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# Peripheral blood leucocyte functional responses to acute eccentric exercise in humans are influenced by systemic stress, but not by exercise-induced muscle damage

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## A B S T R A C T

The effects of comparable lower-limb eccentric exercise that induces high (bench-stepping; STEP) and low (repeated eccentric muscle action; ECC) systemic stress on neutrophil and monocyte phagocytic and respiratory burst activity, and activation antigen (CD11b, CD66b, CD64) expression, were compared in recreationally active subjects (20–37 years old). Leucocyte responses were determined before and 4, 24, 48 and 72 h after exercise using whole-blood flow cytometry. Serum creatine kinase (CK) activity and perceived muscle soreness [delayed-onset muscle soreness (DOMS)] were assessed at the same time points up to 96 h; as a control, measurements were taken during 5 days of rest. DOMS in quadriceps and contralateral triceps surae peaked 24–72 h after STEP ($P < 0.05$) and 48–72 h after ECC ($P < 0.05$), whereas serum CK activity (mean $\pm$ S.E.M.) was only higher than baseline after ECC ($15\,123 \pm 3488$ at 96 h compared with $115 \pm 29$ units $\cdot$ l$^{-1}$ pre-exercise; $P < 0.01$). The total leucocyte count increased from $(5.4 \pm 0.4) \times 10^9 \cdot$ l$^{-1}$ and $(5.7 \pm 0.5) \times 10^9 \cdot$ l$^{-1}$ at baseline to $(7.6 \pm 0.5) \times 10^9 \cdot$ l$^{-1}$ and $(7.0 \pm 0.5) \times 10^9 \cdot$ l$^{-1}$ at 4 h after STEP and ECC respectively; this was largely attributable to changes in the neutrophil count ($P < 0.05$). The proportion of neutrophils undergoing phagocytosis and respiratory burst was unchanged 4 h after ECC and STEP, which, given the increase in neutrophil count after exercise, would suggest an overall improvement in systemic neutrophil microbicidal potential. The intensity of neutrophil ($P = 0.01$) and monocyte ($P < 0.05$) phagocytosis and neutrophil respiratory burst responses ($P < 0.05$) was only increased 24 h after STEP, whereas no changes in these measures were observed after ECC. Activation antigen expression was unchanged in all groups. These findings suggest that systemic stress evoked during an acute bout of eccentric exercise has a greater influence on subsequent leucocyte functional responses than the degree of muscle damage induced.

## INTRODUCTION

Interest in the immunomodulatory effects of exercise has been prompted by evidence that chronic strenuous exercise training can have detrimental effects on immune function in athletes [1], whereas moderate physical activity can boost immune function [2]. A growing number of studies are focusing on the functional responsiveness of leucocyte subpopulations in peripheral blood, in an attempt to provide improved insight into the

effects of exercise-induced immunomodulatory changes on the ability of the immune system to respond to infectious disease.

Exercise that involves a substantial proportion of eccentric muscular activity, in which the active muscles are forcibly lengthened, increases the degree of ultrastructural injury within myofibres [3,4], and this can evoke a significant local inflammatory response [5]. This is characterized by leucocyte infiltration into exercise-damaged tissue [6,7], an increased local production of inflammatory mediators such as cytokines, prostaglandins and leukotrienes [8–10], and a systemic acute-phase response [11]. The inflammatory response to mechanical trauma could account for the progressive release of muscle-specific enzymes into the serum and feelings of soreness that are often observed in untrained subjects in the days after eccentric exercise [5,12].

Neutrophils and monocytes act as the first line of defence against potentially infectious agents, and they also have an important role in the post-exercise inflammatory response to muscle fibre injury [13–15]. The infiltration of neutrophils and monocytes into exercise-damaged muscle as part of the repair/regeneration process is likely to be facilitated by an increased expression of surface adhesion molecules. Adhesion molecules play an important role in leucocyte migration from the circulation to the site of inflammation. Eccentric exercise evoking muscle damage in untrained subjects has been reported to increase the expression of CD11b and CD64 on peripheral blood neutrophils and monocytes [16]. Since there was evidence of a diminished activation response for individuals who were less susceptible to eccentric exercise-induced injury [16], the implication was that leucocyte activation was associated with the degree of exercise-induced muscle damage. Changes in adhesion molecule expression and the involvement of neutrophils and monocytes in post-exercise inflammatory responses might alter the microbicidal potential of these immune cells in peripheral blood.

The functional responsiveness of neutrophils and monocytes in relation to changes in activation antigen expression following eccentric exercise has not been studied previously. Furthermore, no studies have compared the responses between eccentric exercise models that evoke different degrees of systemic stress. The hypothesis of the present study was that changes in activation antigen expression (and immune cell function) are linked to the degree of exercise-induced muscle damage evoked by exercise, irrespective of the level of systemic stress encountered. To test this hypothesis, we compared the responses to serial repetitions of eccentric muscular work performed on an isokinetic dynamometer with those induced following a 40 min bout of intense bench-stepping exercise. Serial repetitions of eccentric muscle actions induce a substantial increase in serum creatine kinase (CK) activity and significant muscle

soreness in the muscle groups involved [12], despite evoking a relatively low level of systemic stress. Bench-stepping, on the other hand, can be used to subject the same muscle groups to eccentric damage, but is a form of exercise that evokes a considerably higher level of systemic (cardiovascular and metabolic) stress.

## MATERIALS AND METHODS

### Participants

Participants were recreationally active, healthy volunteers (age 20–37 years) who regularly undertook two or three exercise sessions per week (involving weight-bearing activity). Subjects were instructed not to perform any exercise training from 48 h before the study until the end of the blood sampling period. Eight participants performed a bout of eccentric exercise involving the quadriceps and triceps surae (ECC), with responses to ECC compared with control measurements taken during 5 days of rest. The order of control and experimental conditions was counterbalanced. A second group of eight age-matched recreationally active participants performed a bout of bench-stepping exercise (STEP), which induces soreness in the same muscle groups [17].

Approval for this study was given by the South Sheffield Research Ethics Committee, and subjects gave their written informed consent according to University guidelines.

### Exercise models

#### ECC

Exercise was performed on an isokinetic dynamometer (Biodex System 3; Biodex Medical), at an angular velocity of $1.05$ rad$\cdot$s$^{-1}$ (quadriceps) or $0.35$ rad$\cdot$s$^{-1}$ (triceps surae), with a 10–12 s rest period between each muscle action. This device consists of a lever arm that rotates around a fixed axis of rotation at the pre-set angular velocity. The limb segment is strapped to the lever arm so that the respective joint centre (i.e. knee, ankle) coincides with the dynamometer axis of rotation, and the participant attempts to resist lever arm motion, thereby performing an eccentric muscle action. A bout of 50 maximum voluntary eccentric muscle actions was performed with the subject in the supine position, using the quadriceps of a randomly selected leg. The exercise bout was split into two sets of 25 repetitions, with a 5 min interpolated rest period between sets. Electrical stimulation (unidirectional 0.5 ms square-wave pulse at 50 Hz), of sufficient intensity to elicit $\sim 25\%$ of the maximum voluntary isometric torque, was superimposed on to the voluntary muscle actions via skin-surface electrodes. Six of the participants performed an additional 50

eccentric muscle actions using a similar protocol for the contralateral triceps surae.

## STEP

The STEP model was based on that used previously by Newham et al. [17] to induce exercise-induced muscle soreness, and comprised 40 min of intense bench-stepping exercise at a rate of 20 steps · min$^{-1}$ using a constant lead leg (chosen randomly). The bench height was set at 0.45 m and the step cadence was controlled precisely by a metronome (verified by observers). This exercise model subjects the triceps surae of the lead leg and the quadriceps of the trail leg to eccentric muscle actions, and evokes evidence of ultrastructural muscle fibre injury in these muscle groups [17]. Heart rate was measured continuously using a heart rate monitor (Sport Tester PE-3000; Polar Electro) and was recorded during the final 1 min of exercise.

## Blood sampling

Blood samples for leucocyte analysis were drawn from an antecubital vein immediately before and 4, 24, 48 and 72 h after both bouts of exercise, and at precisely the same time points in the non-exercise control condition. With the exception of the 4 h sample, all blood was drawn between 08.00 and 08.30 hours. An extra blood sample was taken at 96 h for serum CK analysis, as the increase in serum CK activity after the ECC and STEP protocols often peaks 4–5 days after exercise [17]. Blood was collected into Vacutainer tubes (Becton Dickinson Ltd, Oxford, U.K.) that contained no anticoagulant for serum CK analysis, $K_3$EDTA for leucocyte counts and activation antigen expression, and lithium heparin for leucocyte functional assays by flow cytometry. Serum samples were stored at −80 °C until analysis.

## Criterion measures

### Muscle soreness and serum CK activity

Perceived muscle soreness [delayed-onset muscle soreness (DOMS)] was assessed using a muscle soreness questionnaire that comprised a 10-point soreness scale for six regions of the anterior/posterior thigh and calf. The scale ranged from 1 (normal) to 10 (very, very sore), and the mean ratings for the muscle groups subjected to eccentric exercise (quadriceps and contralateral triceps surae) provided an overall assessment of soreness encountered in these regions. Serum CK activity was assayed using the method of Szasz et al. [18] with an enzymic kit (no. 47-10; Sigma, Poole, Dorset, U.K.); the interassay coefficient of variation was 5.6%.

### Leucocyte analysis

Differential leucocyte counts and other haematological parameters were measured within 6 h of blood sampling using a Coulter STKS automated haematological analyser (Beckman Coulter U.K. Ltd, High Wycombe, U.K.) which was calibrated on a daily basis.

## Leucocyte microbicidal function and activation antigen expression

Neutrophil and monocyte phagocytosis and respiratory burst responses were measured using commercial kits (Phagotest® and Phagoburst®; Orpegen, Heidelberg, Germany). The expression of CD11b, CD64 and CD66b was assessed using a modified three-colour whole-blood flow cytometry technique [19], using FITC-, Cychrome- and phycoerythrin-conjugated monoclonal antibodies. CD11b (Mac-1; complement receptor 3) is the α subunit of the CD11–CD18 heterodimeric complex and plays a key role in localization at inflammatory sites; its expression is up-regulated rapidly upon leucocyte activation [20]. CD64 (FcγR1) is expressed on monocytes and activated granulocytes; it mediates antibody-dependent cytotoxicity and triggers phagocytosis, superoxide production and degranulation [21–23]. CD66b is a member of the carcinoembryonic-like glycoprotein family and is expressed on peripheral blood granulocytes, but not lymphocytes or monocytes [24]. Negative control tubes were stained using appropriate FITC-, Cychrome- and phycoerythrin-conjugated isotype-matched non-reactive negative controls (Becton Dickinson, Serotec Ltd). All samples were stained within 2 h of collection to avoid *ex vivo* up-regulation of CD11b expression [19].

## Flow cytometric analysis

Flow cytometric analysis was performed on a Becton Dickinson FACSort™ flow cytometer (BD Biosciences, Oxford, U.K.). For the functional assays, both the percentage of positively stained cells (cells exhibiting phagocytic/respiratory burst response) and the median channel fluorescence intensity (which correlates with the number of ingested bacteria per cell, or the intensity of the respiratory burst response) were used as the criterion measures of functional status (interassay coefficients of variation were < 2% and < 3% respectively). For analysis of activation antigen expression, the expression of CD11b, CD64 and CD66b by neutrophils ($10^4$ cells) and of CD11b and CD64 by monocytes ($5 × 10^3$ cells) was determined. The percentage of cells expressing each antigen and the median channel fluorescence intensity of expression (which correlates with antigen density) were used as the criterion measures of activation antigen expression (interassay coefficients of variation were < 2% and < 4% respectively).

## Data analysis

Differential leucocyte counts were corrected for changes in plasma volume using the method of Dill and Costill [25]. Differential leucocyte counts, leucocyte functional data and activation antigen expression, and serum CK

activity were analysed using repeated-measures ANOVA. Non-parametric muscle soreness data were analysed using the Wilcoxon signed-ranks test (paired data) and the Mann–Whitney $U$ test (unpaired data). All values are expressed as means$\pm$S.E.M., and differences were considered to be of statistical significance at $P < 0.05$. Statistical analyses were performed using SPSS for Windows (SPSS UK Ltd, Woking, U.K.).

## RESULTS

### Heart rate response to STEP protocol
The STEP protocol was very demanding on the cardiovascular system, even for our recreationally active subjects. Heart rate was measured in the final 1 min of the STEP protocol and was used as an index of cardiovascular stress. The mean$\pm$S.E.M. heart rate in the final 1 min of exercise was $176 \pm 10$ beats $\cdot$ min$^{-1}$, corresponding to a heart rate of $91 \pm 3$% of the predicted maximum (based on $220 -$ age for prediction of maximum heart rate).

### Muscle soreness and serum CK activity
The DOMS score peaked 48 h ($P < 0.05$; Wilcoxon test) and 48–72 h ($P < 0.05$; Wilcoxon test) after ECC in the quadriceps and contralateral triceps surae respectively (Table 1). DOMS in the same muscle groups was also observed after STEP ($P < 0.05$), peaking 24–72 h after exercise (Table 1). The degree of soreness experienced in

**Table 1    Muscle soreness ratings after STEP and ECC**
Data are presented as means, with ranges in parentheses. Statistical significance: $^*P < 0.05$ compared with baseline; $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ between STEP and ECC conditions at that time point.

| | DOMS score | | | |
| --- | --- | --- | --- | --- |
| | Pre-exercise | 24 h | 48 h | 72 h |
| Quadriceps | | | | |
| STEP | 1 (1–1) | 3 (1–6)* | 3 (2–5)* | 2 (1–3) |
| ECC | 1 (1–1) | 5 (2–7)* | 6 (2–9)*† | 5 (2–10)*†† |
| Triceps surae | | | | |
| STEP | 1 (1–1) | 4 (1–6)* | 4 (1–7)* | 4 (1–7)* |
| ECC | 1 (1–1) | 5 (1–7)* | 6 (2–9)* | 6 (2–10)* |

**Table 2    Serum CK activity after STEP, after ECC and in the control condition (CON)**
Data are presented as means$\pm$S.E.M. Statistical significance: $^*P < 0.05$, $^{**}P < 0.01$ compared with CON and STEP conditions.

| | CK level (units $\cdot$ l$^{-1}$) | | | | |
| --- | --- | --- | --- | --- | --- |
| Condition | Pre-exercise | 24 h | 48 h | 72 h | 96 h |
| STEP | 146 $\pm$ 59 | 161 $\pm$ 66 | 106 $\pm$ 43 | 83 $\pm$ 34 | 76 $\pm$ 31 |
| ECC | 115 $\pm$ 29 | 2776 $\pm$ 1597 | 8368 $\pm$ 3080* | 14 673 $\pm$ 3979** | 15 123 $\pm$ 3488** |
| CON | 117 $\pm$ 41 | 90 $\pm$ 32 | 82 $\pm$ 30 | 109 $\pm$ 38 | 117 $\pm$ 40 |

**Table 3    Peripheral blood leucocyte counts after STEP, after ECC and in the control condition (CON)**
Data are presented as means$\pm$S.E.M. Statistical significance: $^*P < 0.05$ compared with baseline.

| | Cell count ($\times 10^9 \cdot$ l$^{-1}$) | | | | |
| --- | --- | --- | --- | --- | --- |
| Condition | Pre-exercise | 4 h | 24 h | 48 h | 72 h |
| Total leucocytes | | | | | |
| STEP | 5.4 $\pm$ 0.4 | 7.6 $\pm$ 0.5* | 5.6 $\pm$ 0.4 | 6.0 $\pm$ 0.4 | 5.8 $\pm$ 0.3 |
| ECC | 5.7 $\pm$ 0.5 | 7.0 $\pm$ 0.5* | 6.1 $\pm$ 0.6 | 6.3 $\pm$ 0.5 | 6.0 $\pm$ 0.4 |
| CON | 6.1 $\pm$ 0.6 | 6.1 $\pm$ 0.4 | 5.9 $\pm$ 0.3 | 5.6 $\pm$ 0.4 | 5.9 $\pm$ 0.4 |
| Neutrophils | | | | | |
| STEP | 2.9 $\pm$ 0.3 | 5.1 $\pm$ 0.4* | 3.0 $\pm$ 0.3 | 3.2 $\pm$ 0.3 | 3.2 $\pm$ 0.2 |
| ECC | 2.9 $\pm$ 0.4 | 4.3 $\pm$ 0.5* | 3.2 $\pm$ 0.3 | 3.4 $\pm$ 0.4 | 3.0 $\pm$ 0.3 |
| CON | 3.1 $\pm$ 0.5 | 3.4 $\pm$ 0.4 | 3.1 $\pm$ 0.3 | 2.8 $\pm$ 0.3 | 2.9 $\pm$ 0.3 |
| Monocytes | | | | | |
| STEP | 0.4 $\pm$ 0.0 | 0.5 $\pm$ 0.1 | 0.5 $\pm$ 0.1 | 0.5 $\pm$ 0.0 | 0.5 $\pm$ 0.1 |
| ECC | 0.5 $\pm$ 0.0 | 0.5 $\pm$ 0.0 | 0.5 $\pm$ 0.0 | 0.5 $\pm$ 0.0 | 0.5 $\pm$ 0.0 |
| CON | 0.5 $\pm$ 0.0 | 0.4 $\pm$ 0.0 | 0.4 $\pm$ 0.0 | 0.4 $\pm$ 0.0 | 0.5 $\pm$ 0.0 |

the quadriceps muscle group was more marked after ECC ($P < 0.01$). Serum CK activity increased progressively from a mean$\pm$S.E.M. baseline value of $115 \pm 29$ units $\cdot$ l$^{-1}$ to $15 123 \pm 3488$ units $\cdot$ l$^{-1}$ ($P < 0.01$; ANOVA) 96 h after ECC, whereas no change in serum CK activity from baseline values was observed after STEP or in the control condition (Table 2).

### Leucocyte counts
The total leucocyte count was increased at 4 h after both ECC and STEP ($P < 0.05$), but had returned to pre-exercise levels after 24 h (Table 3). In both cases, the increase in total leucocyte count was largely attributable to changes in the number of circulating neutrophils ($P < 0.05$). Neither exercise protocol had any effect on the peripheral blood monocyte count (Table 3), and there was no change in leucocyte count at any time point in the control condition.

### Leucocyte phagocytosis and respiratory burst responses
There was no change in the proportion of neutrophils that exhibited phagocytic or respiratory burst responses
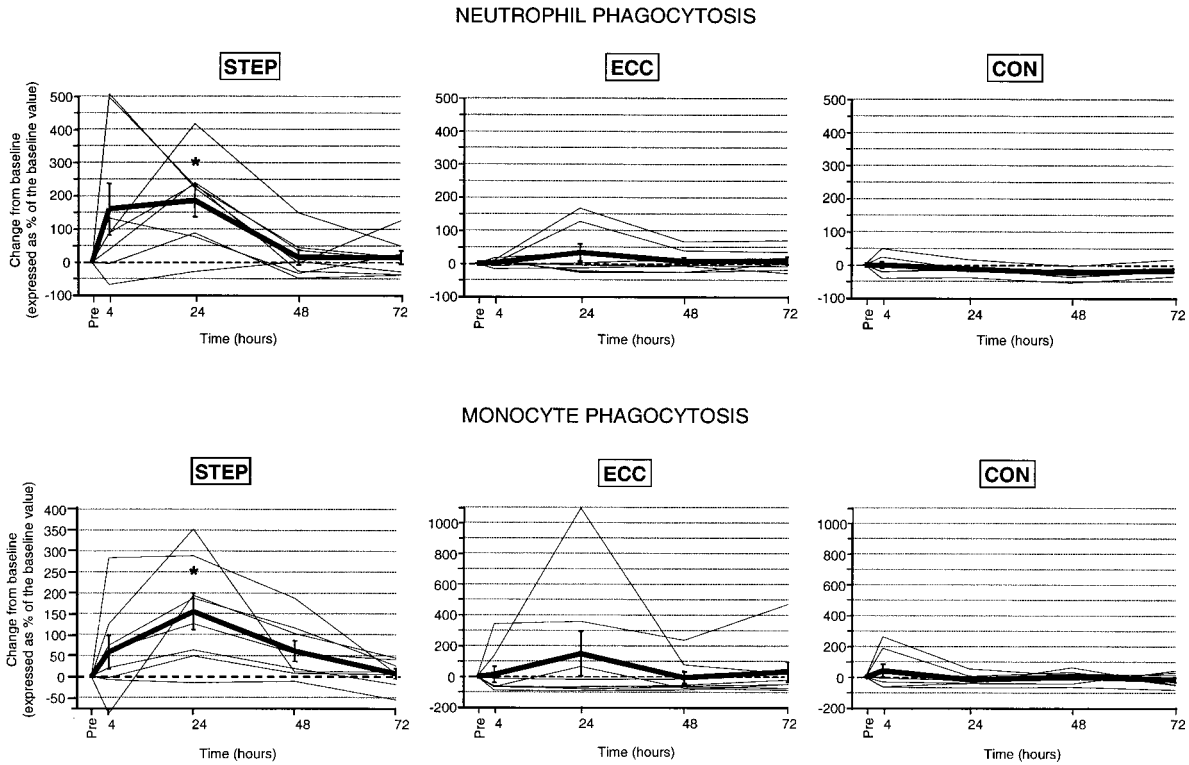
## NEUTROPHIL PHAGOCYTOSIS



## MONOCYTE PHAGOCYTOSIS



**Figure 1 Neutrophil and monocyte phagocytosis after STEP, after ECC and in the control condition (CON)**

Data are presented as individual responses (thin lines) and as mean ± S.E.M. responses (thick lines) for the group; *P < 0.05 compared with baseline.

## NEUTROPHIL RESPIRATORY BURST


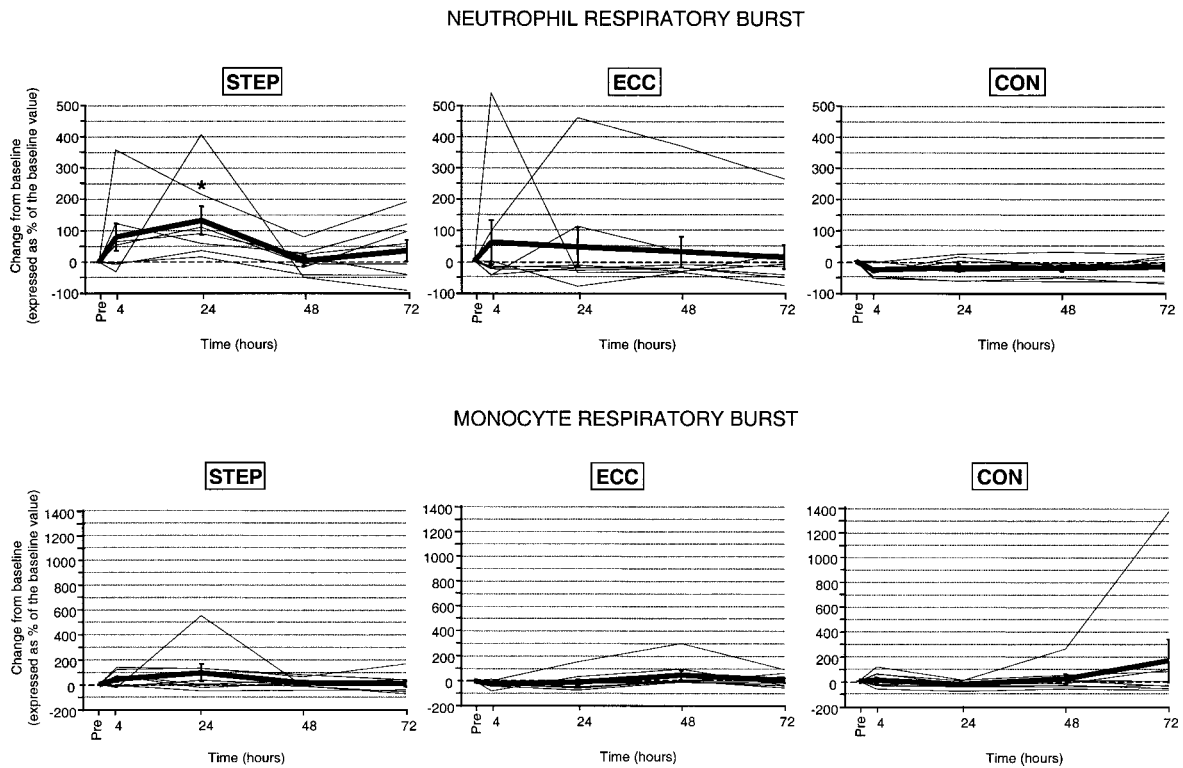
## MONOCYTE RESPIRATORY BURST



**Figure 2 Neutrophil and monocyte respiratory burst after STEP, after ECC and in the control condition (CON)**

Data are presented as individual responses (thin lines) and as mean ± S.E.M. responses (thick lines) for the group; *P < 0.05 compared with baseline.
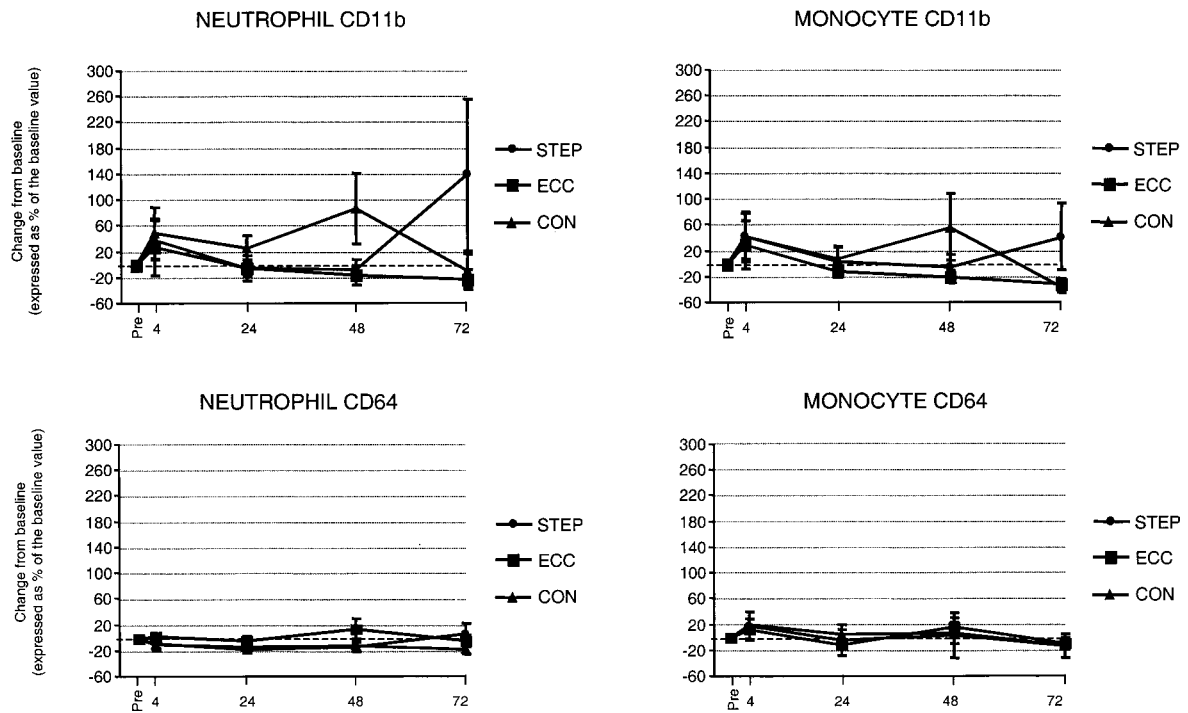
**Figure 3    Neutrophil and monocyte activation antigen expression after STEP, after ECC and in the control condition (CON)**
Data are presented as mean $\pm$ S.E.M. responses.

at any time point after either exercise bout, and 85–95% of neutrophils were functionally responsive throughout the time course of the study. For monocytes, the proportion of cells that exhibited phagocytic (40–50%) and respiratory burst (30–40%) responses was lower than that observed for neutrophils, and this proportion remained unchanged following both exercise bouts and in the control condition.

At the cellular level, there was an increase in the intensity of neutrophil phagocytosis at 24 h after STEP ($P = 0.01$); this was not observed after ECC or in the control condition (Figure 1). This change was mirrored by a concomitant increase in the neutrophil respiratory burst response ($P < 0.05$) at the same time point (Figure 2). A potentiation of monocyte phagocytosis was also observed at the 24 h time point ($P < 0.05$) after STEP, but not after ECC or in the control condition (Figure 1). The intensity of the monocyte respiratory burst response was unchanged after both exercise bouts and in the control condition (Figure 2).

### Leucocyte activation antigen expression
The proportion of peripheral blood neutrophils and monocytes expressing each activation antigen was unchanged after both bouts of exercise and in the control condition. Neutrophils constitutively express CD66b, and the majority of peripheral blood neutrophils were also expressing CD11b. However, the proportion of

neutrophils expressing CD64 was lower, generally in the range 30–40%. Monocytes do not express CD66b, but, as observed for neutrophils, a high proportion of the cells (generally > 80%) were expressing CD11b at all time points. CD64 was also expressed in the majority of peripheral blood monocytes. The intensity of all activation antigens on peripheral blood neutrophils and monocytes was unchanged after both bouts of exercise and in the control condition (Figure 3).

## DISCUSSION

The present study compared phagocytic and respiratory burst responses and activation antigen expression in peripheral blood neutrophils and monocytes before and after acute bouts of exercise associated with low and high systemic stress. Both exercise protocols subjected the quadriceps and contralateral triceps surae to eccentric exercise; however, the level of metabolic and cardiovascular stress evoked by the STEP protocol was considerably greater than that evoked by the ECC protocol. The magnitude of changes in the indirect correlates of exercise-induced muscle damage suggests that a greater degree of temporary, repairable ultrastructural injury to muscle fibres was induced by ECC.

Bench stepping has been shown previously to evoke extensive muscle fibre damage, the release of muscle-

specific enzymes into the serum and significant post-exercise muscle soreness [11,17], as well as a rapid acute-phase response in untrained individuals [11]. In the present study, STEP evoked only moderate post-exercise muscle soreness without any increase in serum CK activity in our recreationally active participants. This attenuated response could reflect the training status of our participants, as trained skeletal muscle is less susceptible to eccentric-induced injury [26].

The total leucocyte count was increased at 4 h after ECC and STEP, but had returned to pre-exercise levels after 24 h. In both instances, the increase in total leucocyte count was largely attributable to changes in the numbers of circulating neutrophils. Given that there was no change in the proportion of neutrophils (85–95 %) that exhibited phagocytic or respiratory burst responses at any time point after either exercise bout, the increase in the number of circulating neutrophils indicates an overall improvement in systemic neutrophil microbicidal potential at this time point.

The increase in the peripheral blood neutrophil count 4 h after STEP was probably attributable to the secondary neutrophil leucocytosis associated with non-specific exercise stress that commonly occurs within 1–3 h of the cessation of intense/prolonged physical exertion. This response is probably due to the effects of elevated plasma cortisol levels on bone marrow [27,28]. It is notable that although a 2-fold increase in neutrophil count was detected 4 h after STEP in the present study, this response was lower than that observed previously after a similar exercise stimulus in untrained subjects [11]. The difference could be explained by a blunted cortisol response to the STEP protocol in our recreationally active participants, or a lower degree of muscle fibre injury, resulting in an attenuated inflammatory response after STEP. The increase in peripheral blood neutrophil count following ECC, on the other hand, could have been indicative of an inflammatory response to muscle fibre injury. This is indicated by the magnitude of the change in the indirect correlates of exercise-induced muscle damage.

At the cellular level, there was an increase in the intensity of the neutrophil phagocytosis and respiratory burst responses 24 h after STEP that was not observed after ECC. A potentiation of monocyte phagocytosis was also observed at the 24 h time point after STEP, but not after ECC. The increase in the intensity of leucocyte functional responses 24 h after STEP could have been induced through the effects of certain cytokines. Studies have demonstrated that strenuous exercise induces the release of interleukin-1 (IL-1) and other cytokines, including IL-6 and tumour necrosis factor α [10,29], that prime the various pathways that contribute to the activation of NADPH oxidase and can amplify immune responses in a concentration-dependent manner [30]. Although exercise-induced increases in circulating levels of IL-6 have usually returned to baseline within 24 h

[9,10], at the tissue level, the release of IL-1β follows a protracted time course after exercise that has a large eccentric component and is associated with high systemic stress [6]. Furthermore, circulating IL-6 levels were recently shown to peak 24 h after eccentric resistance training exercise with large muscle groups that could have induced considerable systemic stress but minimal muscle damage [31]. Unfortunately, this could not be confirmed, because serum CK and the degree of soreness encountered by the subjects, who were described as 'active', were not reported. Neuroendocrine factors such as growth hormone, catecholamines and glucocorticoids and a range of neuropeptides also play a role in modulating immune function in response to exercise [32,33]. However, there is a lack of published data on longer-term changes (12–24 h) in these factors following acute exercise, at the time when potentiation of neutrophil and monocyte microbicidal function was observed in our study.

In contrast with previous studies that have reported an increase in neutrophil and monocyte activation antigen expression in untrained subjects following eccentric exercise with low and high systemic stress [16,34], we observed no changes in activation status after either ECC or STEP. Previous work has reported no change in neutrophil CD11b expression following moderate, sustained cycle ergometry exercise [35] and sub-maximal running exercise [36]. However, there is evidence that increases in neutrophil and monocyte CD11b and CD64 expression and in the concentration of circulating intercellular adhesion molecule-1 (ICAM-1) are influenced by the extent of muscle fibre injury after eccentric exercise [16,34,37]. This seems feasible, as increased expression of cell surface adhesion molecules could mediate the migration and infiltration of neutrophils and monocytes into exercise-damaged tissue, in which they contribute to the repair/regeneration process. Expression of CD11b is increased rapidly and dramatically (300–400 %) upon exposure to chemotactic stimuli by the transport of preformed granular stores of the protein to the surface of the cell [38]. Although most integrins require a conformational change in order to acquire full adhesive function [39], there is evidence that newly mobilized CD11b is capable of functioning in adhesive events if the cells are subsequently exposed to an additional stimulus level [40]. Interestingly, a positive correlation between the number of tissue macrophages and CD11b expression in the days after eccentric cycling exercise has been reported [41].

Our data suggest that exercise-induced muscle damage has no effect on peripheral blood neutrophil and monocyte activation antigen expression. Indeed, the increase in serum CK activity that we observed after ECC was much greater than that reported by Pizza et al. [16] after eccentric forearm flexor exercise. This probably reflects the greater quantity of skeletal muscle subjected to

exercise-induced damage. Furthermore, our study used an established whole-blood flow cytometry technique [42] that is potentially more representative of *in vivo* responses, as cell isolation procedures can affect leucocyte function and the expression of surface antigens [43,44].

In summary, our results show that a potentiation of peripheral blood neutrophil and monocyte phagocytic and respiratory burst responses can occur without any changes in activation antigen expression following acute exercise that has a large eccentric component and that evokes a high level of systemic stress. Exercise-induced muscle damage appears to have no effect on the microbicidal potential of circulating leucocytes, nor on the expression of activation antigens at the cellular level. However, the increased number of functional circulating neutrophils at 4 h after both the ECC and STEP protocols indicates an overall increase in peripheral blood microbicidal potential after eccentric exercise, independent of the level of systemic stress. These findings suggest that the level of systemic stress evoked during an acute bout of eccentric exercise has a greater influence on subsequent leucocyte functional responses than the degree of muscle damage induced.

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