Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage

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1. This study was performed to test the hypothesis that the exercise-induced increase in circulating cytokine levels is associated with muscle damage. Nine healthy young male subjects performed two high-intensity bicycle exercise trials separated by two weeks. The first trial consisted of 30 min of normal bicycle exercise (concentric exercise), whereas the second consisted of 30 min of braking with reversed revolution (eccentric exercise). The work loads were chosen to give the same increases in heart rate and catecholamine levels in the blood during each trial.

2. Significant increases ($P < 0{\cdot}05$) in plasma concentration of creatine kinase (CK), aspartate aminotransferase and alanine aminotransferase were observed only after the eccentric exercise. Furthermore, the level of interleukin-6 (IL-6) in serum increased significantly after the eccentric exercise and was significantly correlated to CK concentration in the following days, whereas no significant changes were found after the concentric exercise.

3. The total concentration of lymphocytes increased significantly ($P < 0{\cdot}05$) as a result of eccentric compared with concentric exercise. This was mainly due to a significantly more pronounced recruitment of natural killer (NK) cells and CD8 positive cells (CD8+ cells) during the eccentric trial. However, no significant differences between the two types of work were found in regard to the circulating concentration of monocytes. The concentration of neutrophils was only significantly increased 2 h after the concentric exercise.

4. The finding that high-intensity eccentric exercise caused a more pronounced increase in the plasma level of IL-6, compared with concentric exercise, supports the hypothesis that the post-exercise cytokine production is related to skeletal muscle damage. The fact that no differences between eccentric and concentric exercise were found in the recruitment of most blood mononuclear cell subsets to the blood supports the hypothesis that the exercise-induced increase in plasma catecholamines is a major determinant of the mobilization of these cells into the blood. However, as eccentric exercise caused a more pronounced increase in the concentration of NK cells and CD8+ cells, factors involved in muscle damage may also contribute to the recruitment of these cells.

Immune function, and natural immunity in particular, is enhanced during muscular activity. Natural killer (NK), B and T cells are recruited to the blood and the composition of lymphocyte subsets is altered because the NK cells increase more than the other lymphocyte subpopulations. Following intense, as opposed to moderate, exercise, the lymphocyte concentration decreases below the baseline value and the duration of this suppression depends on the intensity and duration of the exercise (Hoffman-Goetz & Pedersen, 1994). It has been proposed that the mechanism underlying the recruitment of blood mononuclear cells (BMNCs) during physical exercise may be the increased level of plasma catecholamines (Kappel *et al.* 1991). Infusion of adrenaline resulting in plasma concentrations identical to those observed during concentric exercise has been shown to mimic the exercise effect on BMNC subsets, NK and

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lymphokine-activated killer (LAK) cell activity (Kappel *et al.* 1991; Tvede *et al.* 1994). However, since infusion of adrenaline and noradrenaline does not enhance the concentration of cytokines (M. Kappel, T. Poulsen, H. Galbo & B. K. Pedersen, unpublished observations), the catecholamine response during exercise cannot be responsible for the increased level of cytokines that is detected after some forms of exercise.

Physical exercise can be characterized as being concentric, isometric or eccentric. Most immunological studies have been carried out using concentric exercise or combinations of concentric and eccentric exercise. High-intensity eccentric exercise in untrained subjects is associated with increased levels of myofibre enzymes in plasma, ultrastructural damage of the muscle fibres and an acute inflammatory response in the muscle leading to oedema, infiltration by inflammatory cells and muscle soreness 24–48 h following exercise (Evans *et al.* 1986).

It is possible that inflammatory reactions in the muscles could be responsible for the induction of cytokine production. We hypothesized that high-intensity eccentric exercise causes a more pronounced increase in the plasma levels of cytokines involved in acute inflammatory responses during and following exercise than that produced by concentric exercise. We also hypothesized that the recruitment of the BMNCs to the blood during physical activity is caused by the same mechanism of action in eccentric and concentric exercise. However, since inflammatory cytokines are known to cause proliferation and activation of T and B cells (Roit, 1994), these may also be of importance.

METHODS

Protocol

The experimental protocol was approved by the ethical committee for Copenhagen and Frederiksberg Communities. All subjects were informed of the purposes and risks of the study and written informed consent was obtained. Nine healthy men aged 21–42 years (mean, 26 years) with a maximal oxygen consumption ($V_{O_2,max}$) of 3·01–4·16 l min^{-1} (mean, 3·65 l min^{-1}) corresponding to 51·1 ml kg^{-1} min^{-1} (range, 39·7–58·1 ml kg^{-1} min^{-1}) were studied. The subjects were not taking any medication. They were not elite sportsmen but they were recreationally active, performing exercise 1–5 days a week for 1–2 h (bicycling, football, hiking, aerobics, jogging and table tennis). One week prior to the experiment, $V_{O_2,max}$ and maximal heart rate (HR_{max}) were determined during an incremental exercise test on the same cycle ergometer that was used in the experiments. The ergometer (Bonde-Petersen, 1969) consists of three parts: an electric motor, an electrical induction clutch and a Krogh's bicycle ergometer.

Exercise protocol. Each subject performed two exercise trials separated by 2 weeks. In the first trial the subjects performed 30 min of a two-legged concentric exercise (normal cycling) at 65% of $V_{O_2,max}$. In the second trial (eccentric exercise), the direction of revolution was reversed and the subjects were opposing the rotation of the pedals to a preset number of pedal revolutions per minute for 20 min at 150% followed by 10 min at 100% of the load eliciting concentric $V_{O_2,max}$. Pilot experiments had indicated that with this protocol the plasma catecholamine levels and HR would be the same after 20 and 30 min of each type of exercise. Furthermore, pilot experiments had shown that two different intensities of eccentric exercise had to be used to mimic catecholamine and HR responses to the concentric exercise regimen. The trial of concentric exercise was always performed before the trial of eccentric exercise to avoid prolonged muscle inflammation, damage, protein breakdown, or elevated muscle enzyme levels in the plasma affecting the second exercise bout. However, with the chosen design some order effects cannot be ruled out.

Experimental protocol. The same experimental protocol was used for each trial. The subjects reported to the laboratory after an overnight fast. Subjects rested on the cycle ergometer for 15 min and a blood sample was drawn from an indwelling intravenous catheter in the hand or forearm (baseline t_0). The subjects then performed 30 min of either concentric or eccentric exercise and blood samples were taken and V_{O_2} recorded after 20 (t_{20}) and 30 min (t_{30}) of exercise. HR was continuously monitored throughout the exercise. Further blood samples were obtained 2 h post-exercise (t_{120}) as well as after 2, 4 and 7 days (day-2, day-4 and day-7, respectively). The subjects refrained from any strenuous and/or organized physical exercise for 7 days after the exercise.

Catecholamines. Blood samples for measurements of catecholamines were drawn into ice-cold glass tubes containing EGTA (1·9 mg (ml blood)$^{-1}$; Sigma) and glutathione in reduced form (1·2 mg (ml blood)$^{-1}$; Merck), pH 6–7. Plasma was stored at −80 °C until analysed by a single-isotope radio enzymatic assay (Kjaer, Christensen, Sonne, Richter & Galbo, 1985).

Clinical chemistry tests. Standard laboratory procedures were employed for the estimation of the level of muscle enzymes, leucocyte subsets, haemoglobin and platelets in the blood. Leucocytes, lymphocytes, monocytes and neutrophils were determined using a Cell Counter (Technicon H.1., Miles Inc., Tarrytown, NY, USA). The anticoagulant was EDTA. Muscle enzymes were measured in lithium heparinized plasma using automated enzyme reactions (automated analysis for Hitachi System 717, Boehringer Mannheim Diagnostica, Germany).

Cytokines. Blood samples for measurements of IL-1β and tumour necrosis factor-α (TNF-α) were drawn into ice-cold glass tubes containing EDTA and trasylol (Bayer; 175 KIU (ml aprotinin)$^{-1}$ – where a KIU is a kallikrein inactivator unit, equivalent to 0·14 μg crystalline aprotinin). Plasma and serum for cytokine detection were stored at −80 °C until analysed by commercially available enzyme-linked immunosorbent assay (ELISA) kits (R and D systems, Minneapolis, MN, USA).

Isolation of blood mononuclear cells. BMNCs were isolated by density gradient centrifugation (Lymphoprep Nyegaard, Oslo, Norway) on LeucoSep tubes (Greiner, Frickenhausen, Germany) and washed three times in medium RPMI (1640 Gibco). The cells were frozen in freezing medium (50% RPMI, 30% fetal calf serum (FCS; Gibco), 20% DMSO (Bie & Berntsen, Rødovre, Denmark)) and kept in liquid nitrogen until thawed for analysis.

Flow cytometry analyses. The following antibodies were used. Phycoerythrin-conjugated monoclonal antibodies: IgG1 (mouse) and CD56 (clone MY31) were from Beckton Dickinson (Oxnard, CA, USA); CD45 RO (clone UCHL1), CD16 (clone DJ130c), CD19 (clone HD37), IgG2 (mouse) and CD14 (clone TÛK4) were from DAKO

Table 1. Plasma catecholamine concentrations in relation to eccentric and concentric exercise

	Adrenaline (ng ml^{-1})		Noradrenaline (ng ml^{-1})	
Time	Eccentric	Concentric	Eccentric	Concentric
t_0	0·07 ± 0·03	0·05 ± 0·01	0·22 ± 0·03	0·25 ± 0·04
t_{20}	0·17 ± 0·03*	0·26 ± 0·09*	1·00 ± 0·15*	1·26 ± 0·20*
t_{30}	0·14 ± 0·02*	0·22 ± 0·04*	1·00 ± 0·13*	1·36 ± 0·17*
t_{120}	0·04 ± 0·01	0·03 ± 0·01	0·25 ± 0·04	0·21 ± 0·03

Values are means ± S.E.M. Here, and throughout tables, abbreviations are as follows. t_0, pre-exercise baseline. t_{20} and t_{30}, after 20 and 30 min of exercise, respectively. t_{120}, 2 h after exercise. * $P < 0·05$, significantly different from baseline.

(Glostrup, Denmark). Fluorescein-conjugated monoclonal antibodies: IgG1 (mouse), CD16 (clone DJ130c) and HLA-DR (clone CR3/43) were from DAKO and CD45RA (clone ALB11) was from Immunotech (Marseille, France). Peridinin chlorophyll protein (PerCP)-conjugated monoclonal antibodies: IgG1 (mouse), CD3 (clone SK7), CD8 (clone SK1) and CD4 (clone SK3) were from Beckton Dickinson. Streptavidin PerCP is a single molecule, with a molecular mass of 35 kDa which absorbs 488 nm light from an argon ion laser. It has a sharp emission profile which peaks at approximately 680 nm. Thawed mononuclear cells were washed twice in phosphate-buffered saline (PBS) with 2% FCS. Cells ($1·0 \times 10^5$), were resuspended in 100 μl PBS containing FCS and incubated for 30 min at 5 °C with antibodies. Labelled cells were washed three times and analysed by flow cytometry using a fluorescence-activated cell sorter analyser (Epics XL-MCL, Coulter, FL, USA). The subsequent computer analyses were carried out by PC lysis software from Becton Dickinson. Phycoerythrin-, fluorescein- and PerCP-conjugated mouse IgG were used as negative controls. A lymphocyte gate was used for all the analyses except for the analysis with CD14 where a BMNC gate was used. Lymphocytes were distinguished from monocytes on the basis of their forward *versus* right-angle light scatters. A mean of the double- and triple-stained samples was used to calculate the percentages of CD3, CD8, CD4, CD19, CD14 and HLA-DR. The absolute number of cells per cubic millimetre of a given lymphocyte subpopulation was calculated by multiplying the proportion of lymphocytes with the lymphocyte cell count, whereas the number of monocytes was calculated by multiplying the proportion with the sum of the lymphocyte and monocyte cell count.

Determination of natural killer cell activity. NK cell activity was measured using K562 tumour target cells in a $^{51}Cr^+$ release assay as previously described (Ullum, Goetzsche, Victor, Dickmeiss, Skinhøj & Pedersen, 1995). BMNCs were thawed and incubated for 1 h at 37 °C with: medium; 1000 i.u. ml^{-1} interferon (IFN-α; kindly provided by Dr Robert Jordal, The Blood Bank, Copenhagen County Hospital, Gentofte, Denmark); or 20 i.u. ml^{-1} IL-2 (Boehringer Mannheim). Triplicates of 100 μl effector cells and 100 μl target cells (10^5 cells ml^{-1}) were incubated in microtitre plates for 4 h at 37 °C. Unstimulated effector cells were added in different concentrations giving effector : target ratios of 100 : 1, 50 : 1 and 25 : 1. IL-2 and IFN-α stimulated effector cells were used at an effector : target ratio of 50 : 1. The plates were centrifuged for 10 min; 100 μl supernatant was transferred to new tubes and radioactivity was determined. Spontaneous release was determined by incubation of 100 μl target cells with 100 μl medium and maximum release was determined by incubation of 100 μl target cells plus 100 μl medium with 10% Triton X-100. The percentage $^{51}Cr^+$ release (NK cell activity) was determined by:

$$\text{Percentage lysis} = 100 \times \left(\frac{\text{test } ^{51}Cr^+ - \text{spontaneous } ^{51}Cr^+}{\text{maximum } ^{51}Cr^+ - \text{spontaneous } ^{51}Cr^+} \right),$$

and was given as the mean value of triplicates. Samples from both the eccentric and the concentric exercise series for each subject were analysed on the same day.

Determination of lymphokine-activated killer cell activity. The LAK cell activity was measured as previously described (Ullum *et al.* 1995). After thawing, BMNCs were incubated with IL-2, 6000 i.u. ml^{-1} (Proleukin, Eurocetus B.V., Amsterdam, The Netherlands) for 48 h at 37 °C. LAK activity was measured in a $^{51}Cr^+$ release assay using DAUDI target cells. A LAK cell suspension of 100 μl and 100 μl target cells at a concentration of 2×10^4 cells ml^{-1} were added to each well in microtitre plates. LAK cells were added in different concentrations giving effector : target ratios of 50 : 1, 25 : 1 and 12·5 : 1. The rest of the $^{51}Cr^+$ release assay was performed as described for the NK cell assay.

Statistics

To test whether the measured parameters were influenced by time and the interaction between time and the type of exercise, a three-way analysis of the variance was carried out:

Model parameter = constant + ID + time + work + (time × work).

If a significant effect ($P < 0·05$) was found, for time only, for time and work, and/or the interaction between time and the type of exercise, then Tukey's test was performed. In the first case this was to localize differences during and after exercise compared with pre-exercise values and in the two latter cases Tukey's test was used to compare the two types of exercise at specific times (ID, identification of the subject).

Before carrying out the statistical analysis, the residuals in the ANOVA were tested for a normal distribution. If this was not the case, the data were log transformed and tested again. This was the case for IL-6, creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), adrenaline and noradrenaline. Thus, for these parameters log data were used in the subsequent statistical analysis.

Statistical calculations were performed using SYSSTAT statistical software (SYSSTAT, Inc., Evanston, IL, USA). In all tests $P < 0·05$ was considered significant.

Table 2. Heart rate during exercise (mean and proportion of the maximal HR), oxygen uptake (absolute and as a proportion of $V_{O_2,max}$), and work (absolute and the proportion of the maximal concentric work) in relation to eccentric and concentric exercise

Time	HR (beats min^{-1})	HR/HR$_{max}$ (%)	V_{O_2} (l min^{-1})	$V_{O_2}/V_{O_2,max}$ (%)	Work (W)	Work/work$_{max}$ (%)
$t_{20} + t_{30}$, concentric exercise	146±2	64±2	2·31±0·08	61±1	141±8	66±1
t_{20}, eccentric exercise	152±5	66±2	1·74±0·12	46±3	343±20	159±2
t_{30}, eccentric exercise	146±4	64±2	1·34±3	36±2	215±13	100±2

Values are given as means ± S.E.M.

RESULTS

Catecholamines, heart rate, oxygen consumption, work effect and body temperature

The concentrations of adrenaline and noradrenaline increased during exercise but had returned to pre-exercise values 2 h later with no significant differences between the two types of exercise (Table 1). The HR during exercise with intervals of 3 min was tested by the ANOVA model as previously described. No significant difference was found between trials (Table 2). V_{O_2} was significantly lower during eccentric exercise (Table 2). Rectal body temperature was measured in six subjects but no significant difference was found between trials (data not shown).

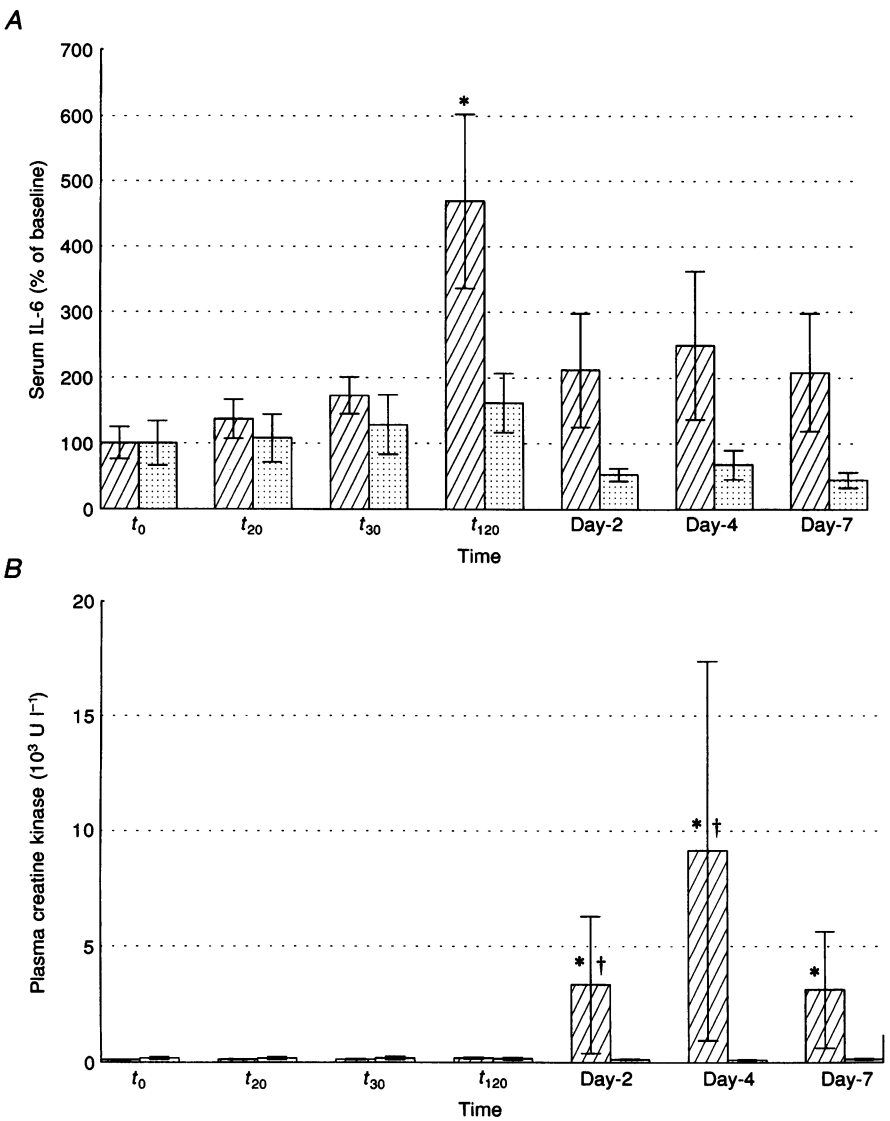

Figure 1. Serum IL-6 (*A*) and plasma CK concentrations (*B*) in relation to eccentric and concentric exercise

Values are given as means ± S.E.M. Here, and in subsequent figures, shadings and abbreviations are as follows. ▨, eccentric exercise; ▩, concentric exercise. t_0, pre-exercise baseline. t_{20} and t_{30}, after 20 and 30 min of exercise, respectively. t_{120}, 2 h after exercise. Day-2, 2 days following exercise; Day-4, 4 days following exercise, Day-7, 7 days following exercise. * $P < 0·05$, significantly different from baseline; † $P < 0·05$, significant difference between the two types of exercise.

Table 3. Plasma concentrations of muscle enzymes in relation to concentric and eccentric bicycle exercise

	ALAT (U l^{-1})		AP (U l^{-1})		LDH (U l^{-1})		ASAT (U l^{-1})	
Time	Eccentric	Concentric	Eccentric	Concentric	Eccentric	Concentric	Eccentric	Concentric
t_0	17±3	24±3	144±8	156±8	299±17	308±14	20±1	23±2
t_{20}	18±2	24±3	160±7*	163±7*	324±17	324±15	21±2	25±2
t_{30}	17±3	24±3	167±8*	163±7*	321±17	332±16	21±1	25±2
t_{120}	17±3	20±3	140±6	146±7	342±26	283±19	21±2	22±2
Day-2	32±12	20±3	142±5	140±7	453±144	281±17	86±54	20±2
Day-4	80±55*	20±3	141±6	145±7	662±318†	286±14	260±216*†	20±2
Day-7	76±48*	20±2	146±6	147±6	406±84	281±11	172±122*†	21±1

ALAT, alanine aminotransferase; AP, alkaline phosphatase; LDH, lactate dehydrogenase; ASAT, aspartate aminotransferase. Day-2, 2 days following exercise; Day-4, 4 days following exercise; Day-7, 7 days following exercise. * $P < 0.05$, significantly different from baseline; † $P < 0.05$, significant difference between the two types of exercise.

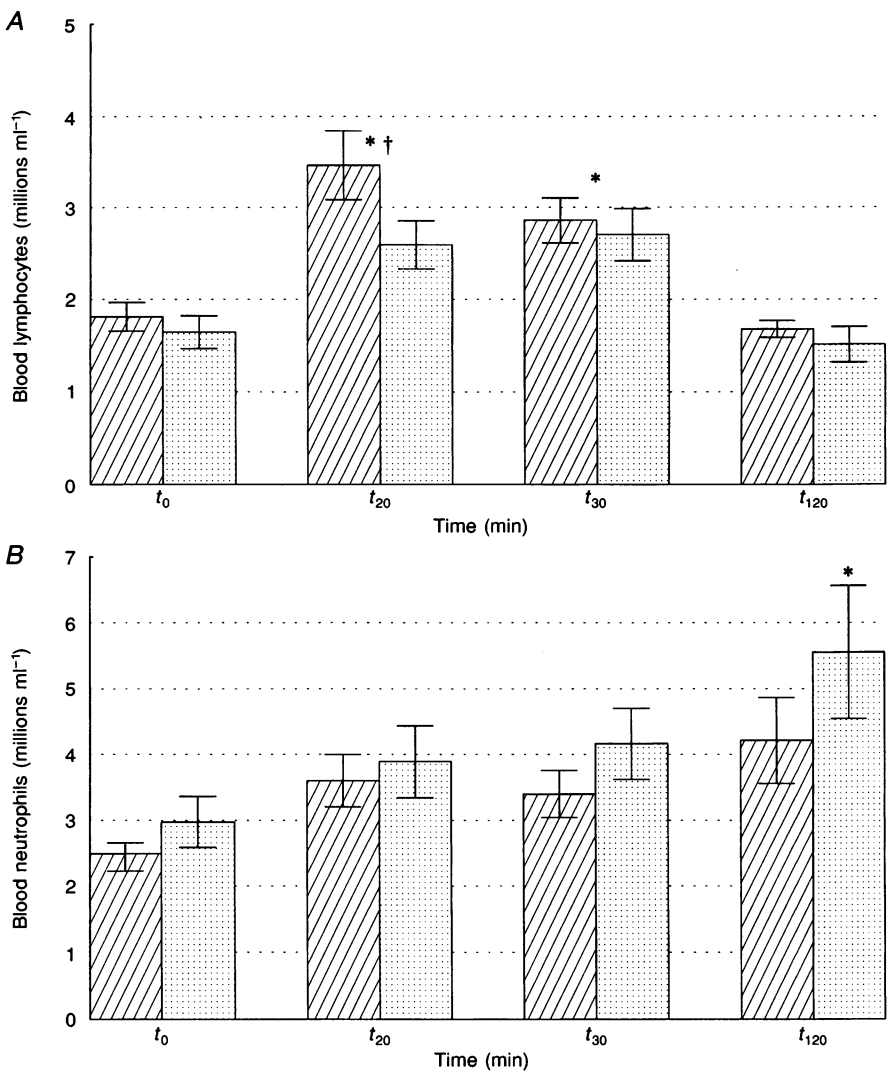

Figure 2. Leucocyte subsets in relation to eccentric and concentric exercise

Blood concentration of lymphocytes (*A*) and neutrophils (*B*). Shading as Fig. 1. Means ± S.E.M. are shown.

Table 4. Flow cytometry analysis

	CD16+CD3−		CD56+CD3−		CD56+CD16+		CD45RA+CD16+		CD8+	
	(%)	(10^6 cells mm^{-3})	(%)	(10^6 cells mm^{-3})	(%)	(10^6 cells mm^{-3})	(%)	(10^6 cells mm^{-3})	(%)	(10^6 cells mm^{-3})
t_0										
Eccentric	7±1	116±17	7±1	119±20	6±1	99±20	7±2	124±31	22±3	414±86
Concentric	5±1	71±22	6±1	88±23	5±1	69±24	8±2	114±35	18±3	278±30
t_{20}										
Eccentric	20±3*	697±154*†	19±3*	700±166*†	16±3*	605±160*†	22±4*	797±208*†	23±2	797±75*†
Concentric	14±2*	340±68*	14±2*	361±70*	11±2*	282±67	14±3*	317±72	22±3	545±73*
t_{30}										
Eccentric	18±3*	514±119*	17±3*	502±123*	15±3*	453±130*	19±4*	551±148	24±2	673±69*
Concentric	15±3*	379±78*	14±2*	379±71*	12±2*	318±75	19±4*	431±107	21±1	581±82*
t_{120}										
Eccentric	6±1	92±11	6±1	92±12	4±1	65±11	6±1	91±18	20±2	339±46
Concentric	5±1	71±14	5±1	77±18	4±1	53±13	5±1	62±18	22±3	298±50

Percentages and concentrations of NK subsets and CD8+ cells in relation to eccentric and concentric bicycle exercise. Values are means ± S.E.M. * $P < 0.05$, significantly different from baseline; † $P < 0.05$, significant difference between the two types of exercise.

Cytokines

The resting values of circulating serum IL-6 (means ± S.E.M.) were 0·748 ± 0·183 in the eccentric trial *versus* 1·530 ± 0·514 pg ml^{-1} in the concentric trial. IL-6 was significantly increased 2 h after eccentric exercise, but not after concentric exercise (Fig. 1*A*). The concentrations of IL-1β and TNF-α were below the detection limit in most subjects.

Muscle enzymes in plasma

Eccentric exercise resulted in a significant increase in plasma CK at day-2, day-4 and day-7 (Fig. 1*B*), and in ASAT and ALAT at day-4 and day-7. In contrast, there was no change in these parameters during or following concentric exercise. Alkaline phosphatase increased during exercise in both types of exercise but was back to pre-exercise values 2 h after exercise (Table 3).

Leucocyte subpopulations

The lymphocyte concentration increased during exercise but returned to baseline level after 2 h in both trials. The level was significantly higher at t_{20} during eccentric compared with concentric exercise (Fig. 2*A*). The concentration of neutrophils was higher 2 h post-exercise compared with baseline, but only after concentric exercise (Fig. 2*B*).

There were no significant differences in the composition (percentages) of BMNC subsets between trials (Table 4). The percentages of all NK cell subsets increased significantly during both types of exercise as did the total concentration of NK subsets. However, the eccentric mode induced a significantly higher concentration in all NK subsets than the concentric mode after 20 min of exercise. This was also the case for the concentration of CD8+ cells (Table 4), whereas no significant differences were found between the two types of exercise regarding the concentrations of CD4+, CD19+, CD14+ and HLA-DR+ cells (data not shown). No change in the proportion CD45RA : CD45RO phenotypes of CD4+ and CD8+ cells was seen (data not shown), indicating that CD45RA and CD45RO phenotypes were recruited to the blood to the same extent. All CD16+ cells expressed CD45RA and no switch to CD45RO was observed during or following exercise.

Haemoglobin, platelets and haematocrit increased during exercise and there was no difference between concentric and eccentric exercise at any time points (data not shown). Thus, changes in plasma volume occurred to a similar extent in both types of work.

NK and LAK cell cytotoxicity

As a result of both types of exercise, NK cell cytotoxicity increased when the cells were unstimulated (effector : target ratio 50:1, 25:1 and 12·5:1) and after stimulation with IL-2 and IFN-α. No differences from the baseline were observed 2 h post-exercise (Fig. 3*A*, *B* and *C*). There were no significant differences between the two types of exercise.

Regarding LAK cell cytotoxicity, there was an increase in lysis of target cells after 20 min of exercise at the effector : target ratio 50:1 as a result of both types of exercise, but there were no differences from pre-exercise values after 30 min of exercise or at 2 h post-exercise. At the effector : target ratios 25:1 and 12·5:1, LAK cytotoxicity did not increase significantly during exercise, but was significantly decreased after exercise compared with the pre-exercise value. There were no significant differences between the two types of work.

Correlation analyses

Pearson's correlation analysis was used to test whether the subjects with the largest IL-6 responses also had the most marked haematological and plasma CK changes. We chose to correlate IL-6 2 h post-exercise with the lymphocyte concentration during exercise and CK concentration in the following days. These particular parameters were chosen as they revealed significant differences between trials. Prior to analyses the CK and IL-6 data were log transformed to obtain a normal distribution. All subjects peaked in plasma CK on day-4 or -7 in relation to the eccentric trial and on day-2, -4 or -7 in relation to the concentric trial. It is not clear whether the highest plasma CK was reached in-between these days or beyond day-7 for some subjects. Therefore, we chose to correlate IL-6 to the mean CK of day-4 and day-7 (log-mean CK_1), the mean CK of day-2, -4 and -7 (log mean CK_2), and the observed peak in CK (CK_{peak}), respectively. A significant correlation ($r = 0{\cdot}722$, $P = 0{\cdot}028$) was found between log IL-6 and log mean CK_1 in the eccentric trial. Similar results were found for log mean CK_2 and log CK_{peak}. A significant correlation was found between log IL-6 and the lymphocyte level after 20 min of eccentric exercise ($r = 0{\cdot}725$, $P = 0{\cdot}042$) but not between log IL-6 and the lymphocyte level after 30 min of eccentric exercise or to the mean of the levels at t_{20} and t_{30}. Furthermore, no significant correlations were found between log IL-6 and the level of CD56+CD16+, CD16+CD3−, CD56+CD3− NK cells, or CD8+ T cells. No significant correlations were found between these parameters in the concentric trial. No correlations were found between log CK concentration and lymphocyte levels at t_{20}, t_{30}, or the mean level of t_{20} and t_{30} in trials.

DISCUSSION

Serum IL-6 was found to be significantly increased 2 h after eccentric exercise, but not after concentric exercise. This may reflect a pronounced inflammation in the muscle and be a factor causing further muscle damage indicated by the increased levels of CK, ASAT and ALAT in the blood during the following week. Supporting this hypothesis is the finding of a significant correlation between the level of IL-6

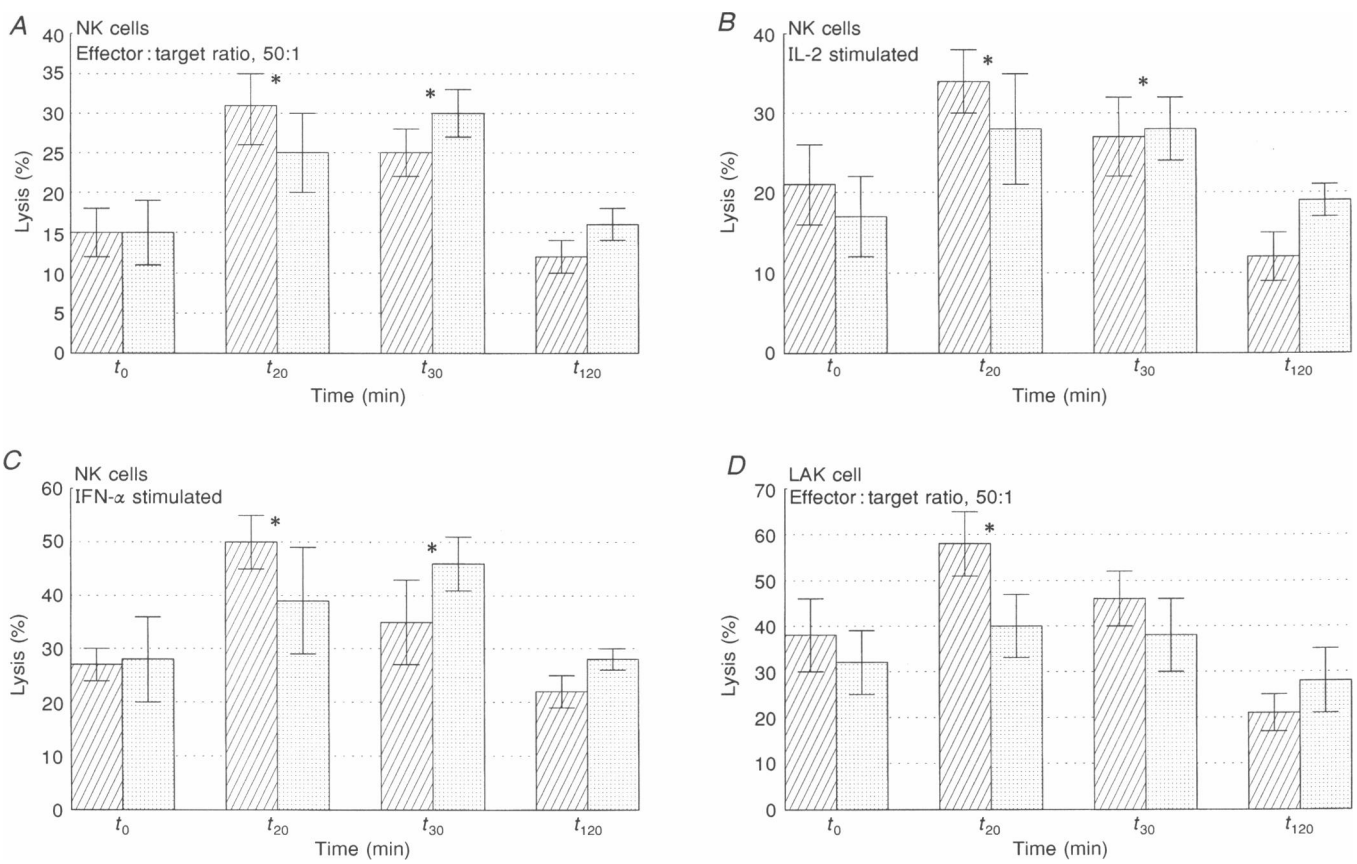

Figure 3. Non-major histocompatibility complex restricted cytotoxity in relation to eccentric and concentric exercise

NK cell cytotoxicity unstimulated (*A*), after 1 h of IL-2 stimulation (*B*) and after 1 h of IFN-α stimulation (*C*). *D*, LAK cell activity. Means ± S.E.M. are shown. Shading as Fig. 1. * $P < 0{\cdot}05$, significantly different from baseline.

2 h after eccentric exercise and plasma CK in the following days. To our knowledge, no comparable observations regarding the effect of eccentric *versus* concentric exercise on cytokines have been carried out before the present study. Furthermore, confusing results have been obtained using different cytokine assays which make direct comparisons between individual studies very difficult. Exercise-induced changes in cytokine levels have mainly been described in relation to eccentric exercise. Increased IL-1β in muscle tissue for up to 5 days (Cannon, Fielding, Fiatarone, Orencole, Dinarello & Evans, 1989) and increased plasma IL-1β (Evans *et al.* 1986), IL-6 (Northoff & Berg, 1991) and TNF-α (Espersen *et al.* 1990) following intense eccentric exercise have been reported. The present study was unable to detect IL-1β and TNF-α in plasma. In agreement with this, Northoff & Berg (1991) were unable to detect IL-1β and TNF-α at the completion of a marathon using an ELISA assay. Regarding increased plasma IL-1β, Evans *et al.* (1986) used a murine thymocyte proliferation bioassay in combination with column chromatography to identify IL-1 activity in plasma after exercise. An anti-IL-1 antibody was employed which effectively neutralized the ability of chromatographed fractions to stimulate thymocyte proliferation. The study was conducted prior to the availability of recombinant IL-1 proteins and the used antibody was produced against a purified antigen. It has been proposed that other cytokines were present in the purified fraction used to immunize rabbits and these might have contributed to the detected activity. Furthermore, the thymocyte proliferation assay also detects IL-6 and the plasma component responsible for the proliferative response in the thymocyte bioassay might have been IL-6 instead of IL-1 (Bagby, Crouch & Shepherd, 1996). Espersen *et al.* (1990) used immunoassays in their study but concern has been expressed (Bagby *et al.* 1996) regarding higher resting values than typically reported. In response to a concentric bicycle exercise at 75% of $V_{O_2,max}$ for 1 h, a small increase in the plasma level of IL-6 has been demonstrated, whereas the plasma levels of IL-1α, IL-1β and TNF-α were below detection level in most subjects (Ullum, Haahr, Diamant, Palmo, Halkjaer-Kristensen & Pedersen, 1994). In that study pre-mRNA for IL-1α, IL-1β, IL-6 and TNF-α could be detected in BMNCs by nuclear run-off analysis, but the amounts did not change in relation to exercise, making it unlikely that the increased plasma level of IL-6 was a result of activated monocytes in the peripheral blood. In the present study, eccentric exercise did not cause a higher increase in the blood concentration of monocytes and neutrophils compared with concentric exercise as would have been expected if these cells were responsible for producing IL-6, or if the increment in these cells was partly a consequence of inflammation in the muscles. It was found that IL-6 2 h after eccentric exercise was positively correlated with the concentration of circulating lymphocytes after 20 min of exercise. Beside being a monokine, IL-6 is also known to be a T helper 2 cytokine (Mosmann & Sad, 1996). It is possible that circulating lymphocytes produce the IL-6. On the other hand, since correlation analysis does not demonstrate whether a causal relation is present, a third factor (i.e. hormonal factors) may cause the increase in both parameters. Thus, the source of the increased blood concentration of IL-6 still needs to be elucidated. It is possible that the source may be macrophages, endothelial cells and fibroblasts in the muscle, or infiltrating leucocytes from the blood. As expected, the blood concentration of monocytes and neutrophils increased during exercise and continued to increase following exercise. The total lymphocyte count increased during exercise and NK cells in particular were recruited to the blood. The lymphocyte concentration returned to baseline 2 h post-exercise. NK and LAK cell cytotoxicity increased during exercise and was back to baseline 2 h after exercise. These data are in agreement with previous observations (Pedersen, Kappel, Klokker, Nielsen & Secher, 1994) and the finding of no post-exercise suppression is consistent with the fact that the exercise was of short duration and moderate intensity. Neither concentric nor eccentric exercise changed the relative fraction of activated lymphocyte phenotypes; all CD16+ cells expressed CD45RA before, during and following exercise and the proportion CD45RA (resting, 'immunologically naive') : CD45RO (activated, 'memory') of CD4+ and CD8+ cells did not change either.

No significant differences were found between eccentric and concentric exercise regarding the composition (percentages) of BMNC subpopulations. With regard to the concentration of BMNCs, eccentric exercise induced a significantly higher recruitment of NK cells and CD8+ cells after 20 min (high workload) than concentric exercise. This is in agreement with the findings of Pizza, Mitchell, Davis, Starling, Holtz & Bigelow (1995), who found a more pronounced increase in circulating lymphocyte level at the end of a 60 min downhill run compared with 60 min of level running at the same HR (Pizza *et al.* 1995). Since there was no significant difference in the catecholamine level between the two forms of exercise in the present study, factors other than catecholamines may also contribute to the recruitment of NK and CD8+ cells during exercise. It is unlikely that IL-6 is such a factor since the peak in lymphocytes preceded the peak in IL-6. Neither was a difference in body temperature responsible.

In addition to exercise, several other physical stressors have been shown to induce similar changes in the cellular immune system. Physical stressors associated with muscle damage and trauma such as burns, surgery and acute myocardial infarction, induce an increased cytokine response, whereas there is no cytokine response to head-up tilt and hyperthermia (Pedersen *et al.* 1994). These data and the present findings regarding differences in cytokine response to eccentric and concentric exercise support the notion that the cytokine response is closely related to muscle inflammation.

In conclusion, high-intensity eccentric exercise caused a more pronounced increase in the plasma level of IL-6 than

did concentric exercise, even when the former was accompanied by a lower oxygen uptake. This finding was probably related to muscle damage and ensuing inflammatory reactions in the skeletal muscle reflected in exaggerated levels of CK and ASAT activities in the week following eccentric exercise. Correspondingly, a significant correlation existed between IL-6 2 h after eccentric exercise and plasma CK activity in the following days. Since eccentric, compared with concentric, exercise caused similar increases in catecholamines but a more pronounced increase in the concentrations of NK and CD8+ cells, factors related to muscle damage and inflammation may add to the effect of catecholamines in the recruitment of these cells.

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