# *Changes in inflammatory mediators following eccentric exercise of the elbow flexors*

**Running Title : Eccentric exercise and cytokines**

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## Abstract

*The aims of this study were to examine the plasma concentrations of inflammatory mediators including cytokines induced by a single bout of eccentric exercise and again 4 weeks later by a second bout of eccentric exercise of the same muscle group. Ten untrained male subjects performed two bouts of the eccentric exercise involving the elbow flexors (6 sets of 5 repetitions) separated by four weeks. Changes in muscle soreness, swelling, and function following exercise were compared between the bouts. Blood was sampled before, immediately after, 1 h, 3 h, 6 h, 24 h (1 d), 48 h (2 d), 72 h (3 d), 96 h (4 d) following exercise bout to measure plasma creatine kinase (CK) activity, plasma concentrations of myoglobin (Mb), interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), IL-4, IL-6, IL-8, IL-10, IL-12p40, tumor necrosis factor (TNF)-α, granulocyte colony-stimulating factor (G-CSF), myeloperoxidase (MPO), prostaglandin $E_2$ ($PGE_2$), heat shock protein (HSP) 60 and 70. After the first bout, muscle soreness increased significantly, and there was also significant increase in upper arm circumference; muscle function decreased and plasma CK activity and Mb concentration increased significantly. These changes were significantly smaller after the second bout compared to the first bout, indicating muscle adaptation to the repeated bouts of the eccentric exercise. Despite the evidence of greater muscle damage after the first bout, the changes in cytokines and other inflammatory mediators were quite minor, and considerably smaller than that following endurance exercise. These results suggest that eccentric exercise-induced muscle damage is not associated with the significant release of cytokines into the systemic circulation. After the first bout, plasma G-CSF concentration showed a small but significant increase, whereas TNF-α and IL-8 showed significant decreases compared to the pre-exercise val-*

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*ues. After the second bout, there was a significant increase in IL-10, and a significant decrease in IL-8. In conclusion, although there was evidence of severe muscle damage after the eccentric exercise, this muscle damage was not accompanied by any large changes in plasma cytokine concentrations. The minor changes in systemic cytokine concentration found in this study might reflect more rapid clearance from the circulation, or a lack of any significant metabolic or oxidative demands during this particular mode of exercise. In relation to the adaptation to the muscle damage, the anti-inflammatory cytokine IL-10 might work as one of the underlying mechanisms of action.*

**Keywords:** cytokines, eccentric exercise

## Introduction

Cytokines are a diverse family of intercellular signaling molecules that are important for haematopoiesis, inflammatory and immune responses. Based on their physiological effects, cytokines are generally classified as „pro-“ and „anti-“ inflammatory cytokines (21, 41). The pro-inflammatory cytokines include tumor necrosis factor (TNF)-α and interleukin (IL)-1β, which are considered to promote inflammatory responses followed by muscle damage (29, 32). In contrast, anti-inflammatory cytokines such as IL-4, IL-10 and IL-1 receptor antagonist (IL-1ra), which blocks the IL-1 activity, inhibit inflammatory responses by reducing the actions of pro-inflammatory cytokines (21, 41). IL-6 and granulocyte colony-stimulating factor (G-CSF) can act as both pro- and anti-inflammatory cytokines, depending on the situation (1, 12-14, 21, 30, 32, 33, 38, 41, 43).

Intense exercise induces muscle damage and subsequent inflammation indicated by muscle soreness and swelling, prolonged loss of muscle function, and the leakage of muscle proteins, such as creatine kinase (CK) and myoglobin (Mb), into the circulation (27, 36, 39, 40). It is well known that eccentric exercise induces greater muscle damage and inflammation than concentric or isometric exercise (27). It has been reported that plasma concentrations of cytokines are affected by the mode of exercise (2, 7, 15, 20, 34, 39-43, 45, 46). The magnitude of the changes in plasma cytokine concentrations depends on the intensity and duration of exercise, in addition to the type of the muscle contraction. As reviewed by previous articles (32, 33, 41, 43), there are large increases in plasma cytokine levels during and after prolonged endurance exercise. Briefly, the plasma levels of TNF-α, IL-1β, and IL-8 increase to 2- to 3-fold, whereas IL-6 increases as much as 100-fold after a competitive marathon race (24, 32, 33, 40-43). Plasma levels of IL-1ra increase to a similar extent, but continue to increase within several hours after exercise (40, 46). Plasma IL-10 concentration also increases by 30-60 times after exercise (23, 25, 43). Thus, there is evidence that intense and prolonged exercise induces changes in the pro-inflammatory/anti-inflammatory cytokine balance within the systemic circulation toward a greater level of anti-inflammatory cytokines.

It is natural to consider that if the muscle damage and inflammation is greater, the alteration of plasma cytokine levels is likely to be larger. However, although eccentric exercise (i.e., elbow/knee flexions) causes greater local muscle damage than prolonged endurance exercise, alterations in plasma cytokine levels appear to be smaller (See review: 40). There is also inconsistency between the profiles of cytokine alteration by the different modes of eccentric exercise. For example, IL-6 increased 20-fold after downhill treadmill running (34), whereas it increased about 3- to 7-fold after eccentric bicycle exercise where local muscle was used (3, 15). Plasma IL-1ra concentration gradually increased 3.5 times up to 2 h after downhill treadmill running (34), but did not change and was not detected after the cycling. IL-1β decreased 6 h and 1 d after exercise when local muscle was loaded during repeated bench press curls (37). With downhill treadmill running and cycling, IL-1β was not detected (3, 5, 15). Thus, the magnitude and profile of cytokine responses to the exercise differ not only between endurance vs. eccentric exercise but also in relation to changes in systemic vs. local factors during exercise, aerobic vs. anaerobic components of metabolic demands, and blood redistribution, etc.



Adaptation to eccentric exercise occurs when the same exercise is repeated using the same muscle. In a previous study (27), it has been demonstrated that muscle soreness, swelling, loss of muscle function, and the myocellular protein release following the second bout is much smaller than those after the first bout. This means that muscles damaged in the first bout of eccentric exercise not only recover but also adapt rapidly to the same exercise, and muscle becomes less susceptible to damage resulting from the same exercise stimuli (27). Cytokines are believed to play important roles in the inflammatory responses to muscle damage and subsequent regeneration (4, 21). Therefore, the profile of inflammatory mediators including cytokines might be different between the first and second bouts. However, it is unknown what effects repeated bouts of eccentric exercise have on inflammatory mediators. In the present study, we hypothesized that an initial bout of eccentric exercise would cause a greater increase in markers of muscle damage and pro-inflammatory cytokines than the second bout of the same exercise. We also hypothesized that the second bout of exercise would increase anti-inflammatory cytokines to a greater extent than the first bout, as one of the adaptive mechanisms to exercise and inflammation. Therefore, we examined alterations in plasma concentration of cytokines and inflammatory markers following eccentric exercise of the elbow flexors, which has been known to induce greater local muscle damage than any other experimental exercise conditions. To investigate the involvement of cytokines in the repeated bout effect, this study compared these variables between two bouts of the eccentric exercise separated by four weeks.

## Methods

### Subjects

Subjects were ten untrained male university students whose characteristics were as follows (mean ± SE): age 20.4 ± 2.0 years, height 171.3 ± 3.7 cm, and body mass 60.8 ± 3.5 kg. None of them performed strenuous exercise for at least one week before the experiment, and had participated in regular resistance training. All subjects were free from any musculoskeletal disorders and diseases during the experimental period. Subjects were requested to avoid any vigorous exercise apart from the exercise performed in the study, and to take no medication or dietary supplements during the experimental period. Subjects were informed of

the experimental procedures and possible risks that were approved in advance by the Institutional Ethics Committee. The experimental study was conducted in conformity with the Declaration of Helsinki.

**Exercise protocol**
Each subject performed two bouts of eccentric exercise of the elbow flexors using the non-dominant arm separated by four weeks (27). Subjects were seated on a bench with the non-dominant arm positioned in front of the body on a padded support, and the forearm was kept supinated throughout the range of motion while holding a dumbbell. The dumbbell was set at 40% of each subject's maximal isometric strength at an elbow joint angle of 90° immediately before each exercise bout. Subjects were asked to lower the dumbbell from an elbow flexed (90°) to a fully extended position (180°) in 3-4 seconds in a controlled manner keeping the velocity as constant as possible. After each eccentric action, an investigator took the dumbbell from the subjects' hand and set the dumbbell to the flexed position to eliminate any concentric actions. Subjects performed 6 sets of 5 eccentric actions with a set interval of 2 minutes.

**Indirect markers of muscle damage**
**Muscle soreness**
Muscle soreness was evaluated by a visual analogue scale (VAS) consisting of a 50mm line with „no pain" at one end and „extremely sore" at the other. With the subject in a seated position and the exercised arm resting on a table, the investigator palpated the elbow flexors (three portions on the upper arm: mid-belly of the biceps brachii and 3-cm above and below the mid belly), then passively extended and flexed the joint to test each subjects perception of soreness. Subjects were asked to indicate the pain level on the line for each of these evaluations.

**Upper arm circumference**
Upper arm circumference was measured by a constant tension tape measure at 3, 5, 7, 9 and 11 cm proximal to the elbow joint. The five measurement sites were marked with a semi permanent ink pen at the first testing session, and remarked at each test. During the measurement, subjects stood with the arm hanging in a relaxed position by the side. Two measurements were taken from each site, and the average of the two was used for further analyses.

**Range of motion (ROM)**
A plastic goniometer was used to measure elbow joint angles for the relaxed (RANG), stretched (SANG) and flexed (FANG) positions. Subjects stood with the arm hanging in a relaxed position at the side and the goniometer was applied twice to measure RANG. Subjects were then asked to extend the elbow as much as they could, and the elbow joint angle was determined for SANG. For FANG, subjects were asked to flex the elbow as much as possible to touch the shoulder with the palm while keeping the elbow joint at the side. These measurements were taken twice, and the average of the two was used for further analyses. ROM was defined as the difference between SANG and FANG.



**Muscle strength**
Maximal isometric strength was measured using a transducer (model 100, Takei Scientific Instruments, Niigata, Japan) connected to an Apple computer (Macintosh Performer 5410, Apple Computer, Cupertino, USA) via a Power Lab system with a provided software programme (Power Lab /8SP, AD Instruments, Castle Hill, Australia). Subjects were seated on a specially designed bench, and the arm was positioned in front of the body with the shoulder joint at 90º flexions and elbow joint angle was also flexed to 90º (27). Subjects were asked to perform 2 maximal isometric contractions for 3 seconds each with 30 seconds between trials. The mean of two trials was used for further analyses.

**Blood sampling**
Approximately 7 ml peripheral blood was collected from an antecubital vein using a disposable needle and vacutainer containing heparin. Peripheral venous blood was drawn immediately before (Pre), immediately after (Post), and 1 h, 3 h, 6 h, 24 h (1 d), 48 h (2 d), 72 h (3 d), 96 h (4 d) after each exercise bout. The blood was centrifuged for 10 min to separate plasma. The samples were stored at -40°C until analyses of CK, Mb and inflammatory mediators; IL-1β, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-12p40, TNF-α, G-CSF, MPO as a marker of neutrophil activation, $PGE_2$ which is the metabolite of arachidonic acids, and HSP 60 and HSP 70 as stress proteins.

**Measurements of plasma substances**
Plasma CK activity was measured spectrophotometrically (VP-Super, Dinabott, Tokyo, Japan) using a test kit (Dinabott, Tokyo, Japan). Plasma Mb concentration was determined on a biochemical analyzer (Model TBA-30A, Toshiba, Tokyo, Japan) using a commercially available assay kit (Denka-Seiken Co, Tokyo, Japan). The normal reference ranges for male adults using these methods are 45-135 IU/l for CK and <85 ng/ml for Mb. For the measurement of cytokines, commercially available enzyme-linked immunosorbent assay (ELISA) kits were used according to the manufacturers' instructions. IL-1β, IL-1ra, IL-4, IL-6 and TNF-α were determined with AN'ALYZA (TECHNE Co, Minnesota, USA). The IL-1β kit does not detect the inactive precursor of IL-1β, and the IL-6 kit directly measures the total amount of IL-6 without the interference of soluble IL-6 receptor. IL-8, IL-10 and IL-12p40 were measured with OptEIA (Becton Dickinson Biosciences, California, USA). G-CSF was measured with Human G-CSF Kit (Immuno-Biological Laboratories Co, Gunma, Japan). MPO was measured with BIOXYTECH MPO-EIA (OXIS International, Inc., Oregon, USA). $PGE_2$ was measured by Correlate-CLIA High Sensitivity Chemiluminescence Enzyme Immunoassay Kit (Assay Designs, Inc., Michigan, USA). HSP60 and HSP70 were measured with StressXpress (Stressgen, British Columbia, Canada). The absorbance was measured spectrophotometrically with a microplate reader (VersaMax, Molecular Devices, California, USA) and the concentration of each substance was calculated by comparison to a calculation curve established in the same measurement.

**Statistical analyses**
Changes in the measures over time were compared between bouts using a two-

Table 1. Changes in some indirect markers of muscle damage after the eccentric exercise bouts.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Muscle soreness assessed by a visual analogue scale (mm) | | | | | | | |
| | Pre | Post | 1 d | 2 d | 3 d | 4 d | |
| 1st bout | 0 | 0±0 | 33.3±2.7 | 37.5±3.8 | 36.2±4.0 | 25.0±3.4 | |
| 2nd bout | 0 | 0±0 | 14.3±2.6 | 20.4±2.8 | 2.0±3.0 | 5.1±2.7 | |
| Circumference (changes from Pre: mm) | | | | | | | |
| | Pre | Post | 6 h | 1 d | 2 d | 3 d | 4 d |
| 1st bout | 0 | 6.5±0.9 | 3.3±0.7 | 5.8±0.8 | 6.4±1.0 | 9.1±1.1 | 13.9±1.3 |
| 2nd bout | 0 | 6.7±0.8 | 1.3±0.6 | 4.4±1.0 | 5.2±0.6 | 5.6±0.7 | 5.3±0.9 |
| Range of motion (changes from Pre in degree: %) | | | | | | | |
| | Pre | Post | 6 h | 1 d | 2 d | 3 d | 4 d |
| 1st bout | 0 | -30.2±2.9 | -21.8±2.6 | -21.4±2.3 | -19.1±2.0 | -16.5±1.5 | -18.0±2.0 |
| 2nd bout | 0 | -33.3±3.1 | -13.4±1.2 | -9.8±2.1 | -7.7±2.0 | -4.7±1.2 | -3.8±1.3 |
| Maximal isometric strength (comparison with Pre: %) | | | | | | | |
| | Pre | Post | 6 h | 1 d | 2 d | 3 d | 4 d |
| 1st bout | 100 | 29.1±5.2 | 37.9±5.6 | 8.9±3.4 | 57.2±3.7 | 60.5±3.1 | 62.8±4.0 |
| 2nd bout | 100 | 31.7±4.7 | 60.5±4.6 | 71.2±4.0 | 81.0±4.2 | 88.3±3.9 | 94.4±3.5 |

way repeated measures analysis of variance (ANOVA). Significance in each bout was assessed by one-way repeated measures ANOVA to obtain the main effect of time. The changes from pre-exercise values were compared using Tukey post-hoc test. Associations among measured variables were analyzed using Pearson's linear regression (coefficient, *r*). The statistical significance was set at P<0.05. Data are presented as means ± SE.

## Results

As shown in Table 1, there were significant alterations in indirect markers of muscle damage. Muscle soreness was significantly greater following the first than the second bout for all soreness assessments. For example, peak soreness while extending the elbow joint, which occurred between 1 d and 3 d after exercise, was approximately one half of that of the first bout after the second bout (Table 1). Increases in upper arm circumferences, which are indicative of swelling, were significantly larger 3 d and 4 d after the second bout compared to the first bout (Table 1). ROM after both exercise bouts reduced, but the magnitude of change was significantly smaller after the second bout compared to the first bout. In addition, maximal isometric strength was still approximately 60% of the baseline 4 d after the first bout, but it recovered to the baseline by 4 d after the second bout. The recovery of the ROM was also significantly faster after the second bout compared to the first bout.

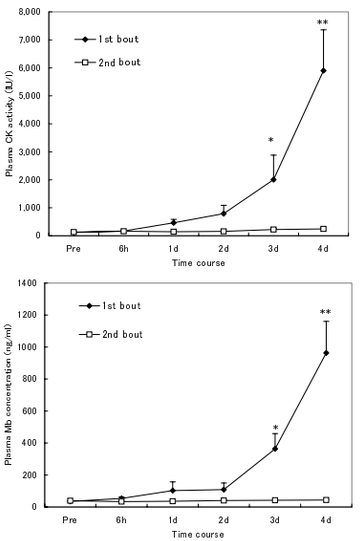


Figure 1. Plasma creatine kinase (CK) activity (A), myoglobin (Mb) concentration (B) before (Pre), 6 h, 1 d, 2 d, 3 d and 4 d after exercise. Data are mean±SE. n=10. Significantly different between the bouts (* P<0.05, ** P<0.01).



There were significant differences in plasma CK and Mb responses between the bouts. In the first bout, the plasma level of CK (Fig. 1A) and Mb (Fig. 1B) increased gradually up to 4 d after exercise and significant differences were observed in both parameters from 3 d to 4 d after exercise. Plasma CK and Mb levels were not significantly altered after the second bout.

On the other hand, there were no significant differences between the two bouts with respect to the inflammatory mediators. However, although the magnitude of change was small, some cytokines showed significant changes after exercise when compared to the pre-exercise values, as summarized in Table 2. G-CSF increased by about 60% 1 h, 3 h and 3 d after the first bout significantly (Fig. 2A). In contrast, TNF-α significantly decreased by about 15% 1 d, 3 d and 4 d after the first exercise bout (Fig. 2B), and IL-8 also decreased by 70-80% from 6 h to 4 d post-exercise (Fig. 2C). After the second bout, IL-10 increased significantly by about 2-fold 1 h and 6 h after exercise. Conversely, IL-8 decreased by 60-70% 1 h, 6 h, 2 d, 3 d, and 4 d after the second bout as compared to the pre-exercise values. No significant changes were found in plasma IL-1β, IL-1ra, IL-6, MPO, $PGE_2$, and HSP 60 and HSP 70 at any time after either exercise bout.

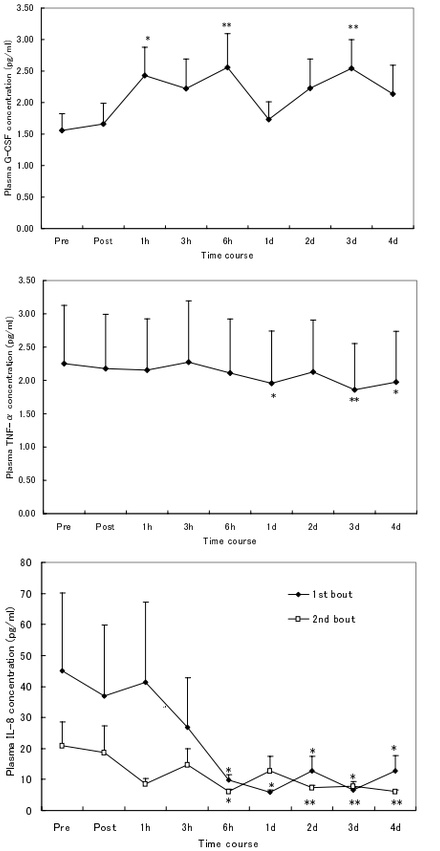


Figure 2. Plasma concentrations of granulocyte colony-stimulating factor (G-CSF) (A), tumor necrosis factor (TNF)-α (B), interleukin (IL)-8 (C) before (Pre), immediately after (Post), 1 h, 3 h, 6 h, 1 d, 2 d, 3 d and 4 d after exercise. Data are mean±SE. n=10. Significantly greater than the Pre values (* P<0.05, ** P<0.01).

Table 3 shows correlations between the post-exercise changes from pre-exercise values in each parameter. After the first bout, G-CSF was positively correlated with CK and Mb. In addition, TNF-α was negatively correlated with CK and Mb, and significant correlations between G-CSF and TNF-α were also shown. There were no significant correlations among parameters in the second bout.

Table 2. Changes in plasma inflammatory mediators after the eccentric exercise bouts (pg/ml, mean±SE).

| 1st bout | Pre | Post | 1 h | 3 h | 6 h | 1 d | 2 d | 3 d | 4 d |
|---|---|---|---|---|---|---|---|---|---|
| G-CSF | 1.56±0.27 | 1.66±0.33 | 2.43±0.45 | 2.22±0.47 | 2.56±0.54 | 1.73±0.28 | 2.22±0.46 | 2.54±0.46 | 2.14±0.46 |
| TNF-α | 2.25±0.87 | 2.18±0.81 | 2.15±0.77 | 2.27±0.92 | 2.11±0.81 | 1.96±0.78 | 2.13±0.78 | 1.86±0.69 | 1.98±0.76 |
| IL-1β | 0.21±0.10 | 0.17±0.05 | 0.13±0.06 | 0.16±0.11 | 0.11±0.04 | 0.10±0.02 | 0.11±0.01 | 0.10±0.02 | 0.13±0.03 |
| IL-1ra | 171.19±32.37 | 182.38±31.00 | 176.69±37.17 | 187.31±38.28 | 170.32±25.57 | 125.18±18.32 | 140.39±22.63 | 114.68±13.08 | 177.43±25.13 |
| IL-4 | 0.12±0.004 | 0.13±0.01 | 0.13±0.01 | 0.12±0.01 | 0.14±0.02 | 0.13±0.01 | 0.12±0.005 | 0.12±0.01 | 0.13±0.01 |
| IL-6 | 1.26±0.45 | 1.32±0.45 | 0.82±0.17 | 0.64±0.14 | 0.57±0.13 | 1.20±0.54 | 0.95±0.31 | 1.10±0.43 | 0.87±0.32 |
| IL-8 | 44.94±25.30 | 37.02±22.76 | 41.45±25.70 | 26.94±15.93 | 9.91±1.57 | 5.89±0.84 | 12.68±4.88 | 6.59±0.80 | 12.81±5.03 |
| IL-10 | 2.71±1.26 | 1.27±0.24 | 1.60±0.60 | 1.67±0.45 | 1.45±0.31 | 2.42±0.54 | 2.19±0.54 | 2.34±0.52 | 3.67±1.53 |
| IL-12p40 | 64.06±16.91 | 76.69±26.92 | 68.64±15.98 | 73.35±17.24 | 73.68±14.68 | 71.84±16.97 | 57.57±12.38 | 66.29±14.50 | 72.87±16.41 |
| $PGE_2$ | 704.10±139.01 | 661.80±115.25 | 602.52±72.95 | 812.41±150.09 | 859.08±95.49 | 541.32±48.79 | 677.00±150.12 | 707.73±120.13 | 528.39±104.43 |
| HSP60 | 2.51±0.91 | 2.41±0.84 | 2.13±0.66 | 2.29±0.71 | 2.55±0.80 | 2.24±0.73 | 2.66±0.84 | 1.72±0.55 | 1.83±0.51 |
| HSP70 | 8.47±0.89 | 12.04±2.58 | 8.07±0.73 | 10.23±1.90 | 7.89±0.53 | 6.84±1.31 | 9.97±2.10 | 7.91±1.42 | 10.28±1.23 |
| MPO | 2.88±0.67 | 2.88±0.61 | 3.08±0.74 | 2.68±0.77 | 2.83±0.61 | 2.52±0.39 | 2.56±0.51 | 2.22±0.40 | 3.11±0.77 |

Pre: before exercise, Post: after exercise, G-CSF: granulocyte colony-stimulating factor, TNF: tumor necrosis factor, IL: interleukin, IL-1ra: IL-1 receptor antagonist, $PGE_2$: prostaglandin $E_2$, HSP: heat shock protein, and MPO: myeloperoxidase

Table 2. Changes in plasma inflammatory mediators after the eccentric exercise bouts (pg/ml, mean±SE).

| 2nd bout | Pre | Post | 1 h | 3 h | 6 h | 1 d | 2 d | 3 d | 4 d |
|---|---|---|---|---|---|---|---|---|---|
| G-CSF | 1.71±0.28 | 2.23±0.28 | 2.24±0.42 | 1.93±0.37 | 2.27±0.40 | 1.72±0.28 | 1.91±0.29 | 2.10±0.43 | 2.82±0.73 |
| TNF-α | 2.21±0.86 | 2.24±0.85 | 2.08±0.74 | 2.19±0.74 | 1.94±0.72 | 2 .09±0.78 | 2.03±0.70 | 1.90±0.70 | 1.95±0.66 |
| IL-1β | 0.17±0.05 | 0.19±0.07 | 0.15±0.04 | 0.14±0.01 | 0.11±0.02 | 0.13±0.01 | 0.11±0.01 | 0.10±0.01 | 0.11±0.02 |
| IL-1ra | 127.92±18.03 | 139.27±19.44 | 152.10±18.66 | 158.67±28.17 | 140.19±21.79 | 140.19±20.56 | 136.93±24.31 | 141.65±23.88 | 111.21±11.68 |
| IL-4 | 0.14±0.01 | 0.13±0.01 | 0.12±0.01 | 0.13±0.01 | 0.13±0.01 | 0.15±0.02 | 0.13±0.01 | 0.13±0.01 | 0.19±0.05 |
| IL-6 | 0.85±0.16 | 1.15±0.45 | 1.20±0.30 | 3.00±1.11 | 1.30±0.38 | 1.57±0.56 | 1.04±0.40 | 0.84±0.35 | 0.88±0.41 |
| IL-8 | 20.95±7.65 | 18.67±8.60 | 8.57±1.71 | 14.70±5.35 | 6.17±0.67 | 12.77±4.65 | 7.27±0.82 | 7.49±1.30 | 6.09±0.54 |
| IL-10 | 1.43±0.29 | 1.25±0.24 | 2.86±1.13 | 1.50±0.29 | 3.36±1.17 | 2.01±0.38 | 2.66±0.59 | 2.49±0.76 | 1.61±0.36 |
| IL-12p40 | 66.36±14.58 | 67.65±16.42 | 57.51±12.33 | 78.05±20.45 | 71.70±17.49 | 63.19±14.87 | 58.27±12.96 | 74.21±16.59 | 83.26±20.51 |
| PGE2 | 598.45±136.07 | 522.12±108.71 | 415.54±56.61 | 658.22±85.11 | 744.70±128.53 | 976.67±345.50 | 817.32±366.38 | 621.60±160.98 | 984.25±490.76 |
| HSP60 | 2.73±1.04 | 2.48±0.91 | 2.01±0.53 | 1.70±0.39 | 1.38±0.28 | 2.43±0.87 | 2.41±0.72 | 2.24±0.88 | 2.01±0.52 |
| HSP70 | 8.02±1.10 | 9.38±3.20 | 12.25±3.92 | 10.10±1.42 | 9.55±2.46 | 8.75±1.10 | 11.11±3.14 | 6.75±0.97 | 8.73±1.97 |
| MPO | 2.96±0.56 | 2.73±0.45 | 3.29±0.88 | 2.65±0.58 | 2.03±0.34 | 3.21±0.72 | 2.26±0.41 | 2.62±0.61 | 2.60±0.33 |

Pre: before exercise, Post: after exercise, G-CSF: granulocyte colony-stimulating factor, TNF: tumor necrosis factor, IL: interleukin, IL-1ra: IL-1 receptor antagonist, $PGE_2$: prostaglandin $E_2$, HSP: heat shock protein, and MPO: myeloperoxidase

## Discussion

The aims of this study were to measure changes in markers of muscle damage and inflammatory mediators after eccentric exercise of the elbow flexors and to determine whether these changes are influenced by a repeated bout of the same exercise. Data from the present study showed that changes in muscle soreness, swelling, ROM, and strength, was significantly larger following the first bout compared to the second bout (Table 1), and CK and Mb increased significantly only after the first bout (Fig. 1). The remarkable increases in CK and Mb together with the large changes in other markers of muscle damage appear to indicate that severe muscle damage occurred in the elbow flexors after the first bout but not after the second bout. These results demonstrate that muscle adapts rapidly to the initial damage, and less damage occurs after a subsequent bout of the same eccentric exercise. Contrary to our hypothesis that the initial bout of eccentric exercise would cause a greater changes in the plasma cytokine levels than those of the second bout, this study showed that no significant differences between the two bouts were evident for the changes in any of the plasma cytokine concentrations, although some cytokines showed significant alterations following exercise as compared to the pre-exercise values in each bout. Therefore, it seems reasonable to state that severe muscle damage by eccentric exercise does not induce significant alterations in plasma levels of cytokines.

Comparing endurance exercise with eccentric exercise, it has been demonstrated that larger changes in plasma cytokine levels occur after endurance exercise. Since the magnitude of increase in plasma CK activity after exercise in the present study was larger than that after endurance exercise that were shown to result in larger changes in plasma cytokines, it seems reasonable to assume that the eccentric exercise should show larger, or at least similar, changes in cytokines, because muscle inflammation is much more severe. However, it was not the case. It would appear that factors other than muscle damage involve in the changes in cytokines in the blood in case of endurance exercise. This difference may be due to the facts that eccentric exercise is unlikely to induce energy crisis, oxidative stress, metabolic and hormonal alterations, and endotoxaemia discussed later in detail, all of which are known as stimuli for systemic cytokine release (41). Catecholamines (adrenergic stimulation) are considered to be an inducer of IL-6 production, and it is reported that IL-6 release was promoted by adrenaline (38, 41). Also,

Table 3. Correlations between the differences from the pre-exercise values in the first bout.

| Parameters | | | Coefficient | Significance |
|---|---|---|---|---|
| G-CSF 3d-Pre vs. | CK | 2d-Pre | r=0.6699 | P<0.05 |
| | CK | 3d-Pre | r=0.7259 | P<0.05 |
| | CK | 4d-Pre | r=0.6451 | P<0.05 |
| | Mb | 1d-Pre | r=0.6502 | P<0.05 |
| | Mb | 3d-Pre | r=0.6954 | P<0.05 |
| TNF-α 1d-Pre vs. | Mb | 6h-Pre | r=-0.8009 | P<0.01 |
| TNF-α 3d-Pre vs. | Mb | 6h-Pre | r=-0.7767 | P<0.01 |
| | CK | 6h-Pre | r=-0.8032 | P<0.01 |
| TNF-α 4d-Pre vs. | CK | 6h-Pre | r=-0.8080 | P<0.01 |
| G-CSF 6h-Pre vs. | TNF-α | 3d-Pre | r=0.7070 | P<0.05 |
| | TNF-α | 4d-Pre | r=0.8718 | P<0.01 |
| G-CSF 3d-Pre vs. | TNF-α | 4d-Pre | r =0.6674 | P<0.05 |

Pre: pre-exercise value, G-CSF: granulocyte colony-stimulating factor, TNF: tumor necrosis factor, CK: creatine kinase, and Mb: myoglobin.



sympathetic activation promotes the production of chemokines such as IL-8 (41). It has been reported that stress hormone secretion increases the plasma concentrations of TNF-α, IL-6, IL-12 and IL-1ra (35, 38, 41). Moreover, it has been demonstrated that the mRNA levels of IL-6 and IL-8 in skeletal muscle increased more under conditions of low glycogen compared to normal levels of glycogen (6, 38). Endurance exercise is likely to affect all of these factors, and endurance exercise produce large changes in plasma cytokines. On the contrary, it seems reasonable to assume that the eccentric exercise of the elbow flexors does not influence any of the cytokine-stimulating factors. The relatively short exercise time and the small muscle groups used in the exercise, together with less chance of oxidative stress and energy demand in the exercise, are factors that may account for the smaller magnitude of changes in plasma cytokine levels reported in the present study.

Clearance is also thought to affect the plasma concentrations of cytokines followed by exercise. It has been reported that cytokines are removed from the systemic circulation into the urine after exercise (41, 42). In explanation of the smaller increase in plasma cytokine concentrations in the present study compared to strenuous endurance exercise, it is possible to assume that the higher plasma cytokine levels after endurance exercise are related to slower clearance of cytokines from the circulation. Since renal blood flow is likely inhibited during prolonged strenuous exercise by the exercise-induced redistribution of systemic blood flow, the clearance of plasma cytokines may also be reduced during endurance exercise (41). In contrast, it is less likely that renal blood flow is reduced during eccentric exercise using a small muscle group. Therefore, plasma cytokines may be cleared more rapidly from the circulation after this form of exercise, which might account for the lower plasma cytokine concentrations measured in the present study. Further studies on clearance of cytokines are under investigation.

Another possible reason for the smaller plasma cytokine response after eccentric exercise compared to endurance exercise may be the difference in reduction of blood flow in the internal organs (ischaemia) resulting from an increase in the blood flow into the muscle (9). Moreover, physical stimuli during endurance exercise could damage the gut mucosa, and allow bacteria to invade into the systemic circulation (4, 41). This might lead to an increase in plasma endotoxin lipopolysaccharide (LPS) levels. In endurance exercise, ischaemia is more likely to be induced and the damage for the gut mucosa by physical stimuli is larger. In support of this notion, it is reported that the blood concentration of specific anti-LPS antibodies (IgG) in the triathletes decreased (4). Since LPS is a potent stimulus of cytokine production (6, 22), it is suggested that bacterial translocation caused by exercise may induce the rise in plasma cytokine concentrations. On the other hand, it is unlikely that the localized eccentric exercise affects the splanchnic blood flow and induces endotoxaemia.

Moreover, we need to consider the involvement of lymphatic transport of the muscle-derived substances. It is suggested that cytokines spill over from the muscle tissue not only into the blood, but also into the lymph as well. Lymphatic ducts eventually join into blood flow; however, if cytokines enter into the lymphatic system, the plasma cytokine levels may be modified. In addition, lymph fluid goes through the lymph nodes, where a lot of immune cells exist. Therefore, the cytokine production can be modulated when some components draining from

the damaged muscle pass through the lymph nodes. As a consequence, this source of cytokine production and removal may alter plasma cytokine levels.

We observed a significant correlation between the plasma concentrations of G-CSF and TNF-α after the first bout of exercise (Table 3). Plasma G-CSF concentration increased prior to the decrease in the plasma level of TNF-α (Fig. 2). This would suggest that G-CSF might inhibit the production of pro-inflammatory cytokines such as TNF-α. It has been reported that G-CSF acts as an anti-inflammatory cytokine by reducing the release of pro-inflammatory cytokines such as TNF-α and IL-1β and by inducing anti-inflammatory cytokines such as IL-4 and IL-6 (7, 14). One group reported that G-CSF attenuated the lipopolysaccharide (LPS)-induced release of the TNF-α and IL-1β (30). Furthermore, G-CSF induced the release of soluble TNF receptors (sTNF-R) and IL-1ra, which reduce the activity of TNF-α and IL-1β by down regulation of their formation and secretion (1, 12-14, 30). Another group also demonstrated that G-CSF decreased the level of IL-8 and attenuated the release of TNF (13). Taken together, it is possible that G-CSF may act as an anti-inflammatory cytokine in response to exercise-induced muscle damage.

In this study, as shown in Fig. 2 (c), we observed that the exercise decreased plasma IL-8 concentration following both exercise bouts. However, other studies reported that moderate-intensity endurance exercise caused no change or a borderline significant rise in plasma IL-8 concentration (16, 39), whereas there are consistent reports of increased plasma IL-8 concentration after marathon races (23-26, 29, 33, 40, 42). These findings suggest that IL-8 is released into the circulation under prolonged severe exercise conditions involving multiple muscle groups. Therefore, the alterations in plasma IL-8 concentration appear to depend on the conditions of exercise (systemic vs. local; endurance vs. eccentric exercise).

The main function of IL-10 is to suppress the production of TNF-α, IL-1, IL-6, IL-8 and G-CSF and enhance IL-1ra production (17, 18, 21). One previous study suggested that IL-10 reduces serum levels of TNF-α, IL-1β, IL-6 and IL-8 (30). Many studies have reported that intense and prolonged exercise increases plasma IL-10 concentration (21, 41, 42). Malm et al. suggested that IL-10 is important for the muscle tissue restoration (21). Therefore, it is possible to consider that increases in plasma IL-10 level help the reduction of muscle damage and the muscle recovery. Our data showed that plasma IL-10 concentration increased within several hours after the second bout, suggesting that this response may reflect a role of IL-10 in reducing inflammation and promoting adaptation to muscle damage in the repeated bout of eccentric exercise.

In the present study, IL-6 did not change significantly but it is considered that IL-6 may have a role as an anti-inflammatory cytokine (32). The previous study showed that, unlike IL-1 and TNF-α, increases in the plasma level of IL-6 seems to be the primary inducer of two anti-inflammatory cytokines IL-1ra and IL-10 together with hepatocyte-derived acute-phase proteins, which show anti-inflammatory activity (32, 38). IL-6 inhibits the expression of TNF-α and IL-1 (21, 32). Furthermore, in support of our study, some previous studies demonstrated that the kinetics of IL-6 responses differ between those induced by eccentric exercise with muscle damage and those induced by non-damaging exercise with concentric muscle contractions (32). It has been demonstrated that in the case of concentric exercise, increased IL-6 levels decrease to the pre-exercise values



within a few hours. In contrast, eccentric exercise induces a modest increase in the plasma IL-6 and elevates it for a few days (28). Thereby, the alteration of plasma IL-6 levels is greater with concentric exercise than it is with eccentric exercise. Thus, the production of IL-6 is more largely induced by the muscle contractions than by the muscle damage, or it is independent of muscle damage (32). In support of this idea, Croisier et al. showed that training could decrease the muscle damage and delayed-onset muscle soreness (DOMS), whereas the increase in IL-6 was not affected by training (8). Thus, it is possible to think that IL-6 has a role of anti-inflammatory cytokine rather than inflammatory cytokine and that the alteration in IL-6 is affected by the mode of exercise and other factors.

Heat shock proteins (HSPs) are proteins presented in the cells (47). They are induced on exposure to the pathological, physiological and environmental stressors including radiation, heat, cold, high-intensity exercise, surgery, hypoxia, ischaemia, protein degradation, acidosis, endotoxaemia and reduced glucose availability (11, 22, 47). It has been demonstrated that physical exercise increases serum HSP72 concentration (11). For instance, cycling for 120 minutes progressively increased serum HSP72 levels (9) and treadmill running for 60 minutes also elevated serum HSP72 concentration and skeletal muscle HSP72 mRNA expression (47). Although Febbraio et al. observed the increase in expression of HSP72 gene and protein in the skeletal muscle following exercise, the increase was very small. They also showed that hepatosplanchnic tissue releases HSP72 into the circulation during exercise (9). Their study supported another previous study, which indicated that HSP72 was not significantly increased in the contracting skeletal muscle, though it was released into the circulation from other tissue or organs (47). In the present study, no significant changes of the plasma HSP60 and HSP70 levels were observed. It is possible to state that HSPs produced by eccentric exercise were much smaller and did not change the plasma levels.

In conclusion, the present study demonstrated that the alterations in plasma cytokine levels after eccentric exercise were relatively small, although severe muscle damage occurred and that the adaptation of skeletal muscle to the repeated bouts of eccentric exercise was induced. The results of this study suggest that after the first bout, pro-inflammatory cytokines were suppressed possibly by the anti-inflammatory effect of G-CSF, as indicated by the decreases in the plasma levels of TNF-α and IL-8 after the exercise. After the second bout, the increase in IL-10 may be involved in the adaptation against muscle damage and inflammation. In addition, it is possible that the clearance of plasma cytokines from the systemic circulation, involvement of the lymphatic flow in the production and removal of cytokines, and endotoxaemia caused by ischaemia may affect the plasma cytokine concentrations following by exercise.

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