Immune Alterations, Lipid Peroxidation,
and Muscle Damage Following a Hill Race

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Catalogue Data

Key words: acute phase response, cytokines, antioxidant capacity, creatine kinase, field study
Mots-clés: phase aiguë, cytokines, capacité antioxydante, créatine kinase, étude de terrain

Abstract/Résumé
Hill races usually include large downhill running sections, which can induce significant degrees of muscle damage in a field setting. This study examined the link between muscle damage, oxidative stress, and immune perturbations following a 7-km mountainous hill race with 457 m of ascent and 457 m of descent. Venous blood samples were taken from 7 club level runners before, immediately after, and 48 hrs postrace. Samples were analysed for total and differential leukocyte counts, markers of muscle damage (CK), lipid peroxidation (MDA), and acute phase proteins (CRP; fibrinogen; α-1-ACT). The total antioxidant status (TEAC) and plasma levels of the proinflammatory cytokines IL-6, IL-8, and TNF-α were also determined. Subjective pain reports, and plasma activities of CK, MDA, and circulatory monocytes reached peak values at 48 hrs postrace (p < 0.05). TEAC and the cytokine IL-8 increased immediately after the race (p < 0.05). Plasma TNF-α remained unchanged (p > 0.05). Despite the reports of muscle damage and soreness, no evidence of an acute phase response was observed (p > 0.05), which may be explained by the failure of the race.
to induce a plasma TNF-α response. Future studies should examine the link between muscle damage, oxidative stress, and the acute phase response following hill races of longer duration with larger eccentric components.

Les courses en montagne avec leurs bonnes portions de course vers le bas comportent un potentiel de lésions musculaires importantes. Cette étude analyse la relation entre les lésions musculaires, le stress par oxydation, et les modifications au système immunitaire après une course de 7 km en montagne comprenant 457 m de montée et autant de descente. Des échantillons de sang veineux sont prélevés chez 7 coureurs d’un club: avant, immédiatement après la course, et 48 hr plus tard. Dans les échantillons, on fait le dénombrement des globules blancs (global et par catégories), on analyse les marqueurs de lésion musculaire (CK), on observe la peroxydation lipidique (MDA) et les protéines en phase aiguë (CRP; fibrinogène; α-1-ACT). Le statut antioxydant global (TEAC) et les taux de cytokines pro-inflammatoires IL-6, IL-8, et TNF-α sont également pris en compte. Les sensations de douleur, les activités plasmatiques de CK, et les monocytes en circulation atteignent un sommet 48 hr après la course (p < 0,05). Le TEAC et les cytokines IL-8 augmentent immédiatement après la course (p < 0,05). Les TNF-α plasmatiques ne sont pas modifiées (p > 0,05). Malgré des sensations reportées de lésion et de douleur musculaire, on n’observe pas de manifestation d’une phase aiguë (p > 0,05), et ce, probablement à cause de l’absence de TNF-α plasmatiques engendrées par la course. Il importe que d’autres études se penchent sur la relation entre les lésions musculaires, le stress par oxydation, et la manifestation d’une phase aiguë à la suite de courses en montagne de plus longue durée et présentant plus d’actions pliométriques.

**Introduction**

The sport of hill running has become increasingly popular in the U.K. (Creagh et al., 1998). Many hill races consist of large downhill running sections which require the working muscles to contract eccentrically. Unaccustomed eccentric exercise has consistently been shown to result in muscle inflammation, delayed-onset muscle soreness (DOMS), and the release of the muscle-derived enzyme creatine kinase (CK), an indicator of muscle damage. Muscle soreness is commonly accompanied by increased plasma CK levels and both tend to reach peak values during the subsequent days of recovery following eccentrically biased exercise protocols (Maughan et al., 1989; Schwane et al., 1983; Smith et al., 1998). The importance of the immune system following muscle damaging exercise is evident, as this type of activity has been shown to trigger an acute phase response similar to that seen as a result of bacterial infection, surgical trauma, or inflammatory disease states (Fielding and Evans, 1997; Mackinnon, 1999).

Although many factors might explain the etiology of muscle pain and damage, free-radical-mediated oxidative stress is believed to play a causative role (Child et al., 1999; Goodman et al., 1997; Radak et al., 1999). Activation of the immune system aids in the repair and regeneration of damaged tissue by releasing acute phase proteins and inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) to stimulate migration and margination of neutrophils and monocytes to areas of inflammation (Tidball, 1995). Paradoxically, these phagocytic cells have the potential to generate free radicals and associated reactive oxygen species (ROS) which could cause subsequent dam-
age to the tissue in a process termed secondary muscle damage (Child et al., 1999). It is by this mechanism that the delayed elevation of CK and consequent muscle soreness has been hypothesised.

Among the most consistent effects of oxidative stress in response to exercise is the peroxidation of lipid cell membranes (Goodman et al., 1997; Mastaloudis et al., 2001). This cytotoxic process is marked by the presence of potentially harmful aldehydes and has been shown to occur during times of muscle damage following eccentrically biased exercise protocols (Kanter et al., 1988; Maughan et al., 1989; Sacheck et al., 2003). Both Maughan et al. (1989) and Sacheck et al. (2003) found a CK correlating increase in lipid peroxidation markers after 45 minutes of downhill treadmill running, suggesting a possible consequence of ROS production by infiltrating phagocytes. Although the cytotoxic effects of ROS may be attenuated by antioxidant defences, by-products of lipid peroxidation have been found to increase following exercise despite a concomitant increase in total antioxidant status (Child et al., 1998; Kaikkonen et al., 1998; 2002).

The acute phase response associated with exercise has been suggested to relate to skeletal muscle damage (Fallon, 2001). During infection, this response elicits potentially beneficial effects including complement activation and stimulation of phagocytosis, and is mediated by glycoproteins from the liver. C-reactive protein (CRP) is the most abundant of these acute phase proteins and has been reported to increase following exercise, particularly when muscle damage is evident (Fallon, 2001; Gleeson et al., 1995). Other researchers, however, have failed to observe any evidence of an acute phase response following exercise despite a concomitant elevation in plasma CK activity (Hubinger et al., 1997; Pyne et al., 1997).

It appears there is still a need to determine whether exercise-induced immune perturbations are associated with changes in markers of muscle damage, lipid peroxidation, and total antioxidant status (Nieman et al., 2003). Many studies to date have been restricted to laboratory based protocols such as downhill treadmill running (Maughan et al., 1989; Sacheck et al., 2003; Smith et al., 1998). Despite its increasing popularity and the potential to induce extensive muscle damage in a field setting, no data are currently available on the immune and biological responses of athletes participating in hill races. The aim of this study was to examine the effects of a competitive hill race on markers of oxidative stress, muscle damage, and the acute phase response of the immune system.

**Methods**

**SUBJECTS AND DESIGN**

Seven club level runners (6 M; 1 F) volunteered to participate in this study. Each one provided informed consent, and approval for the study was granted by the institution. Subjects were required to refrain from any strenuous activity for 48 hrs before and 48 hrs after the race. Their physical characteristics are presented in Table 1.

Subjects underwent a maximal exercise test to volitional exhaustion on a treadmill (Woodway, ergo ELG 55, Weil am Rhein, Germany) following the Bruce protocol (Bruce et al., 1973). Maximum oxygen uptake (VO₂max), maximum heart rate (HRmax), and maximum blood lactate concentration (La max) were deter-
mined from the test. Oxygen uptake (breath by breath) was measured using online gas analysis (CPX MedGraphics, Oldham, U.K.), and heart rate was recorded every 5 sec (S610, Polar Electro, Kempele, Finland). A fingertip blood sample was collected in a capillary tube before and immediately after the test to determine blood lactate concentration (Analox P-G15, London, U.K.).

Each subject competed in the 18th Scottish Hill Runners Association Tinto Hill Race (total distance = 7 km; 3.5 km/457m ascent; 3.5 km/457m descent). Environmental conditions during the race were 8.5 ºC and 66% humidity. Measurements were collected in a mobile laboratory situated next to the racecourse. Heart rate was measured during the race and blood lactate concentrations were determined before and after the race. All laboratory materials were purchased from Sigma-Aldrich, Dorset, U.K., unless otherwise stated. Intravenous blood samples were collected in 6-ml vacuum tubes anticoagulated with lithium heparin (Becton-Dickinson, Oxford, U.K.) at prerace, immediately post, and 48 hrs postrace. Whole blood was mixed (1:20) with white blood cell stain diluent (crystal violet in 10% acetic acid) and total leukocyte counts were estimated using a haemocytometer. Blood smears were stained with a Romanowsky stain (Raymond A. Lamb, London, U.K.) to determine differential leukocyte counts.

An individual who was not aware of the sample identity performed the cell counts. Neutrophils were assessed and quantified for their level of maturity based on morphological appearance. Whole blood was centrifuged for 10 min at 1,000 g and the removed plasma was stored at –80 ºC until analysis. Subjects graded their sensations of muscular pain using the CR10 scale (Borg, 1998) at each blood sampling time point with an additional assessment made at 24 hrs postrace.

**BIOCHEMICAL ANALYSIS**

The plasma activity of the muscle damage marker CK, the lipid peroxidation marker malondialdehyde (MDA), and the trolox equivalent antioxidant capacity (TEAC) were measured using spectrophotometric analysis (Dynatech MRX, Billinghurst, Kent, U.K.). CK was measured using a standard laboratory kit (CK-NAC, Instrumentation Laboratory, Lexington, MA) and the TEAC was determined following an adapted method of a previously described assay (Re et al., 1999). Briefly, 2,2'-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>32 ± 0.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.8 ± 1.1</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>68.6 ± 1.3</td>
</tr>
<tr>
<td>VO2max (ml·kg⁻¹·min⁻¹)</td>
<td>54 ± 1</td>
</tr>
<tr>
<td>HRmax (beats·min⁻¹)</td>
<td>190 ± 1.4</td>
</tr>
<tr>
<td>La max (mmol·L⁻¹)</td>
<td>10.2 ± 0.2</td>
</tr>
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</table>
azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation (ABTS⁺) was produced by reacting a 14-mM concentration of ABTS with an equal volume of 4.9 mM of potassium persulfate (final concentration = 7 mM ABTS in 2.45 mM potassium persulfate).

The mixture was incubated in the dark at room temperature for 12–16 hrs before use. The ABTS⁺ solution was diluted with 5.5 mM phosphate buffer saline (PBS; pH 7.4) to an absorbency of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. An aliquot of 10 µl of plasma or Trolox standard (6-hydroxy-2,5,7,8-tetramethylocroman-2-carboxylic acid) was added to 1 ml of diluted ABTS⁺ solution and the absorbance was read at 30 °C exactly 1 min after the initial mix and up to 6 min afterward. The percentage inhibition of the blank absorbency (0.70 ± 0.02) was calculated for each Trolox standard and plasma sample for the standard reference data and the sample TEAC, respectively.

MDA was assayed by diluting three volumes of the reagent (10.3 mM N-methyl-2-phenylindole, in acetonitrile) with one volume of 100% methanol (HPLC grade) prior to use. MDA (10 mM 1,1,3,3-tetramethoxypropane, in 20 mM Tris-HCl, pH 7.4) standards were prepared to final concentrations of 0 to 20 µM. An aliquot of 10 µl 0.5 M BHT (in acetonitrile) was added to 200 µl of plasma or MDA standard followed by 650 µl of the diluted reagent or 75% acetonitrile/25% methanol for each sample blank. The solution was gently vortexed before adding 150 µl 12 N (37%) HCl. The solution was mixed and incubated at 45 °C for 60 min. Samples were centrifuged at 15,000 g for 10 minutes and absorbance of the supernatant was read at 586 nm.

The plasma content of the acute phase proteins C-reactive protein (CRP), fibrinogen, and alpha-1-antichymotrypsin (α-1-ACT) were measured by double antibody sandwich ELISA. All rabbit antihuman coating and HRP detection antibodies were purchased from DakoCytomation, Glostrup, Denmark. Briefly, a 96-well plate was coated overnight with the capture antibody for each respective acute phase protein. The plate was washed with PBS + 0.1% Tween 20, and 100 µl of standards or plasma were added to the wells in duplicate. Plasma samples were diluted 1:50 (CRP), 1:20,000 (Fibrinogen), and 1:2,000 (α-1-ACT) in PBS + 0.1% Tween 20.

Following 2-hr incubation at room temperature, the plate was washed and 100 µl of the detection antibodies were added to each well and incubated at room temperature for 1 hr in the dark. The plate was washed and the colour was developed by adding 100 µl chromogenic substrate and incubated for 15 min in the dark. Colour development was stopped by adding 100 µl 0.5-M sulphuric acid and the absorbance was read at 490 nm using a 96-well plate reader (Dynex MRX II, West Sussex, U.K.). The proinflammatory cytokines IL-6, IL-8, and TNF-α were measured by ELISA using commercially available CytoSet kits (BioSource, Nivelles, Belgium) in accordance with the manufacturer’s instructions. All samples were analysed in triplicate.

Variables measured in plasma were analysed with and without correction for changes in plasma volume. Measured variables were divided by the albumin ratio of plasma, calculated as the albumin concentration at a given time point divided by the albumin concentration at prerace (Dall et al., 2001). All data are presented without albumin correction unless stated otherwise.
Table 2  Race Performance Measures (mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race time (min/sec)</td>
<td>43:08 ± 0:32</td>
</tr>
<tr>
<td>Ascent time (min/sec)</td>
<td>29:24 ± 0:16</td>
</tr>
<tr>
<td>Descent time (min/sec)</td>
<td>13:44 ± 0:48</td>
</tr>
<tr>
<td>Race mean HR (beats·min⁻¹)</td>
<td>181 ± 1</td>
</tr>
<tr>
<td>Ascent mean HR (beats·min⁻¹)</td>
<td>185 ± 1</td>
</tr>
<tr>
<td>Descent mean HR (beats·min⁻¹)</td>
<td>178 ± 1</td>
</tr>
<tr>
<td>Blood lactate concentration (mmol·L)</td>
<td>8.5 ± 0.4</td>
</tr>
</tbody>
</table>

All results are presented as mean ± SEM. A repeated-measures ANOVA was used to detect changes from prerace through to 48 hrs postrace. Significant changes detected by the ANOVA were analysed using multiple pairwise t-tests with Bonferroni correction for post hoc analysis. Statistical significance was accepted at $p < 0.05$.

**Results**

All subjects successfully completed the hill race. Race performance measures are listed in Table 2. Figure 1 shows the subjective sensations of muscle soreness reported by the runners from prerace through to 48 hrs postrace. All postrace values were significantly greater than prerace values. Signs of DOMS were evident by the intensity of the soreness reported reaching peak values at 48 hrs postrace.

Changes in circulatory leukocytes from prerace through to 48 hrs postrace are shown in Table 3. Total leukocytes increased significantly following the race and remained elevated at 48 hrs postrace. There were no significant changes in neutrophil number, whereas lymphocytes increased immediately after the race before returning to baseline 48 hrs later. The number of circulatory monocytes reached significant peak values at 48 hrs postrace. Neutrophils and monocytes combined (phagocytes) increased significantly after the race and remained elevated at 48 hrs post. The percentage of immature neutrophils in the circulation changed from prerace values of $15 ± 1.4\%$ to $14 ± 1.1\%$ at postrace, and to $34.7\%$ at 48 hrs postrace. The percentage of immature neutrophils at 48 hrs postrace was significantly greater than the percentage observed at both pre- and postrace, $p < 0.05$.

Figure 2 shows the changes in plasma activities of CK, MDA, and TEAC in response to the hill race. CK and MDA showed similar kinetic responses increasing to reach statistically significant peak values at 48 hrs postrace. MDA values seen at 48 hrs postrace were significantly greater than values observed immediately after the race, $p < 0.05$. A significant increase in TEAC was observed immediately after the race and returned to baseline at 48 hrs post, $p < 0.05$. Both CK and MDA results were not influenced by albumin correction. However, the TEAC when
Figure 1. Subjective sensations of muscle soreness, as graded by CR10 scale (Borg, 1998), before and after the hill race (mean ± SEM). The CR10 scale required subjects to grade their sensations of muscle soreness from 0 = no pain (“nothing at all”) relative to 10 = maximum level of pain ever experienced (“extremely strong, maximum pain”). Significant differences: *from prerace and † from postrace, p < 0.05.

Table 3 Total White Blood Cell and Differential Counts (∗10^9/L) at Pre, Post, and 48 hrs Postrace (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Prerace</th>
<th>Postrace</th>
<th>48 hrs Postrace</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>4.2 ± 0.2</td>
<td>8.2 ± 0.2*</td>
<td>6.2 ± 0.22*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.5 ± 0.2</td>
<td>4.0 ± 0.13</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.5 ± 0.06</td>
<td>3.5 ± 0.2*</td>
<td>1.9 ± 0.1†</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.26 ± 0.01</td>
<td>0.65 ± 0.06</td>
<td>1.1 ± 0.08*</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.10 ± 0.01</td>
<td>0.08 ± 0.0</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Phagocytes</td>
<td>2.8 ± 0.16</td>
<td>4.7 ± 0.13*</td>
<td>4.1 ± 0.15*</td>
</tr>
</tbody>
</table>

Note: Statistically significant difference: *from prerace values; † from postrace values, p < 0.05.

corrected for albumin tended to decrease from prerace values of 2.23 ± 0.01 mM/L to 2.01 ± 0.04 mM/L at postrace and 1.93 ± 0.03 mM/L at 48 hrs postrace. Values at 48 hrs postrace relative to prerace values were close to being statistically significant in accordance with Bonferroni correction.

The plasma activity of the acute phase proteins CRP, fibrinogen, and α-1-ACT are presented in Figure 3. CRP and α-1-ACT showed no significant changes in response to the hill race. In contrast to what might be expected, fibrinogen activity decreased in a linear fashion, falling to statistically significant levels at 48 hrs
Figure 2. Alterations in plasma levels of creatine kinase (CK), malondialdehyde (MDA), and trolox equivalent antioxidant capacity (TEAC) following the hill race (mean ± SEM). Significant differences: * from prerace and † from postrace, $p < 0.05$. 
postrace. This result, in conjunction with the CRP and α-1-ACT responses, was not affected by albumin correction. Albumin correction, however, did result in a significant reduction in fibrinogen activity at postrace, \( p < 0.05 \).

Changes in the plasma levels of IL-8 and TNF-α in response to the hill race are shown in Figure 4. IL-8 was significantly increased immediately after the hill race. Values observed at 48 hrs postrace were significantly reduced from postrace values but remained elevated from prerace values. No significant plasma changes were observed for TNF-α, \( p > 0.05 \). The proinflammatory cytokine, IL-6, was not detected in any of the samples (data not shown).

**Discussion**

This study examined the effects of a competitive hill race on markers of muscle damage, lipid peroxidation, total antioxidant status, and the acute phase response. It was found that the hill race induced significant levels of DOMS and muscular damage, as subjective pain reports and plasma CK activity reached peak values at 48 hrs postrace. Plasma MDA, IL-8, circulatory monocytes, and total leukocytes were also elevated at this time. IL-8 and the free-radical scavenging ability of plasma increased immediately after the race. Despite the reports of muscle damage and soreness, no evidence of an acute phase response was observed, as the plasma glycoproteins CRP, α-1-ACT, and fibrinogen did not increase following the race.

The current findings corroborate previous studies reporting an increased number of phagocytic cells in the circulation during times of muscle damage (Pizza et al., 1995; Smith et al., 1998). The increase in plasma IL-8 immediately after the race may have acted as a chemotactic signal to stimulate the recruitment of neutro-
phils to the newly damaged tissue. IL-8 remained elevated 48 hrs later; however, the increase in circulatory monocytes observed at that time is likely to be explained by other chemotactic factors, as IL-8 is predominantly involved in neutrophil trafficking (Geiser et al., 1993). Although there was no neutrophil elevation in the peripheral blood at this time, it is possible that these cells had already infiltrated the tissue. Neutrophils are the first cells to respond to inflammation, have a half-life of less than 10 hrs, and reach peak values 1 to 2 hrs in blood and up to 6 hrs in muscle following injury (Pizza et al., 1995; Tidball, 1995). The greater number of immature neutrophils observed at 48 hrs postrace supports this. The loss of mature neutrophils to tissue infiltration or possibly cell death may have prompted a bone marrow response to release additional neutrophils into the circulation.

The hill race induced a significant elevation in MDA, which is an end product of lipid peroxidation. This manifestation of oxidative stress has been linked to the pathogenesis of several disease states such as atherosclerosis and Parkinson's disease (Mylonas and Kouretas, 1999). Although lipid peroxidation marked in the form of MDA can represent damage from all body cells, it is likely to be of skeletal muscle origin when exercise is involved. Strenuous exercise markedly increases the rate of oxygen uptake in active skeletal muscle by as much as 100- to 200-fold, leading to an increase in the generation of ROS (Davies et al., 1982). Davies et al. reported that the levels of ROS in the muscle doubled after exhaustive exercise, leading to the proposal that active muscle fibres may be susceptible to oxidative damage.

It is possible that infiltrating phagocytes may be partially responsible for the production of these damaging free radicals. Neutrophils and monocytes migrate from the blood to the damaged muscle tissue to aid in repair and regeneration via

![Figure 4. Changes in plasma activities of the cytokines interleukin-8 (IL-8) and TNF-α (mean ± SEM). Significant differences: *from prerace and †from postrace, p < 0.05.](image)
phagocytosis. These cells have the potential to release superoxide anions, hydrogen peroxide, and hypochlorous acid, causing subsequent damage to the local tissue in an action known as the “oxidative burst” (Mylonas and Kouretas, 1999). In the present study, plasma MDA and CK increased over time in a similar manner, suggesting a possible relationship between muscle damage and the presence of ROS. Similar observations have been reported following a bout of sustained endurance running and downhill treadmill running (Goodman et al., 1997; Kanter et al., 1988; Maughan et al., 1989).

Interestingly, other studies have failed to find an increase in lipid peroxidation markers despite clear evidence of muscle damage. Child et al. (1999) found no change in muscle or plasma MDA to accompany the increase in serum CK observed up to 5 days after a bout of 70 maximal eccentric contractions of the knee extensors. This suggests that lipid peroxidation may only accompany markers of muscle damage following activity that requires a sustained level of oxygen uptake using large muscle groups, as opposed to localised resistance exercises that are more brief and anaerobic in nature. Thus, electron spillover in the mitochondria, which accompanies increases in aerobic metabolism, may be the primary site responsible for generating the ROS that damage lipid cell membranes in an exercise context. The source of ROS production leading to exercise-induced lipid peroxidation and its association with muscle damage requires further examination.

The results of the present study are consistent with others which found a significant elevation in total antioxidant capacity immediately following a bout of sustained endurance exercise (Child et al., 1998; Liu et al., 1999). There was no significant increase in plasma MDA immediately after the hill race when the TEAC was elevated, suggesting a possible protective role of plasma antioxidants against exercise-induced lipid peroxidation. Although other exercise studies have reported an elevation of lipid peroxidation markers in serum despite a concomitant increase in total antioxidant status (Child et al., 1998), it is unclear at present whether the extent of lipid peroxidation would have been greater had there been no increase in the ability to scavenge free radicals in plasma.

It is important to note, however, that an almost significant reverse effect was seen when the TEAC was corrected for albumin, suggesting that the increase in plasma antioxidant status observed after the hill race may simply be a result of haemoconcentration as opposed to the exercise per se. In contrast, other studies using haemoglobin and haematocrit to estimate and correct for changes in plasma volume have still reported an increased antioxidant status in response to exercise (Child et al., 1998). However, it is difficult to obtain accurate estimates of plasma volume change because measurements of haemoglobin, haematocrit, total protein, and serum albumin are based on a few key assumptions and thus have limitations (Johansen et al., 1998; Lundvall and Lindgren, 1998). For this reason, and the fact that it is the actual concentration of a variable to which the body responds (Mastaloudis et al., 2001), we decided to present the data without correcting for estimated plasma volume changes. Until a reliable method is devised in order to accurately estimate exercise-induced fluid shifts, it will be difficult to establish whether subtle changes in TEAC, and indeed other biological variables, have any physiological significance following exercise.
No evidence of an acute phase response was observed following the hill race despite an increase in CK and muscle soreness. This may be explained by the failure of the hill race to induce significant elevations in plasma of TNF-α, as this proinflammatory cytokine is involved in the release of acute phase proteins from the liver (Mackinnon, 1999). Similarly, Nosaka and Clarkson (1996) reported no change in plasma TNF-α and CRP despite elevated CK levels following eccentric muscle contractions. In addition, Smith et al. (2000) and Suzuki et al. (2000) failed to find any significant changes in plasma TNF-α despite evidence of muscle damage. It is plausible then that localised inflammation of skeletal muscle following certain forms of exercise may differ from the inflammatory response caused by other bodily insults such as bacterial infection and tissue injury of a different form (Nosaka and Clarkson, 1996).

It is possible that the duration of this particular hill race was insufficient to cause the levels of tissue damage that would trigger an acute phase response by the immune system. Serum CRP levels have been found to increase following a 160-km triathlon, a 42-km marathon, and 2 and 3 hours of treadmill running (65–85% velocity of lactate threshold) in the subsequent days of recovery (Liesen et al., 1977; Taylor et al., 1987; Weight et al., 1991). However, Durstine et al. (2001) found that neither short- nor long-duration exercise altered the expression of the acute phase protein lipoprotein(a) despite increases in CK activity following both exercise sessions.

Additional more recent studies have questioned whether and to what extent there exists an acute phase response following exercise. Hubinger et al. (1997) found no increase in CRP immediately or up to 7 days after level or downhill running, despite increased CK levels following the eccentrically biased protocol. Pyne et al. (1997) made similar observations using uphill, downhill, and near level running, while we failed to find a significant increase in CRP or α-1-ACT following a marathon race (unpublished data).

The lack of an exercise-induced alteration of α-1-ACT in the present study has been echoed elsewhere. Fallon (2001) found no change in α-1-ACT during and immediately following a 6-day ultraendurance event, although CRP levels increased from the first day onward. Thus, alterations of plasma α-1-ACT activity may only occur in cases of chronic inflammation and over a longer time period (Thompson et al., 1992). Advances in the methods used to measure acute phase proteins, the protein measured, the protocols employed, and the training status of the subjects examined may explain some of the discrepancies between studies. Mattusch et al. (2000) found baseline CRP levels to decline following a 9-month training program in a group of subjects preparing for a marathon. Liesen et al. (1977) showed that a 9-week training program reduced the CRP expression 1 and 3 days following a 2-hr treadmill run at 90% individual anaerobic threshold. This may suggest a systemic anti-inflammatory effect in response to regular training. Five of the 7 subjects in the present study were habitual hill runners and therefore likely to be accustomed to the stresses of the race.

The significant reduction in plasma fibrinogen expression seen at 48 hrs postrace was unexpected. Studies on the effects of acute exercise on plasma fibrinogen activity have been inconsistent (El-Sayed et al., 2000). The reduction of
plasma fibrinogen concentration observed in the present study may be attributed to an enhanced rate of fibrinogen catabolism, removal from the plasma to the interstitial spaces, or increased fibrin clot formation (El-Sayed et al., 1999). Thus a more frequent sampling strategy may be required in order to find the peak time of fibrinogen expression and its biological significance following muscle-damaging exercise protocols. Despite previous reports of IL-6 production and exercise (Nieman et al., 2003; Steensberg et al., 2001; Vassilakopoulos et al., 2003), no circulating IL-6 activity was detected in the plasma of the hill runners participating in this study.

Other studies have reported significant increases in plasma IL-6 following exercise to levels above and below 8 pg/ml (Nieman et al., 2003; Steensberg et al., 2001; Vassilakopoulos et al., 2003). As the detection limit of the assay used in the present study was 7.8 pg/ml, it is possible that any increase in plasma IL-6 activity following the hill race occurred below this limit of detection.

In conclusion, the hill race induced significant levels of oxidative stress, muscle pain, and muscle damage to the subjects involved. Conversely, there was no evidence of an acute phase immune response, which may be explained by the lack of a proinflammatory cytokine response. The intensity, duration, and mode of exercise may influence the activation of an acute phase response that mirrors the inflammatory response seen following various other bodily insults including bacterial infections and surgical trauma. Future studies should examine the link between muscle damage, oxidative stress, and the acute inflammatory response following hill races of longer duration with larger eccentric components.

Acknowledgments
The authors acknowledge the laboratory assistance of Dr. D.M. Brown and Ms. S.J. Brown.

References


*Received July 13, 2004; accepted in final form September 13, 2004.*