsequently rejected (Schwane et al. 1983; Bobbert et al. 1986) since no significant increases in leukocytes were found after negatively biased exercise. We did however, see these increases between 1.0 and 2.0 h after exercise, supporting Tullson and Armstrong's (1978) theory.

In conclusion, the evidence presented in this study suggests that eccentric exercise in the form of downhill running produced significant neutrophilia relative to concentric-uphill walking. Neutrophils were significantly elevated above baseline between 1.0 and 2.0 h after exercise. At mild workloads (< 50%) cortisol did not appear to be responsible for the increase in neutrophils noted. This suggests that it may be prudent to examine neutrophil responses during the 5 h following eccentric exercise.

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