Eur J Appl Physiol (1999) 80: 452–460 

ORIGINAL ARTICLE

**I.K.M. Brenner · V.M. Natale · P. Vasiliou**
**A.I. Moldoveanu · P.N. Shek · R.J. Shephard**

# Impact of three different types of exercise on components of the inflammatory response

Accepted: 2 May 1999

**Abstract** It was hypothesized that muscle injury would be greater with eccentric than with all-out or prolonged exercise, and that immune changes might provide an indication that supplements the information provided by traditional markers such as creatine kinase (CK) or delayed-onset muscle soreness. Eight healthy males [mean (SE): age = 24.9 (2.3) years, maximum oxygen consumption ($\dot{V}O_{2\,max}$) = 43.0 (3.1) ml · kg$^{-1}$ · min$^{-1}$] were each assigned to four experimental conditions, one at a time, using a randomized-block design: 5 min of cycle ergometer exercise at 90% $\dot{V}O_{2\,max}$ (AO), a standard circuit-training routine (CT), 2 h cycle ergometer exercise at 60% $\dot{V}O_{2\,max}$ (Long), or remained seated for 5 h. Blood samples were analyzed for CK, natural killer (NK) cell counts ($CD3^-/CD16^+56^+$), cytolytic activity and plasma levels of the cytokines interleukin (IL)-6, IL-10, and tissue necrosis factor α (TNF-α). CK levels were only elevated significantly 72 h following CT. NK cell counts increased significantly during all three types of exercise, but returned to pre-exercise baseline values within 3 h of recovery. Cytolytic activity per NK cell was not significantly modified by any type of exercise. Prolonged exercise induced significant increases in plasma IL-6 and TNF-α. We conclude that the lack of correlation between traditional markers of muscle injury (plasma CK concentrations and muscle soreness rankings) and immune markers of the inflammatory response suggests that, for the types and intensities of exercise examined in this study, the exercise-induced inflammatory response is modified by humoral and cardiovascular correlates of exercise.

**Key words** Natural killer cell · Cytolytic activity · Cytokines · Creatine kinase · Aerobic and resistance exercise

I.K.M. Brenner · P.N. Shek · R.J. Shephard
Defence and Civil Institute of Environmental Medicine, Toronto, Ontario, Canada

V.M. Natale
Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo, Brazil

P. Vasiliou · A.I. Moldoveanu
Programme in Exercise Sciences,
Graduate Department of Community Health,
University of Toronto, Ontario, Canada

P.N. Shek · R.J. Shephard (✉)
University of Toronto, Faculty of Physical Education and Health,
P.O. Box 521, Brackendale, BC., VONIHO, Canada
e-mail: royjshep@mountain-inter.net,
Tel.: +604-898-5527, Fax: +604-898-5724

R.J. Shephard
Department of Public Health Sciences, University of Toronto, Ontario, Canada

P.N. Shek
Department of Laboratory Medicine and Pathobiology,
University of Toronto, Ontario, Canada

## Introduction

Interest in the regulation of inflammation has increased rapidly in recent years, due to several factors that include: the emergence of immunocompromised populations (e.g., the frail elderly, those receiving immunosuppressant drugs following organ transplantation, and those suffering from HIV infections), the appearance of new strains of antibiotic-resistant micro-organisms, and a growing incidence of sepsis amongst surgical and trauma patients (Cross 1996). However, progress in the understanding of inflammatory dysregulation, and in the development of appropriate remedies has been hampered by a lack of appropriate human experimental models. Detailed studies in our own laboratories (Gannon et al. 1995; Shek et al. 1994; Shinkai et al. 1993) and elsewhere (Northoff et al. 1995) have noted that there are some striking parallels between the complex regulatory and counter-regulatory responses to surgical trauma and the reactions to either a single bout of exhausting exercise (Gannon et al. 1995; Shek et al. 1994; Shinkai et al. 1993) or a prolonged and systematic period of heavy training (Verde et al. 1992a, b).

Strenuous physical activity can cause mild sub-clinical injury, with the potential for an excessive inflammatory reaction and immunosuppression (Cross 1996; Shek et al. 1994) that in many respects mimics the reactions observed in clinical sepsis. Muscle damage, complement system activation and/or endotoxemia are some of the factors that have been suggested to trigger an inflammatory response to exercise (Camus et al. 1993, 1994, 1997). Specific changes that have been observed following strenuous exercise and in infectious disease states include: the acute phase response, leukocyte mobilization and activation, release of inflammatory mediators (cytokines), tissue damage and cellular infiltration, the production of free radicals and activation of the complement, coagulation and fibrinolytic pathways (Camus et al. 1993, 1994).

These observations support the view that the well-defined microtrauma that is associated with vigorous exercise can induce immune reactions that can serve as an experimental model of the inflammatory response. Exercise immunologists have examined the effects of various types of exercise on immune responses, but comparisons have rarely been conducted on the same panel of subjects. In order to identify the most effective form of exercise for such studies, we compared the impact of strenuous all-out exercise, circuit training and prolonged exercise on accepted markers of the inflammatory response. Our hypotheses were that the activity with the largest resistance component (circuit training) would generate the greatest increase in the traditional markers of muscle injury [creatine kinase (CK) release and delayed onset muscle soreness (DOMS) and that changes in immune function might provide an alternative experimental measure of the inflammatory response. The specific components of the immune system that were examined include elements that are dominant in sepsis and its complications [natural killer (NK) cell count and activity, interleukin (IL)-6, tumour necrosis factor-$\alpha$ (TNF-$\alpha$), and IL-10 production].

## Methods

### Subjects

The subjects were eight healthy, non-smoking, moderately fit males aged 20–40 years [mean (SE) = 24.9 (2.3) years who were recruited from a pool of university students in accord with a protocol approved by the Human Experimentation Review Committees of our Institute and the University of Toronto. Written informed consent to participate in this study was obtained and subjects were each medically screened by a physician prior to entry into the study. Exclusionary criteria included physical illnesses, manifestations of allergy, psychiatric diagnoses and use of medications. Subjects were of average height [1.74 (2.4) m], body mass [75.1 (1.9) kg], percent body fat [16.6 (2.2)%] and aerobic power [maximum oxygen consumption, $\dot{V}O_{2\,max} = 43.0$ (3.1) ml $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$].

### Study design

Each subject visited the laboratory on 13 occasions. At entry, anthropometric measurements were made (height, body mass and the thickness of the triceps, biceps, subscapular and suprailiac skinfolds). The percentage body fat was predicted from the equations of Durnin and Womersley (1974) and Siri (1956). During this visit, we determined the one-repetition maximal voluntary force of contraction (1-RM) at each of five different resistance stations, and we also measured the subjects' $\dot{V}O_{2\,max}$ using a progressive cycle ergometer test (Jones 1988).

Subjects were then assigned to one of four different experimental conditions, according to a randomized-block design. In one experiment, each subject undertook "all-out" cycling (AO) at a pace that could be sustained for 5 min (equivalent to some 90% of the directly measured $\dot{V}O_{2\,max}$). In another experiment, each subject performed a standard circuit-training routine (CT), involving five different types of exercise (biceps curl, knee extension, hamstring curl, bench press and leg press). Three sets of ten repetitions at 60–70% of the 1-RM were completed at each station; oxygen consumption was not measured during these tests, but from the post-exercise heart rate it was found to be approximately 50% of $\dot{V}O_{2\,max}$. In a third experimental condition, subjects performed 2 h of cycle ergometer exercise (Long) at 60–65% $\dot{V}O_{2\,max}$. Subjects remained seated for a 3-h recovery period following each of the experimental conditions. For the control condition, the subject sat at rest for 5 h. Subjects returned to the laboratory for follow-up examinations at 24 and 72 h after participation in each experimental protocol. All experiments were conducted on the same day of the week, at the same time of day, and with a 2-week interval between experiments.

### Physiological measurements

Heart rate was monitored continuously throughout each of the experimental sessions, using a heart rate telemetry system (Polar CIC, Port Washington, N.Y., USA). Oxygen consumption was measured intermittently during the AO and Long exercise, using a metabolic cart (model 2900c, Summit Technologies, Oakville, Ontario, Canada). Body temperature was assessed prior to, at the end of each exercise routine and 3 h post-exercise, using an infrared, tympanic non-contact temperature probe (Braun Thermoscan HM2, Lynfield, Mass., USA). At similar time points, blood pressures (systolic and fifth-phase diastolic) were measured by auscultation, using a standard sphygmomanometer cuff (Tycos, Arden, N.C., USA). During the AO and Long exercise bouts, subjects rated their overall perceived exertion (RPE), using Borg's original 6- to 20-point categoric scale (Borg 1970). On the days following each experiment (24 and 72 h post-exercise), subjects were asked to describe the extent of muscular soreness/stiffness (DOMS) they experienced in response to each of the exercise bouts.

### Blood sampling

During the AO, Long and control conditions, venous blood samples were collected from an indwelling venous catheter (Deseret Medical, Sandy, USA) that had been inserted into the medial antecubital vein 30 min prior to each experiment. The catheter was maintained patent between sampling with a 0.6-ml heparin-saline lock (100 units $\cdot$ ml$^{-1}$). Venipuncture was performed to obtain blood samples during and following CT as well as for the 24-h and 72-h recovery samples. Blood samples of 24 ml each were taken at times corresponding to pre-exercise, immediately post-exercise, and 3, 24 and 72 h post-exercise. The first 1.0 ml of each blood sample was discarded. Hemoglobin, red blood cells and reticulocyte count were checked prior to each experiment. All values proved normal, but the study design provided for more detailed hematological evaluation if a persisting hematological deficit had developed as a result of earlier blood sampling.

At the specified times, aliquots of blood were drawn into vacutainers (Becton-Dickinson, Oakville, Ontario, Canada). Heparinized whole blood (143 USP units sodium heparin) was used for the cytolytic functional assay. Tripotassium ethylenediamine tetraacetate ($K_3$EDTA)-treated whole-blood specimens were used for the determination of NK cell ($CD3^-$, $CD16^+56^+$) number (by

monoclonal antibody, mAb, staining) and for hematology. A separate vacutainer containing $K_3$EDTA-treated blood was used to obtain plasma samples for cytokine analyses. Vacutainers without additive were used to obtain serum samples for the CK analysis. Lymphocyte counts, hemoglobin and hematocrit were determined using a Coulter JT Automatic Hematology System (Coulter Electronics, Hialeah, Fla., USA). Lymphocyte counts, cytokine and CK concentrations were adjusted for changes in blood and plasma volumes relative to respective pre-exercise values, using the method of Dill and Costill (1974).

## Immunophenotyping

Direct two-color immunofluorescence was performed using 100-µl whole-blood samples. Samples were first washed with 2 ml of phosphate buffered saline containing 0.1% sodium azide (0.1% $NaN_3$-PBS). The cell pellet was then stained with 10 µl of the selected mAb. Non-specific staining was assessed using control tubes containing whole blood and mouse immunoglobulins $IgG_{2a}$ (FITC) and $IgG_1$ (PE), where FITC is fluorescein isothiocyanate and PE is phycoerythrin. NK cells were identified by staining cell surfaces with the anti-CD3 mAb (FITC)/anti-CD16,56 mAb (PE) (Becton-Dickinson, Mississauga, Ontario, Canada). After 30 min incubation on ice in the dark, 2 ml of 10% fluorescence activated cell sorter (FACS) lysing solution (Becton Dickinson) was added and the tubes were vortexed. The tubes were then kept in the dark at room temperature for a further 10 min to lyse the red cells. Non-lysed were centrifuged at 300 *g* for 5 min at 4°C. They were then washed with 2 ml of cold 0.1% $NaN_3$-PBS and centrifuged for 5 min at 4°C and 300 *g*. The resultant pellet was re-suspended in 0.3 ml of cold 0.1% $NaN_3$-PBS, vortexed and immediately analyzed by flow cytometer.

A FACS flow cytometer (Becton-Dickinson Immunosystems, Mountainview, Calif., USA) was used to examine the mAb-stained cell suspensions. First, using FACScomp software (Becton-Dickinson Immunocytometry Systems). The flow cytometer was calibrated with a mixture of mono-sized FITC- and PE-conjugated and unconjugated latex particles (≈6.0 µm Calibrite beads, Becton Dickinson). For each subject, an isotype negative control was used to optimize the setting of the fluorescence detectors. Fluorescence compensation was adjusted using an anti-CD4 mAb (FITC)/anti-CD8 mAb (PE) dual-stained sample. Non-staining control samples were used to determine gates for the lymphocyte population and boundaries for fluorescence intensity. NK ($CD3^-/CD16^+56^+$) cell numbers were calculated from total lymphocyte number, as quantified by the Coulter counter, using the equation: total number of NK cells per l blood = [(% $CD3/CD16^+56^+ \times 100^{-1}$) × (total number of lymphocytes per l blood)].

## Determination of NK cell activity

A $^{51}$Cr-release assay assessed the total cytolytic activity of isolated peripheral blood mononuclear cells (PBMC). Firstly, PMBC were isolated by Ficoll-Hypaque centrifugation. Heparinized venous blood (10 ml) was diluted with an equal volume of Dulbecco's phosphate-buffered saline (PBS; Sigma, St. Louis, Mo., USA). For each blood sample, three 15-ml centrifuge tubes were used to layer 7 ml of diluted blood carefully over 5 ml of Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). The suspension was then centrifuged for 30 min at 20°C and 450 *g*. The mononuclear layer was removed and washed twice, firstly with PBS and then with RPMI-1640 culture medium containing L-glutamine (Gibco, Burlington, Ontario, Canada). The cell suspension was centrifuged for 10 min at 10°C and 275 *g* after each wash. The washed PBMC were resuspended in 1 ml of RPMI-1640 supplemented with 10% fetal calf serum (Gibco, 10% FCS RPMI-1640). The cell count was determined with the aid of an electronic cell counter (Coulter Counter Model ZM, Luton; Bedfordshire, UK) and was then adjusted to $2 \times 10^6$ cells · $ml^{-1}$, using 10% FCS as the diluent.

A human erythroleukemic cell line (K562: American Type Culture Collection, Rockville, Md, USA), maintained in suspension in our laboratory, was used for the $^{51}$Cr-release assay. One million K562 cells, maintained in 10% FCS RPMI-1640 medium (Gibco) were labelled by mixing with 100 µl (3.7 MBq) of sodium chromate-51 ($^{51}$Cr; New England Nuclear, Boston, Mass., USA) for 60 min at 37°C and 4% $CO_2$. The radiolabelled cells were washed three times with 4 ml of cold 10% FCS RPMI-1640 medium and then diluted with 10 ml of medium to achieve a final concentration of $1 \times 10^5$ cells · $ml^{-1}$. The labelled cells were kept on ice until the assay was performed.

Triplicates of 100 µl of PBMC at concentrations of $2 \times 10^6$, $1 \times 10^6$, and $0.5 \times 10^6$ cells · $ml^{-1}$ were mixed with a 100-µl suspension of radiolabelled ($^{51}$Cr) target cells at $1 \times 10^5$ cells · $ml^{-1}$ by centrifugation (1 min at 37°C and 160 *g*) in a 96-well round-bottom microtiter plate (Sarstedt, St. Leonard, Quebec, Canada). After incubation for 4 h at 37°C, 4% $CO_2$, the cell mixture was centrifuged for a further 5 min at 4°C and 225 *g*. Supernatant was then withdrawn (100 µl) and transferred to polystyrene, round-bottom tubes (12 × 75 mm; Falcon, Lincoln Park, N.J., USA). The radioactivity of the supernatant was determined by a Cobra Automated Gamma Counter (Model 5002, Packard Instruments, Downers Grove, Ill., USA). Spontaneous release of $^{51}$Cr was assessed by incubating 100 µl of medium with 100 µl of target cells. The maximum potential release of radioactive material was determined by incubating 100 µl of 1% Triton X-100 (a biodegradable non-ionic surfactant; Sigma) with 100 µl of target cells. The percentage of $^{51}$Cr release (cytolytic activity) was calculated using the formula: percentage lysis = [(test − spontaneous) cpm + (maximum − spontaneous) cpm] × 100%. Lytic units were determined from the values obtained for percent lysis at three different effector-target ratios (20:1, 10:1 and 5:1) by the exponential curve-fitting method of Pross et al. (1981). The lytic unit is defined as the number of effector cells required to lyse 20% of 10,000 target cells. The results are expressed as the number of lytic units contained in $1 \times 10^6$ PBMC (LU · $10^6$ $PBMC^{-1}$; Pross et al. 1981). Lytic units were also calculated on a per-NK-cell basis, using the formula: single cell cytolytic activity = lytic units + [% NK cells × ($10^6$ PBMC − monocytes)].

## Biochemical analysis

$K_3$-EDTA-treated blood was obtained for cytokine analysis. Tubes were mixed by gentle inversion, placed in an ice-water bath for no more than 30 min (to prolong the half-life of the cytokines), and then were centrifuged for 15 min at 4°C and 2250 *g*. The plasma samples were separated from the packed red cells, transferred to Eppendorf tubes and immediately frozen and stored at −70°C. Plasma concentrations of IL-6, TNF-α and IL-10 were determined on all samples using enzyme immunoassay kits (R & D Systems, Minneapolis, Minn., USA); in our experience, these kits meet the makers' claims of a sensitivity of 0.094 pg · $ml^{-1}$ for IL-6, 0.11 pg · $ml^{-1}$ for TNF-α and 0.5 pg · $ml^{-1}$ for IL-10. Blood samples that were obtained without additive were kept at 37°C for 1 h to allow for clotting and were then centrifuged for 15 min at 37°C and 2250 *g*. The serum samples were separated from the packed red cells, transferred to Eppendorf tubes and immediately frozen and stored at −70°C. A quantitative colorimetric determination kit (Sigma) and spectrophotometer (Spectronic 1001, Bausch & Lomb, Rochester, N.Y., USA) were used to determine levels of serum CK.

## Statistical analysis

Results are expressed throughout as means (SE). For data on NK cell counts, NK cell activity and activity per cell, a 4 × 5 (four conditions, five times of measurement) repeated-measures analysis of variance was performed with SuperAnova (Abacus Concepts, Berkeley, Calif., USA). Data obtained on cytokines and CK were analyzed using a 3 × 5 (three conditions, five times of measurement) repeated-measures analysis of variance. Specific post-hoc contrasts explored significant interactions. A probability (*P*) value of $< 0.05$ was set as the level of statistical significance.

## Results

### Physiological responses

Mean physiological responses recorded from times corresponding to pre-exercise, immediately post-exercise and recovery (3 h, 24 h and 72 h post-exercise) are presented in Table 1. During the AO exercise bout, the oxygen consumption averaged 38.3 (4.3) ml · $g^{-1}$ · $min^{-1}$, corresponding to approximately 89% of $\dot{V}O_{2\,max}$. Subjects exercised at an average of 63% $\dot{V}O_{2\,max}$ [27.1 (1.7) ml · $kg^{-1}$ · $min^{-1}$] during the prolonged exercise bout. The heart rates immediately following the CT exercise suggested that the oxygen consumption during this experiment amounted to no more than 50% of $\dot{V}O_{2\,max}$.

In terms of the immediate physiological response, the intensity of effort was ranked AO > Long > CT. The AO exercise led to a significant rise in heart rate and systolic blood pressure (Table 1) and subjects perceived the exercise intensity as being "very hard" [corresponding to an RPE of 17.5 (0.8)]. Similarly, prolonged exercise led to a significant rise in heart rate, systolic blood pressure and tympanic temperature immediately post-exercise, and a significant drop in systolic blood pressure occurred after 3 h of recovery. In contrast, diastolic blood pressure was significantly reduced at the end of the Long exercise and remained lower than normal after 3 of recovery. Subjects reported the intensity of effort during the prolonged exercise as "somewhat hard" to "hard" [RPE = 14.2 (1.5)]. The CT exercise induced significant elevations in heart rate, systolic blood pressure and tympanic temperature. Heart rate and tympanic temperature had returned to pre-exercise baseline levels after 3 h of recovery, whereas at the same stage, systolic blood pressure was significantly below baseline values.

**Table 1** Physiological responses to the different exercise bouts. Values are given as the mean (SE). (*Rec* Recovery)

| Condition | Heart rate (beats · $min^{-1}$) | Blood pressure (mmHg) | | Tympanic temperature (°C) |
|---|---|---|---|---|
| | | Systolic | Diastolic | |
| All-out exercise | | | | |
| Pre | 80 (4) | 124 (6) | 77 (2) | 36.3 (0.1) |
| Post | 180 (6) | 137 (6) | 70 (4) | 36.5 (0.1) |
| Rec 3h | 72 (4) | 103 (6) | 75 (3) | 36.3 (0.1) |
| Circuit training | | | | |
| Pre | 84 (4) | 121 (2) | 77 (2) | 36.1 (0.2) |
| Post | 123 (11)* | 136 (6)* | 73 (3) | 36.6 (0.2)* |
| Rec 3h | 66 (3) | 107 (4)* | 72 (3) | 36.1 (0.1) |
| Prolonged exercise | | | | |
| Pre | 81 (4) | 118 (2) | 77 (2) | 36.2 (0.1) |
| Post | 149 (7)* | 143 (6)* | 62 (2)* | 36.5 (0.2)* |
| Rec 3h | 75 (4) | 101 (5)* | 65 (4)* | 36.4 (0.1) |

* Significant difference, $P < 0.05$

### NK cell counts

All three types of exercise induced a significant rise in circulating NK cell counts (Fig. 1a). In accordance with the physiological data, the rise was greatest for AO exercise [AO = 1.74 (0.25) cells · $10^9$ $l^{-1}$ vs sit = 0.27 (0.08) cells · $10^9$ $l^{-1}$]. Values were slightly lower for the Long exercise [Long = 1.10 (0.15) cells · $10^9$ $l^{-1}$ vs sit = 0.26 (0.04) cells · $10^9$ $l^{-1}$], and the weakest response was seen with the CT exercise [CT = 0.65 (0.13) cells · $10^9$ $l^{-1}$ vs sit = 0.21 (0.03) cells · $10^9$ $l^{-1}$]. NK cell counts had returned to pre-exercise baseline values within 3 h recovery. At no point during our observations did the NK cell count drop below the pre-exercise baseline.

### Cytolytic activity

Total cytolytic activity measured in lytic units (LU · $10^6$ $PBMC^{-1}$) increased significantly in response to the AO and Long exercise conditions [AO = 19.0 (2.0) vs sit = 5.5 (1.1), and Long = 19.6 (2.1) vs sit = 8.7 (2.0); Fig. 1b]. Lytic activity tended to rise in response to the CT routine, but this trend was not statistically significant [CT = 11.8 (2.2) vs sit = 6.1 (0.9)]. When cytolytic activity was expressed on a single-cell basis relative to NK cell counts there were no longer any significant differences in response between the different experimental conditions (Fig. 1c), suggesting that changes in cytolytic activity were determined primarily by changes in NK cell count.

### Cytokines

Cytokine responses were more marked with the Long exercise than with the other two experimental conditions. Although plasma IL-6 concentrations tended to rise following both AO exercise and CT, levels were significantly elevated only following the Long exercise, peaking at 3 h post-exercise [recovery 3 h = 6.06 (1.95) pg · $ml^{-1}$ vs pre-exercise = 1.14 (0.31) pg · $ml^{-1}$; Fig 2a]. Levels of TNF-α were also significantly increased, but only in response to Long exercise, peak values being attained after 72 h of recovery [recovery 72 h = 1.87 (0.27) pg · $ml^{-1}$ vs pre-exercise = 1.05 (0.13) pg · $ml^{-1}$; Fig 2b]. Plasma concentrations of IL-10 were significantly reduced following AO exercise, but were not influenced by CT or Long exercise (Fig. 2c).

### CK and DOMS

In terms of traditional markers of muscle damage, the largest response was to the CT exercise. Seven of the eight subjects reported muscular soreness in the chest, arms and/or legs 24–48 h following the CT routine, and two of the eight subjects reported muscular soreness in the legs following the Long exercise. Serum concentra-

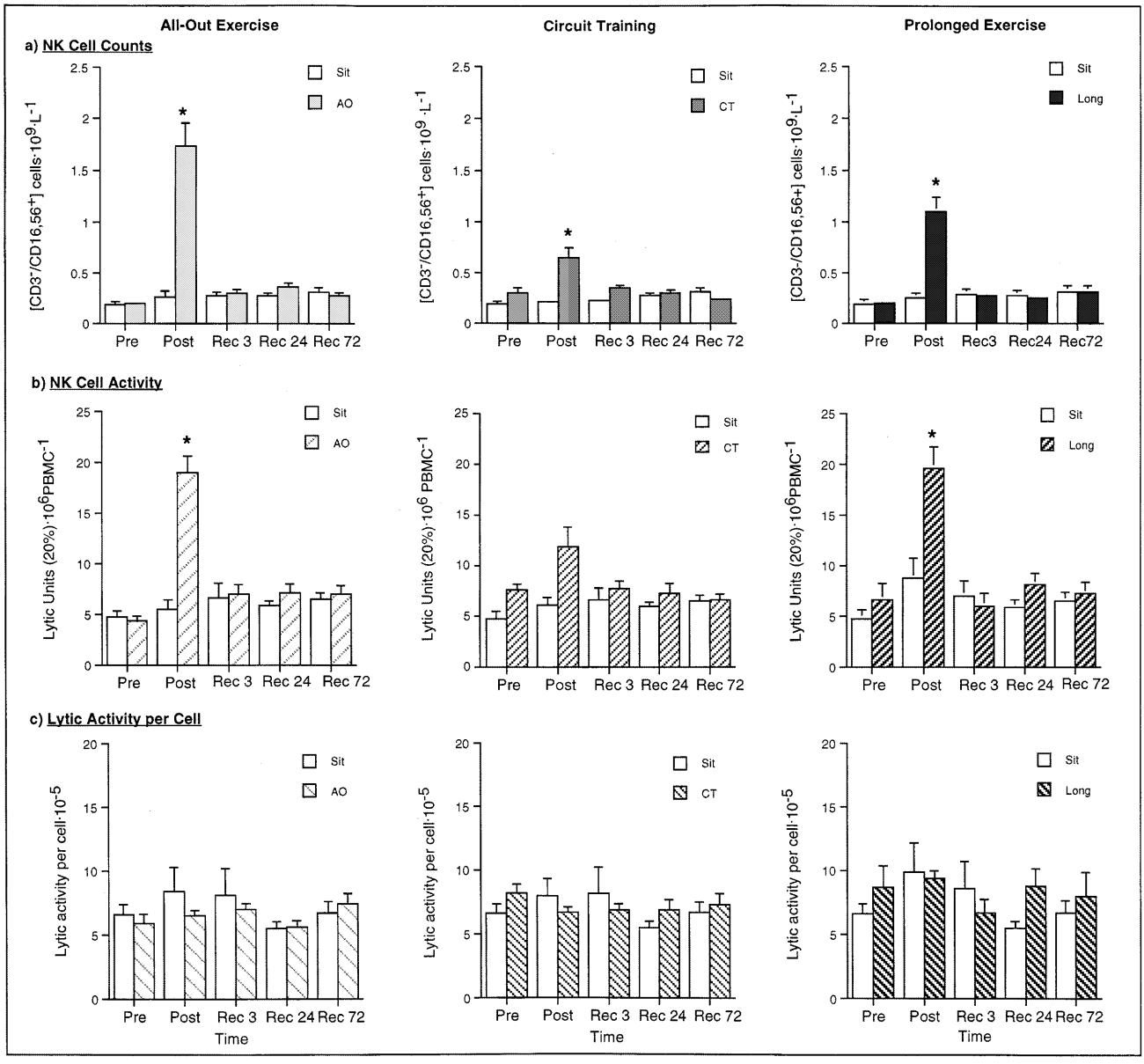


**Fig. 1a–c** Natural killer (NK) cell response to the different experimental conditions. **a** Changes in $CD3^-/CD16^+56^+$ cell counts during all-out (AO) exercise and control sit condition, circuit training (CT) and control sit condition and prolonged exercise (Long) and control sit condition. **b** Changes in total NK cell activity (NKCA) during the three different exercise conditions. **c** Changes in single-cell NKCA during the three different exercise conditions. (*Pre* Pre-exercise, *Post* post-exercise, *Rec* recovery). *Level of significance of $P < 0.05$

tions of CK tended to rise 24 h following both the CT and Long exercise, but levels were only significantly elevated 72 h following CT [CT recovery 72 h = 290.5 (127.5) Sigma Units · $ml^{-1}$ vs pre-exercise = 22.1 (3.0) Sigma Units · $ml^{-1}$; Fig. 3].

## Discussion

Although similar experiments have been carried out by other investigators, this is the first study in which the same subjects performed three different types of exercise (all-out exercise, circuit training and prolonged exercise) at close to the maximum level that they could tolerate. Responses were contrasted in terms of an accepted index of sub-clinical muscle injury (CK production) and readily measured components of the inflammatory reaction that are dominant in sepsis (for example, NK cell count and cytolytic activity, IL-6, TNF-α and IL-10 production).

### Impact of different types of exercise on levels of CK

CK is an enzyme that is involved in muscle metabolism; leakage of this enzyme into the plasma is widely accepted as a semi-quantitative indicator of muscle injury. Muscle injury seems more likely to occur following unaccustomed eccentric contractions than after concentric

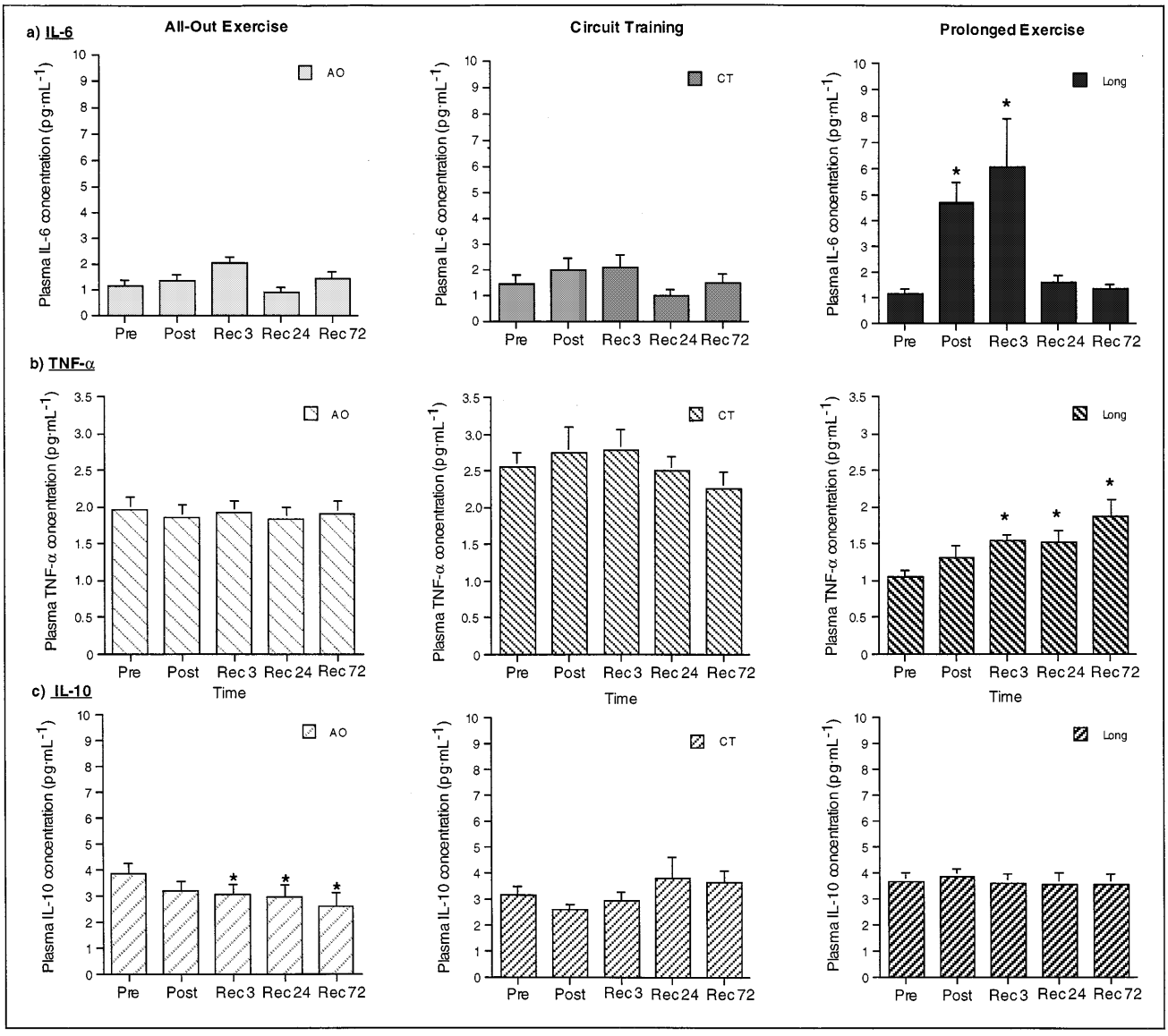


**Fig. 2a–c** Cytokine response to the three different experimental conditions. **a** Plasma levels of interleukin (II)-6 during the AO, CT and Long exercise conditions. **b** Plasma levels of tissue necrosis factor (TNF)-α during the three different exercise conditions. **c** Plasma levels of IL-10 during the three different conditions. *Level of significance of $P < 0.05$

contractions of similar vigor (Smith 1991), and we thus hypothesized that this form of exercise would be the most likely to induce muscle injury.

In accordance with our hypothesis, there was a significant increase in serum CK concentrations 72 h following CT. In addition, there was a weaker tendency for levels of CK to increase 24 h following prolonged exercise. We thus concluded that the CT regimen (which involved both concentric and eccentric contractions) had induced more muscle injury than the Long cycle ergometry (which was primarily a concentric form of exercise). As in other reports, there was a temporal separation of DOMS and CK changes, but nevertheless,

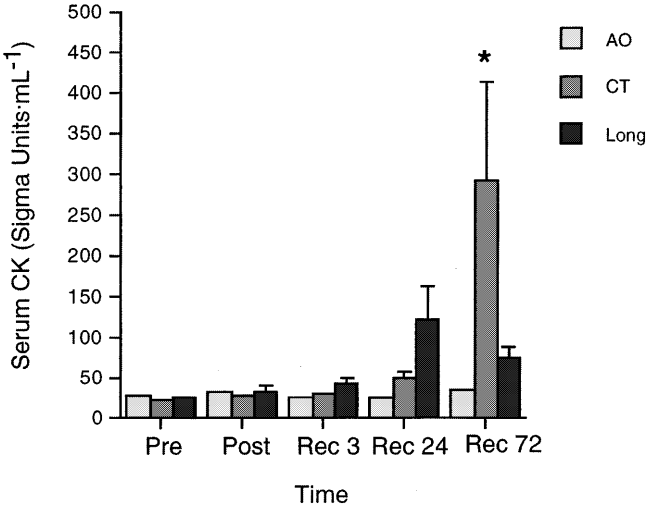


**Fig. 3** Levels of serum creatine kinase (CK) in response to the AO, CT and Long exercise bouts. *Level of significance of $P < 0.05$

and in support of the injury ranking, seven of our eight subjects reported muscle soreness (24–48 h) following CT, whereas only two of the eight subjects complained of muscle soreness 24 h following Long exercise condition.

## Impact of three different types of exercise on NK cells

It is well established that circulating NK cells counts increase in proportion to the intensity of aerobic exercise (Gannon et al. 1995) and that resistance exercise also recruits NK cells into the circulation (Nieman et al. 1995; Stock et al. 1995). Similarly, we observed a significant rise in NK cell count in response to all three types of exercise (greatest for AO exercise, which was performed at 89% $\dot{V}O_{2\,max}$, slightly less for Long exercise, which was performed at 63% $\dot{V}O_{2\,max}$, and least for the CT routine), with a return to baseline levels after 3 h of recovery. In contrast, Shek et al. (1995) exercised subjects on a treadmill, at 65% $\dot{V}O_{2\,max}$ for 90–120 min and reported a post-exercise reduction in NK cell counts that persisted for at least 7 days. The conflicting post-exercise findings may be attributed to differences in the timing of blood sampling as well as differences in both exercise intensity and modality; both heart rate and RPE values suggest that the Long exercise adopted in our present study was vigorous but not exhausting.

Total NK cell activity (NKCA) was significantly increased in response to the AO and Long exercise sessions, returning to pre-exercise baseline levels by 3 h post-exercise. Like Nieman et al. (1995), we did not observe a significant change in total NKCA immediately following CT, although there was a tendency for it to be increased. Strenuous one-legged eccentric exercise has been shown to increase total NKCA (Palmø et al. 1995). With the exception of Shek et al. (1995), who reported a significant reduction in total NKCA that lasted 7 days following 2 h of treadmill exercise at 65% $\dot{V}O_{2\,max}$, total and single-cell cytotoxic activity have been shown to decrease 1–2 h following exercise (Berk et al. 1990; Nieman et al. 1995; Shinkai et al. 1992; Tvede et al. 1993). In our study, total NKCA may have shown a transient fall below pre-exercise baseline values in the first 3 h post-exercise, but we cannot comment on this possibility since we did not collect blood samples during that time.

Increases in cardiac output (via shear stress) and/or changes in levels of circulating hormones (leading to alterations in adhesion molecules and recruitment of cells from reservoirs) account for the exercise-induced change in cell counts. The immediate post-exercise increase in NKCA is primarily due to the exercise-induced rise in circulating NK cell counts (Mackinnon 1992). However, several humoral factors released into the circulation during exercise can also modulate NKCA. NKCA is stimulated by increased concentrations of certain circulating cytokines (IL-2, IL-6, TNF-$\alpha$ and IFN-$\gamma$; Benzcur et al. 1984; Henney et al. 1981; Jewitt et al. 1996; Luger et al. 1989), $\beta$-endorphin (Fiatarone et al. 1989) and dopamine (Basu et al. 1995; Deleplanque et al. 1994), but is reduced in response to prostaglandin $E_2$ (Pedersen et al. 1990). By expressing total cytolytic activity per NK cell, we have demonstrated that the single-cell cytolytic activity was unaltered by any of the three different types of exercise adopted here. This lends support to the general hypothesis that most of the exercise-induced changes in NKCA are attributable to changes in cell counts, rather than to alterations in cellular activity or the recruitment of more active cell types.

## Impact of different types of exercise on plasma cytokine concentrations

Although brief bouts of maximal exercise do not appear to alter levels of IL-6 (Natelson et al. 1996), strenuous resistance (eccentric) exercise (Anwar et al. 1997) and prolonged exercise have induced significant increases in IL-6 (Drenth et al. 1995; Gannon et al. 1997; Nehlsen-Cannarella et al. 1997). In agreement with others (Drenth et al. 1995; Gannon et al. 1997; Nehlsen-Cannarella et al. 1997), the Long exercise bout induced five- to sixfold increase in levels of IL-6, both immediately post-exercise and after 3 h of recovery. In contrast to the results of Anwar et al. (1997), the CT routine had no effect. This discrepancy may be due to differences in the emphasis of the muscle contraction and the intensity of effort. Our subjects performed both the concentric and eccentric components of various resistance exercises at 60–70% of their 1-RM force (three sets, ten repetitions per set, 1 min rest between sets), whereas Anwar et al. (1997) had their subjects perform only the eccentric phase of a bench press and hamstring curl at 100% 1-RM voluntary contraction (four sets, ten repetitions per set, 2 min rest between sets).

The acute exercise-induced elevation in TNF-$\alpha$ reported in this study is consistent with the results of other researchers who examined prolonged exercise (Camus et al. 1997; Gannon et al. 1997; Rhind unpublished data). Although TNF-$\alpha$ is elevated within 2 h of cycle ergometer exercise at 65% $\dot{V}O_{2\,max}$ (Rhind unpublished data), substantial increases have also been observed following a marathon run (Camus et al. 1997) and a 250-km cycling road race (Gannon et al. 1997). At low concentrations, TNF-$\alpha$ can be difficult to detect accurately in the plasma. In this study, both baseline and exercise-induced levels of TNF-$\alpha$ were higher in the AO exercise and CT conditions than in the Long exercise condition (in which an exercise-induced rise in levels of circulating TNF-$\alpha$ occurred). These differences across conditions may also be attributed to variations in the sensitivities of the plates (reagents), since each condition was analyzed in separate plates.

The exercise-induced mechanisms of IL-6 and TNF-$\alpha$ release have not been clearly defined. Changes in the numbers of circulating leukocytes and leukocyte subsets

may play a role. Activated T-cells and tissue macrophages are well known producers of IL-6 and TNF-α (Hagiwara et al. 1995). There is also evidence that cell debris released from injured muscles (Bruunsgaard et al. 1997; Northoff et al. 1995) and/or endotoxin leakage from the gut (Bouchama et al. 1991) may stimulate the release of these pro-inflammatory cytokines. However, for the different exercise conditions that were examined in the study presented here, the ranking of CK release did not parallel the response of the pro-inflammatory cytokines, suggesting that other, activity-related mechanisms contribute to the release of these cytokines during prolonged exercise.

Little attention has been focused on examining the impact of different types of exercise on the secretion of anti-inflammatory cytokines (Anwar et al. 1997; Gannon et al. 1997; Rhind unpublished data). A few studies have shown that levels of IL-10 are either increased (Anwar et al. 1997; Rhind unpublished data) or remain the same after strenuous exercise (Gannon et al. 1997). We hypothesized that the exercise-induced elevations in pro-inflammatory cytokines (IL-6 and TNF-α) would, in turn, stimulate the release of anti-inflammatory cytokines. Surprisingly, levels of circulating IL-10 were reduced following AO exercise. Moreover, CT and Long exercise did not alter levels of circulating IL-10. Anwar et al. (1997) reported elevated plasma levels of IL-10 48 and 72 h following the eccentric phase of a bench press and hamstring curl at 100% 1-RM (four sets, ten repetitions per set, two min rest between sets). Our subjects performed their resistance exercise at a much lower intensity (60–70% 1-RM) than in the experiments of Anwar et al. (1997). In contrast to an unpublished report by Rhind, who used a similar exercise design to us, we did not observe any increase in plasma IL-10 concentrations following prolonged exercise. Our subjects exercised at a slightly lower exercise intensity (63% $\dot{V}O_{2\,max}$ vs 65% $\dot{V}O_{2\,max}$), and perhaps a threshold exercise intensity of at least (65% $\dot{V}O_{2\,max}$ is required before increases in this cytokine can be detected. However, our findings are consistent with those of Gannon et al. (1997), who reported that levels of IL-10 did not change, despite significant increases in levels of IL-6 and TNF-α, following a 6.5-h competitive cycling race. Inconsistencies in levels of circulating IL-10 following exercise may be due to differing collection times, assay sensitivities and the short half-lives of circulating cytokines. The decrease in IL-10 concentration following AO exercise may also have reflected clearance/removal rates that exceeded the rate of production.

In summary, this study was designed with the intent of determining which type of exercise would be best suited to study inflammatory response. Our reasoning was that vigorous exercise would induce tissue injury and trigger inflammation. The evidence of CK levels and DOMS support the traditional view that muscle injury is greatest with eccentric exercise. However, contrary to our initial hypothesis, there are substantial discrepancies between such rankings of muscle injury and the extent of pro-inflammatory changes in the immune system, emphasizing that other humoral and circulatory factors unique to exercise contribute to the observed pattern of immune response. Of the three different types of exercise studied, the prolonged exercise routine induced significant immune changes that would be considered typical of a pro-inflammatory cytokine response. Thus, if exercise were to be adopted as a model for study of the inflammatory response, this might seem to be the most effective type of activity to choose. The significant increase in plasma levels of IL-6 and TNF-α, the mobilization of cytotoxic cell populations, and increased NK cell cytotoxic activity all suggest that prolonged exercise activates several components of the inflammatory response. Further research is required in order to examine the usefulness of adopting a more strenuous exercise protocol (e.g., marathon running) as a model with which to study the inflammatory response.

**Acknowledgements** This study was supported in part by a research grant from the Defence and Civil Institute of Environmental Medicine.

## References

Anwar A, Smith LL, Holbert D, Sorensen T, Fragen M (1997) Serum cytokines after strenuous exercise (abstract). Med Sci Sports Exerc 29:S72

Basu S, Dasgupta PS, Chowdhury JR (1995) Enhanced tumour growth in brain dopamine-depleted mice following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. J Neuroimmunol 60:1–8

Benzcur M, Matsushima K, Oppenheim JJ, Herberman RB, Ortaldo JR (1984) Stimulatory role of purified IL-1 on human NK cells (abstract). J Leukocyte Biol 36:396

Berk LS, Nieman DC, Youngberg WS, Arabatzis K, Simpson-Westerberg M, Lee JW, Tan SA, Eby WC (1990) The effect of long endurance running on natural killer cells in marathoners. Med Sci Sports Exerc 22:207–212

Borg G (1970) Perceived exertion as an indicator of somatic stress. Scand J Rehabil Med 23:92–98

Bouchama A, Parhar RS, El-Yazigi A, Sheth K, Al-Sedairy S (1991) Endotoxemia and release of tumour necrosis factor and interleukin 1α in acute heat stroke. J Appl Physiol 70:2640–2644

Bruunsgaard H, Galbø H, Halkjaer-Kristensen J, Johansen TL, Maclean DA, Pedersen BK (1997) Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol (Lond) 499:833–841

Camus G, Deby-Dupont G, Deby C, Juchmes-Ferir J, Pincemail J, Lamy M (1993) Inflammatory response to strenuous muscular exercise in man. Mediat Inflamm 2:335–342

Camus G, Deby-Dupont G, Duchateau J, Deby C, Pincemail J, Lamy M (1994) Are similar inflammatory factors involved in strenuous exercise and sepsis? Intensive Care Med 20:602–610

Camus G, Poortmans J, Nys M, Deby-Dupont G, Duchateau J, Deby C, Lany M (1997) Mild endotoxemia and the inflammatory response induced by a marathon race. Clin Sci 92:415–422

Cross A (1996) Novel approaches to the treatment of sepsis: lessons learned and future directions. Shock 6:S71–S74

Deleplanque B, Vitello S, Le Moal M, Neveu PJ (1994) Modulation of immune reactivity by unilateral striatal and mesolimbic dopaminergic lesions. Neurosci Lett 166:216–220

Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration. J Appl Physiol 37:247–248

Drenth JPH, Van Uum SMH, Van Deuren M, Pesman GJ, Van der Ven-Jongekrijg J, Van Der Meer JWM (1995) Endurance run increases circulating IL-6 and IL-1rα but downregulates ex vivo TNF-α and 1L-1β production. J Appl Physiol 79:1497–1503

Durnin JVGA, Womersley J (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32:77–97

Fiatarone MA, Morley JE, Bloom ET, Benton D, Solomon GF, Makinodan T (1989) The effect of exercise in natural killer cell activity on young and old subjects. J Gerontol 44:M37–M45

Gannon GA, Shek PN, Shephard RJ (1995) Natural killer cells: modulation by intensity and duration of exercise. Exerc Immunol Rev 1:26–48

Gannon GA, Rhind SG, Suzui M, Shek PN, Shephard RJ (1997) Circulating levels of peripheral blood leucocytes and cytokines following competitive cycling. Can J Appl Physiol 22:133–147

Hagiwara E, Abbasi F, Mor G, Ishigatsubo Y, Klinman D (1995) Phenotype and frequency of cells secreting IL-2, IL-4, IL-6, IL-10, IFN and TNF-α in human peripheral blood. Cytokine 7:815–822

Henney CS, Kuribayashi K, Kern DE, Gillis S (1981) Interleukin-2 augments natural killer cell activity. Nature 291:335–338

Jewitt A, Gan X, Lebow LT, Bonavida B (1996) Differential secretion of TNF-α and IFN-γ by human peripheral blood-derived NK subsets and association with functional maturation. J Clin Immunol 16:46–54

Jones NL (1988) Clinical exercise testing. Saunders, Philadelphia, pp 135–144

Luger TA, Krutman J, Kirnbauer R, Urbanski A, Schwarz T, Klappacher G, Kock A, Micksche M, Malejczyk J, Schauer E, May LT, Sehgal PB (1989) IFNB2/IL-6 augments the activity of human natural killer cells. J Immunol 143:1206–1209

Mackinnon LT (1992) Exercise and immunology. Human Kinetics Champaign, Ill

Natelson BH, Zhou X, Ottenweller JE, Bergen MT, Sisto SA, Drastal S, Tapp WN, Gause WL (1996) Effect of acute exhausting exercise on cytokine gene expression in men. Int J Sports Med 17:299–302

Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Schmitt RL, Bailey EM, Warren BJ, Utter A, Davis JM (1997) Carbohydrate and the cytokine response to 2.5 h of running. J Appl Physiol 82:1662–1667

Nieman DC, Henson DA, Sampson CS, Herring JL, Suttles J, Conley M, Stone MH, Butterworth DE, Davis JM (1995) The acute immune response to exhaustive resistance exercise. Int J Sports Med 16:322–328

Northoff H, Enkel S, Weinstock C (1995) Exercise, injury and immune function. Exerc Immunol Rev 1:1–25

Palmø J, Asp S, Daugaard JR, Richter EA, Klokker M, Pedersen BK (1995) Effect of eccentric exercise on natural killer cell activity. J Appl Physiol 78:1442–1446

Pedersen BK, Tvede N, Klarlund K, Christensen LD, Hansen FR, Galbø H, Kharamzi A, Halkjaer-Kristensen J (1990) Indomethacin in vitro and in-vivo abolishes post-exercise suppression of NK cell activity in peripheral blood. Int J Sports Med 11:127–131

Pross HF, Baines MG, Rubin P, Shragge P, Patterson MS (1981) Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells IX. The quantitation of natural killer cell activity. J Clin Immunol 1:51–63

Shek PN, Sabiston BH, Paucod JC, Vidal D (1994) In: Buguet A, Radomski M (eds) Physical exercise, hyperthermia, immune system and recovery from sleep in man. C.R.S.S.A., La Tronche France, pp 119–137

Shek PN, Sabiston BH, Buguet A, Radomski MW (1995) Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response. Int J Sports Med 16:466–474

Shinkai S, Shore S, Shek PN, Shephard RJ (1992) Acute exercise and immune function: relationship between lymphocyte activity and changes in subset counts. Int J Sports Med 13:452–461

Shinkai S, Kurokawa Y, Hino S, Hirose M, Torii J, Watanabe S, Shirachi S, Oka K, Watanabe T (1993) Triathlon competition induced a transient immunosuppressive change in the peripheral blood of athletes. J Sports Med Phys Fitness 33:70–78

Siri WE (1956) The gross composition of the body. Adv Biol Med Phys 4:239–280

Smith LL (1991) Acute inflammation: the underlying mechanism in delayed onset muscle soreness. Med Sci Sports Exerc 23:542–551

Stock C, Schaller K, Baum M, Liesen H, Weib M (1995) Catecholamines, lymphocyte subsets, and cyclic adenosine monophosphate production in mononuclear cells and CD4+ cells in response to submaximal resistance exercise. Eur J Appl Physiol 71:166–172

Tvede N, Kappel M, Halkjær-Kristensen J, Galbø H, Pedersen BK (1993) The effect of light, moderate and severe bicycle exercise on lymphocyte subsets, natural and lymphokine activated killer cells, lymphocyte proliferative response and interleukin 2 production. Int J Sports Med 14:275–282

Verde T, Thomas S, Moore RW, Shek PN, Shephard RJ (1992a) Immune response and increased training of the elite athlete. J Appl Physiol 73:1494–1499

Verde T, Thomas S, Shephard RJ (1992b) Potential markers of heavy training in highly trained distance runners. Br J Sports Med 26:167–175