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Original article

# Changes in the number of circulating CD34<sup>+</sup> cells after eccentric exercise of the elbow flexors in relation to muscle damage

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## Abstract

**Background:** It has been reported that strenuous exercise increases the number of bone marrow-derived progenitor cells such as CD34<sup>+</sup> cells in the blood, but no previous studies have investigated the changes in circulating CD34<sup>+</sup> cells following resistance exercise. This study tested the hypothesis that the number of CD34<sup>+</sup> cells in the blood would increase after eccentric exercise of the elbow flexors, but decrease in recovery, and the magnitude of the changes would be dependent on the magnitude of muscle damage.

**Methods:** Nine men (28.0 ± 6.6 years) performed exercises consisting of 10 sets of six maximal voluntary eccentric contractions of the elbow flexors with their non-dominant arm. Six of them performed the same exercise with the same arm 4 weeks later. Changes in indirect markers of muscle damage were measured before, within 10 min after, and at 24, 48, 72, and 96 h after eccentric exercise. Differential leukocyte counts (total leukocytes, neutrophils, lymphocytes, monocytes) and CD34<sup>+</sup> cells in the blood were measured before, immediately after, and at 2, 24, 48, 72, and 96 h following the exercises.

**Results:** After eccentric exercise, significant ( $p < 0.05$ ) decreases in maximal voluntary isometric contraction torque and increases in delayed onset muscle soreness and plasma creatine kinase activity were observed. However, no significant changes in leukocytes and CD34<sup>+</sup> cells were evident. The changes in muscle damage markers were significantly ( $p < 0.05$ ) smaller following the second exercise session as compared with the first exercise session, but the changes in leukocytes and CD34<sup>+</sup> cells were not significantly different between sessions.

**Conclusion:** These results did not support the hypothesis, and showed that eccentric exercise-induced muscle damage to the elbow flexors did not influence the number of circulating CD34<sup>+</sup> cells.

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**Keywords:** Creatine kinase; Hematopoietic progenitor cells; Lengthening contractions; Leukocytes; Repeated bout effect

## 1. Introduction

Skeletal muscle fibers have the ability to regenerate after damage induced by chemical, physical, or mechanical stimuli.<sup>1</sup> Satellite cells are mainly responsible for the regeneration

process,<sup>2</sup> but bone marrow-derived progenitor cells may also be involved in muscle regeneration.<sup>3,4</sup> One such progenitor cell is identified by the CD34 molecule on the cell surface (CD34<sup>+</sup>),<sup>5,6</sup> and CD34<sup>+</sup> cells are normally found in the bone marrow as hematopoietic or endothelial progenitor cells. However, small numbers of CD34<sup>+</sup> cells can be detected in the circulation.<sup>7,8</sup> Palange et al.<sup>7</sup> reported that circulating CD34<sup>+</sup> cells in the peripheral blood were involved in tissue repair processes. Quyyumi et al.<sup>9</sup> showed a reduction in myocardial

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infarct size following a coronary infusion of CD34<sup>+</sup> bone marrow cells, which implies that the infused CD34<sup>+</sup> cells played a role in the regeneration process of the damaged muscle.

Several studies have reported changes in circulating CD34<sup>+</sup> cells following exercise.<sup>10–14</sup> For example, Bonsignore et al.<sup>10</sup> examined circulating CD34<sup>+</sup> cells and their subpopulations in endurance athletes, and reported that compared with sedentary subjects, trained runners showed 4-fold higher circulating CD34<sup>+</sup> cell counts at baseline, which decreased one day after a marathon race, suggesting the peripheral use of these cells. Morici et al.<sup>11</sup> reported that supramaximal rowing exercise (1000 m) doubled the number of circulating CD34<sup>+</sup> cells in young, well-trained athletes. In a more recent study, the post-exercise changes in several bone marrow-derived hemopoietic and angiogenic progenitors were compared between a marathon and a 1500-m run, and Bonsignore et al.<sup>12</sup> reported that CD34<sup>+</sup> cells did not change after a marathon, but increased after a 1500-m run. In contrast, Adams et al.<sup>13</sup> found significant decreases in CD34<sup>+</sup>, CD117<sup>+</sup>, and CD133<sup>+</sup> cells immediately after a marathon run, and suggested that this was probably due to a systemic inflammatory response. It is important to note that all of these studies investigated endurance-type exercises, and no previous studies have investigated changes in bone marrow-derived progenitor cells following resistance exercise.

It is possible that endurance exercises influence systemic factors (e.g., hormones, metabolism, and circulation) that could affect the levels of circulating progenitor cells. It seems likely that the increases in systemic blood flow are smaller for resistance exercise, especially when a small muscle group (e.g., elbow flexors) is used, as compared with endurance exercises that were investigated in previous studies.<sup>10–14</sup> The investigation of strenuous resistance exercise would shed light on the physiological significance of progenitor cell release from the bone marrow into the circulation. It is possible that the changes in circulating CD34<sup>+</sup> cells were associated with muscle damage and inflammation, and the decreases in circulating CD34<sup>+</sup> cells found after the marathon<sup>13</sup> were due to the migration of the cells into the damaged tissues. Thus, the effect of muscle damage on the number of circulating progenitor cells should be clarified by minimizing the influence of systemic factors. To do so, resistance exercise consisting of eccentric contractions of the elbow flexors appears to be suitable.

It has been well-documented that eccentric exercise results in muscle damage characterized by the disruption of myofibers and intermediate filaments,<sup>15</sup> connective tissue,<sup>16</sup> and microvessels,<sup>17</sup> and by symptoms such as delayed onset muscle soreness and the loss of muscle function.<sup>18,19</sup> It is known that eccentric exercise of the elbow flexors induces muscle damage when it is performed by untrained subjects; however, if the same exercise is repeated within several weeks (e.g., 4 weeks), muscle damage is attenuated.<sup>18</sup> Thus, repeated sessions of the elbow flexor eccentric exercise will clarify better whether the magnitude of the muscle damage affects the number of circulating CD34<sup>+</sup> cells.

The purpose of this study was therefore to examine the effect of muscle damage on the number of circulating CD34<sup>+</sup> cells in order to test the hypothesis that the number of CD34<sup>+</sup> cells would change (increase initially and then decrease in recovery) after the first session of eccentric exercise of the elbow flexors, but the changes would be smaller after the second exercise session performed 4 weeks later as compared with the first session.

## 2. Materials and methods

### 2.1. Subjects

Nine healthy men who had not been involved in a resistance-training program for at least 6 months prior to the present study were recruited as subjects. Their age, height, and body mass were  $28.0 \pm 6.6$  years,  $171.7 \pm 7.0$  cm, and  $67.4 \pm 10.1$  kg (mean  $\pm$  SD), respectively. All subjects provided their written informed consent, and answered a medical questionnaire before participating in this study, which had been approved by the Edith Cowan University Human Research Ethics Committee. No subjects had current or previous musculoskeletal injuries to the upper limbs or neuromuscular disorders, and were not taking any medications during the experimental period. None of them had any symptoms of sickness during the experimental period. They were requested and reminded to refrain from unaccustomed exercise and/or vigorous physical activity, to maintain their normal dietary habits, to refrain from alcohol and caffeine, and to not have any treatments (e.g., massage, stretching) of the exercised muscles during the study. The subjects were asked to have a similar breakfast during the experimental period, and to drink enough water before coming to the laboratory.

### 2.2. Study design

All subjects ( $n = 9$ ) performed a bout of maximal eccentric exercise of the elbow flexors in the morning (between 7:00 am and 11:00 am) with their non-dominant arm. Six of them performed the second exercise session with the same arm 4 weeks later at the same time in the morning. The number of subjects used in the present study was estimated from the magnitude of the changes in CD34<sup>+</sup> cells reported in previous studies in which endurance exercises were used.<sup>10–14</sup> Since previous studies have shown large differences between the first and second eccentric exercise sessions of the elbow flexors for changes in indirect markers of muscle damage,<sup>18,19</sup> it was expected that a smaller number of subjects (i.e.,  $n = 6$ ) would suffice for a comparison between sessions. However, the small sample size for the repeated session investigations should be considered a limitation of the study. Maximal voluntary isometric contraction (MVC) torque, muscle soreness and plasma creatine kinase (CK) activity were measured as markers of muscle damage before, within 10 min after, and at 24, 48, 72, and 96 h after eccentric exercise. Venous blood samples were collected before, immediately after, and at 2, 24, 48, 72, and 96 h after exercise to assay for circulating leukocytes (neutrophils, lymphocytes, monocytes, eosinophils) and CD34<sup>+</sup>

cells. The blood samples before exercise and at 24–96 h following exercise were taken in the morning at a similar time of day ( $\pm 2$  h). This study was carried out in October–February (summer in Australia).

### 2.3. Eccentric exercise

Each subject was seated on an arm preacher curl bench, and the exercised arm was placed on the padded arm support of the bench with a supinated forearm position, and the elbow was aligned with the axis of rotation of an isokinetic dynamometer (Cybex6000; Lumex, Inc., Ronkonkoma, NY, USA) operated by HUMAC2004 software (Computer Sports Medicine, Inc., Stoughton, MA, USA). The exercise consisted of 10 sets of six maximal voluntary eccentric contractions of the elbow flexors over the range of motion from a half flexed ( $90^\circ$ ) to a fully extended position ( $0^\circ$ ) at a constant velocity of  $90^\circ/\text{s}$  with a 2-min rest between sets. The subjects were verbally encouraged to maximally resist against the extension of the elbow joint through the whole range of motion. After each contraction, the isokinetic dynamometer returned the arm to a flexed position at a constant velocity of  $30^\circ/\text{s}$ , creating a 3-s passive recovery between contractions.

### 2.4. Markers of muscle damage

#### 2.4.1. MVC torque

The MVC torque of the elbow flexors was measured by the isokinetic dynamometer that was used for the exercise. The subjects performed two maximal voluntary isometric contractions at an elbow angle of  $90^\circ$  flexion for 3 s with a 60-s rest between contractions. Verbal encouragement was given to the subjects during contractions. The peak torque of each contraction was obtained from the recorded force by the data acquisition system, and the higher torque of the two measurements was used for further analysis.

#### 2.4.2. Muscle soreness

Muscle soreness was assessed using a 100-mm visual analog scale (VAS) where 0 mm indicates “no pain” and 100 mm indicates “extremely painful”. The subjects were instructed to place a mark on the VAS when the corresponding elbow joint was extended maximally by the investigator.

#### 2.4.3. Plasma CK activity

Approximately 5 mL of blood was drawn from an antecubital vein of the dominant arm (non-exercised arm) by a standard venipuncture technique using a disposable needle and a vacutainer containing ethylenediaminetetraacetic acid (EDTA). The blood sample was immediately analyzed using a Reflotron spectrophotometer (Boehringer-Manheim, Pöde, Czech Republic) for plasma CK activity. The normal reference range for CK activity using this method is 50–220 IU/L, according to the information provided by the manufacturer. Based on our previous studies, the measurement error of plasma CK activity using this method is less than 6% for the coefficient of variation (CV).

### 2.5. CD34<sup>+</sup> cells and leukocytes

A portion of the collected blood sample was used for analyses of the circulating CD34<sup>+</sup> cells by flow cytometry (FACSCanto II Flow Cytometer; BD Biosciences, San Jose, CA, USA) with FACSDiva software (version 6.1.1; BD Biosciences). The cells were stained with an R-phycoerythrin (RPE) conjugate of an anti-CD34 antibody (clone 581; Coulter/Immunotech, Beckman Coulter, Fullerton, CA, USA). All antibodies were used at the manufacturer’s recommended concentration after verification of their immunoreactivity in-house. The erythrocytes were lysed with Pharmlyse (BD Biosciences), and were then analyzed within the hour. The dual platform CD34 analysis was performed using the absolute leukocyte count performed on an LH750 Hematology Analyzer (Beckman Coulter, Fullerton, CA, USA), and the leukocyte count was used to calculate the count from the percentage of CD34<sup>+</sup> cells. The gating procedure followed that described by the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines.<sup>20</sup> The calculated result is the number of CD34<sup>+</sup> positive cells  $\times 10^6/\text{L}$  of whole peripheral blood. The CD34 analysis has a CV = 7.4% for the performing laboratory. The differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils) were determined by an automatic blood cell counter (Beckman Coulter). The hematocrit was also assessed for all blood samples. The reliability of these measurements that were established in this laboratory was less than 6% for the CV.

### 2.6. Statistical analysis

A Shapiro–Wilk test was used to confirm that all dependent variables were normally distributed. The changes in the muscle damage markers, leukocytes and CD34<sup>+</sup> cells, and hematocrit over time after the first eccentric exercise were analyzed by a one-way repeated measures ANOVA, followed by a Tukey’s *post-hoc* test when a significant time effect was found to locate the time points that were different from the baseline values. To compare between the first and second sessions for changes in muscle damage markers, leukocytes and CD34<sup>+</sup> cells, and hematocrit, a two-way repeated measures ANOVA was used. When a significant session  $\times$  time interaction effect was observed, a Tukey’s *post-hoc* test was performed to compare the first and second sessions for each time point separately. All analyses were conducted with SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA), and a  $p < 0.05$  was considered statistically significant. The results were presented as means  $\pm$  SD.

## 3. Results

### 3.1. Markers of muscle damage

The changes in MVC torque, muscle soreness, and plasma CK activity are shown in Table 1. After the first eccentric exercise session, the MVC torque decreased more than 50%

immediately and 24 h post-exercise, and did not recover to the baseline by 96 h post-exercise. Muscle soreness developed after the exercise, and peaked at 48–72 h post-exercise. These changes after the first session were similar between the entire subject group ( $n = 9$ ) and those subjects in the sub-group ( $n = 6$ ) who performed the second eccentric exercise session 4 weeks later. After the second exercise session, the MVC torque recovered significantly faster to the baseline, and the muscle soreness was significantly less as compared with the first session. No significant changes in plasma CK activity were evident after the first session because of the large variability in the responses among subjects. However, all subjects showed increases from the baseline (peak–baseline value = 155–3562 IU/L). No significant changes in plasma CK activity were evident after the second session.

### 3.2. Hematocrit and leukocytes

No significant changes in hematocrit were observed over time, and the average value was  $0.44 \pm 0.02$ . Changes in the number of total leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils are shown in Fig. 1. No significant changes in the number of any cells were observed after the first and second eccentric exercise sessions.

### 3.3. CD34<sup>+</sup> cells

The number of CD34<sup>+</sup> cells per mL of blood ranged from 1400 to 7000 (average  $3200 \pm 1497$ ) at baseline. No significant changes in this number were evident over time for both sessions, and no significant differences in the changes were evident between sessions. As shown in Fig. 2, no significant changes in the percentage of CD34<sup>+</sup> cells to the total number of leukocyte cells were evident either after exercise.

## 4. Discussion

The results showed that the number of circulating leukocytes and CD34<sup>+</sup> cells did not change significantly after eccentric exercise, although the exercise resulted in muscle damage as evidenced by the large decreases in MVC torque,

the development of muscle soreness and increased plasma CK activity. Furthermore, the results from the sub-group showed significantly faster recovery of MVC torque, less muscle soreness and no increases in plasma CK activity after the second session as compared with the first one, indicating less muscle damage after the second session than after the first. However, no significant differences between sessions in the changes of leukocytes or CD34<sup>+</sup> cells were observed. These results did not support the hypothesis that muscle damage to the elbow flexors would change the number of circulating CD34<sup>+</sup> cells, and that the magnitude of muscle damage would influence the number of circulating CD34<sup>+</sup> cells.

It is well-documented that eccentric exercise induces muscle damage as indicated by large and prolonged decreases in muscle function, the development of muscle soreness, and increases in muscle specific proteins such as CK in the blood.<sup>18</sup> As shown in Table 1, the MVC torque decreased more than 50% immediately after exercise, and was still 30% lower than the baseline at 4 days post-exercise. These changes were similar to those reported in previous studies,<sup>18,19</sup> and suggested that the muscle damage was induced by the first eccentric exercise session. However, the magnitude of the increase in plasma CK activity was not as large as that reported in previous studies,<sup>18,19,21</sup> in which a similar eccentric exercise of the elbow flexors was used. In the present study, a relatively large increase in plasma CK activity from the baseline to the peak (e.g., 3000 IU/L) was seen in only two subjects. This might indicate that the muscle damage induced in the present study did not result in muscle necrosis for most of the subjects. This may be the reason why non-significant changes in leukocytes and CD34<sup>+</sup> cells were observed in the present study. In the two subjects whose peak CK activity was relatively high, no increases in CD34<sup>+</sup> cells in the circulation were observed. However, it is possible that the changes in CD34<sup>+</sup> cells could have been detected if greater increases in plasma CK activity (e.g., >10,000 IU/L) had been induced. The responses of the six subjects who performed the second eccentric exercise 4 weeks after the first session showed the typical indications of the repeated bout effect characterized by a faster recovery of muscle strength, less muscle soreness, and smaller increases in plasma CK activity (Table 1). The sample

Table 1  
Changes in maximal voluntary isometric contraction torque (MVC), muscle soreness assessed by a visual analog scale (VAS), and plasma creatine kinase (CK) activity before (pre), immediately after (0), and at 24, 48, 72, and 96 h following exercise for the first session performed by nine subjects, and for the first and second sessions performed by six subjects (mean  $\pm$  SD).

Parameter	Subject No.	Pre	0 h	24 h	48 h	72 h	96 h
MVC (Nm)	1st ( $n = 9$ )	51.1 $\pm$ 7.4	23.4 $\pm$ 3.7*	25.8 $\pm$ 5.1*	26.7 $\pm$ 5.0*	31.6 $\pm$ 4.3*	35.6 $\pm$ 5.6*
	1st ( $n = 6$ )	49.8 $\pm$ 6.9	21.8 $\pm$ 1.6*	23.6 $\pm$ 4.4*	24.1 $\pm$ 3.9*	29.6 $\pm$ 2.9*	34.3 $\pm$ 6.3*
	2nd ( $n = 6$ )	45.7 $\pm$ 6.3	27.3 $\pm$ 3.6*	35.2 $\pm$ 3.5	37.0 $\pm$ 4.8	39.5 $\pm$ 5.2	41.2 $\pm$ 5.6
VAS (mm)	1st ( $n = 9$ )	–	–	32.4 $\pm$ 23.1*	48.3 $\pm$ 19.2*	50.7 $\pm$ 19.4*	38.8 $\pm$ 16.7*
	1st ( $n = 6$ )	–	–	31.3 $\pm$ 18.7*	52.8 $\pm$ 23.4*	56.5 $\pm$ 20.1*	39.4 $\pm$ 19.8*
	2nd ( $n = 6$ )	–	–	26.5 $\pm$ 23.2	33.8 $\pm$ 22.4*	29.2 $\pm$ 19.7	17.5 $\pm$ 15.3
CK activity (IU/L)	1st ( $n = 9$ )	118.7 $\pm$ 126.5	130.6 $\pm$ 127.5	243.2 $\pm$ 137.5	604.9 $\pm$ 1125.5	427.6 $\pm$ 379.7	812.1 $\pm$ 1107.4
	1st ( $n = 6$ )	135.5 $\pm$ 155.5	140.3 $\pm$ 159.4	254.5 $\pm$ 149.2	766.6 $\pm$ 1388.5	360.9 $\pm$ 376.1	401.0 $\pm$ 412.9
	2nd ( $n = 6$ )	82.6 $\pm$ 42.3	89.8 $\pm$ 43.1	89.9 $\pm$ 43.1	76.1 $\pm$ 29.6	68.6 $\pm$ 38.5	81.4 $\pm$ 46.6

\*indicates a significant difference from the pre-exercise value.

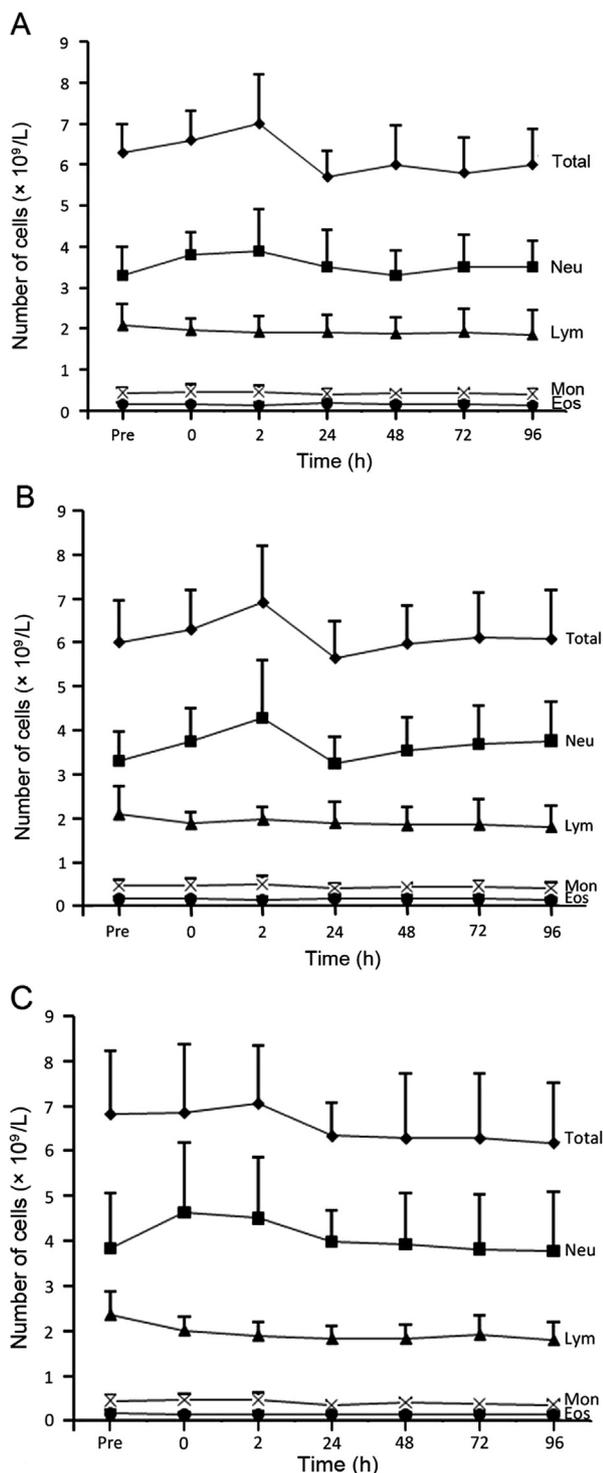


Fig. 1. Changes in the number of total leukocytes (Total), neutrophils (Neu), lymphocytes (Lym), monocytes (Mon), and eosinophils (Eos) before (pre), immediately after (0), and at 2, 24, 48, 72, and 96 h after the first eccentric exercise session performed by nine subjects (A), and after the first (B) and second (C) eccentric exercise sessions performed by six subjects.

size for this comparison was small, which is a limitation of the study; however, in spite of the large differences between sessions for the changes in muscle damage markers, the changes in CD34<sup>+</sup> cells were not significantly different between

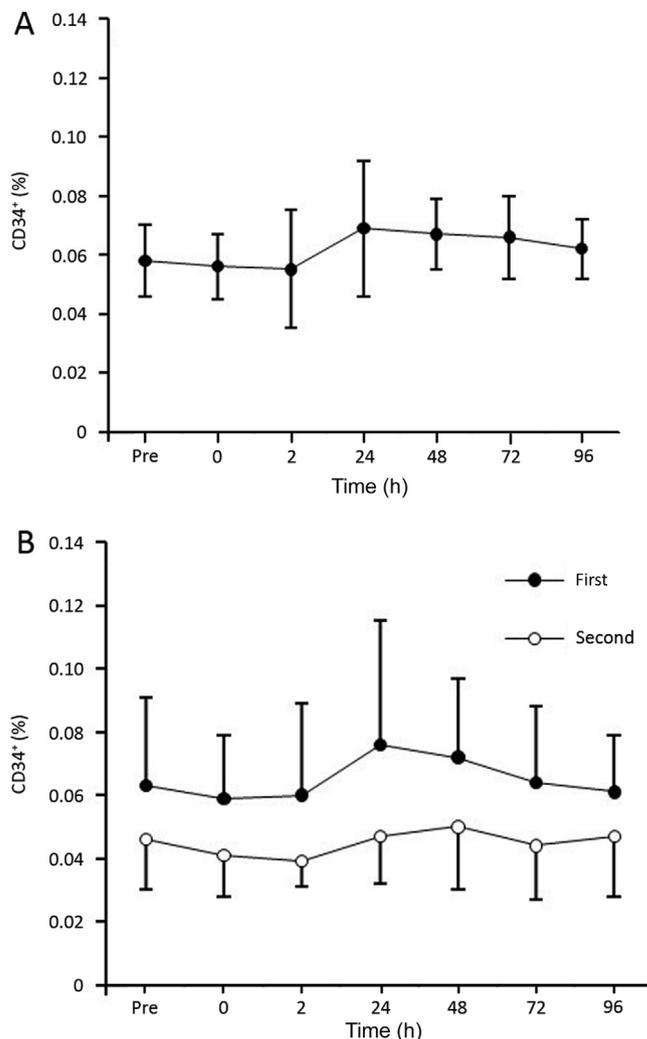


Fig. 2. Changes in the number of CD34<sup>+</sup> cells relative to the total number of leukocytes before (pre), immediately after (0), and at 2, 24, 48, 72, and 96 h after the first eccentric exercise session performed by nine subjects (A), and after the first and second eccentric sessions bouts performed by six subjects (B).

sessions (Fig. 2). Thus, it seems unlikely that the magnitude of the muscle damage to the elbow flexors induced by eccentric exercise affected the number of circulating CD34<sup>+</sup> cells, at least for the muscle damage in which the peak plasma CK activity was not very high.

The present study did not find any significant changes in the number of leukocytes (neutrophils, lymphocytes, monocytes, eosinophils) after exercise, and confirmed the findings of the previous study showing that the changes in leukocytes were more due to a circadian rhythm rather than to muscle damage.<sup>21</sup> Although some studies have reported increases in the number of circulating leukocytes after eccentric exercise, it should be noted that most of them used eccentric exercise with an aerobic component or with larger muscles.<sup>22,23</sup> Many studies have reported that exercise induced mobilization of circulating leukocytes and progenitor cells;<sup>10–14,24–26</sup> however, it is important to note that all of these studies examined endurance exercises. The present study was the first to investigate the changes in circulating CD34<sup>+</sup> cells following

resistance exercise, more precisely resistance exercise consisting of pure eccentric contractions. The number of circulating CD34<sup>+</sup> cells in the present study appears to be comparable to the baseline values (1000–10,000 cells/mL) reported in previous studies.<sup>10–14</sup> No significant changes in hematocrit were evident, and the time of the day for the blood sampling was standardized, so the changes observed should have been due to the number of CD34<sup>+</sup> cells induced by the eccentric exercise. However, no significant changes in any blood cells were found in the present study (Figs. 1 and 2). It seems likely that one of the reasons why the eccentric exercise did not change the leukocytes or CD34<sup>+</sup> cells in the present study was the smaller effects on systemic blood flow as compared with endurance exercise. It seems likely that not muscle damage, but rather the changes in hormones, metabolism, and circulation due to endurance exercise were associated with the increased circulating CD34<sup>+</sup> and other progenitor cells reported in the previous study.<sup>12</sup>

We hypothesized that the number of CD34<sup>+</sup> cells would increase immediately to 2 h after eccentric exercise due to the increased release of cells from the bone marrow, but would decrease in the recovery days, because they would be mobilized to the damaged muscles. Otto et al.<sup>5</sup> stated in their review that bone marrow-derived progenitor cells could differentiate into myotubes *in vitro*, and potentially form skeletal muscle; however, when compared to skeletal muscle satellite cells, bone marrow-derived progenitor cells were less efficient at myotube fusion. Pisani et al.<sup>6</sup> reported that both CD34<sup>+</sup> and CD34<sup>-</sup> cells exhibited equivalent myogenic potential, but only CD34<sup>-</sup> cells did not differentiate into adipocytes, and proposed that the CD34<sup>-</sup> cell fraction could be a promising alternative to the current use of a total myoblast population for muscle cell therapy. Ieronimakis et al.<sup>27</sup> showed that CD34<sup>-</sup> cells became the major myogenic population following acute muscle injury induced by cardiotoxin, whereas the percentage of CD34<sup>+</sup> cells remained constant, since the activated CD34<sup>-</sup> cells reversed their activation to maintain the pool of CD34<sup>+</sup> reversed cells. Alfaro et al.<sup>28</sup> showed that Cd34(-/-) mice displayed a defect in muscle regeneration after acute or chronic muscle injury, and that this defect was caused by impaired entry into proliferation and delayed myogenic progression of satellite cells. Thus, CD34 plays an important function in modulating satellite cell activity. However, it is not known whether circulating CD34 cells are involved in the muscle regeneration process in humans. It cannot be denied that the effect of local muscle damage on CD34<sup>+</sup> cells was not detected. It is possible that if the magnitude of muscle damage had been greater and/or the amount of damaged muscle had been greater than in the present study, then significant changes in circulating CD34 cells could have been observed, and thus this warrants further study.

Rehman et al.<sup>24</sup> stated that exercise-induced endothelial progenitor cells could promote angiogenesis and vascular regeneration. Laufs et al.<sup>25</sup> demonstrated that nitric oxide played an important role in the vascular endothelial growth factor (VEGF)-mediated regulation of circulating progenitor cells. Möbius-Winkler et al.<sup>14</sup> also reported that increases in

hematologic and endothelial progenitor cells (CD34<sup>+</sup>, CD133<sup>+</sup>) were related to VEGF and IL-6. Eccentric exercise affects not only muscle fibers, but also the capillary structure such that the capillary luminal area was increased by more than 20% at 1 and 3 days after 300 eccentric contractions of the gastrocnemius muscles in rats, although the capillary endothelial cells retained their normal structure.<sup>17</sup> It is not known whether the eccentric exercise in the present study affected the capillaries, but it is reasonable to assume that endothelial repair was not required. Eccentric exercise of the elbow flexors has a minimal effect on the circulation, because it does not affect the heart rate or blood pressure during exercise.<sup>21</sup> Together with the results from previous studies, the present data indicate that the changes in the number of circulating CD34<sup>+</sup> cells are not necessarily related to muscle damage, but that other factors such as increased shear stress may be involved in the larger increases in circulating CD34<sup>+</sup> cells after endurance exercise reported in previous studies.<sup>10–14</sup>

In the present study, we examined only one subfraction of leukocytes based on the presence or absence of the CD34 antigen. This is a definite limitation of this study. It is possible that certain subpopulations within the CD34<sup>+</sup> cell fraction, for example, CD34<sup>+</sup>/VEGFR2(KDR)<sup>+</sup>, CD34<sup>+</sup>/CD133<sup>+</sup>, or CD34<sup>+</sup>/CD45<sup>+</sup> EPCs, might have changed following eccentric exercise. It would have been better if other bone marrow-derived progenitor cells were also investigated. In the present study, a control group that did not perform any exercise was not included, and the baseline measures were performed only immediately before the eccentric exercise, which was also a limitation. Since the number of circulating CD34<sup>+</sup> cells was small, it is possible that a small change was not detected if the change was within the measurement error (CV was 7.4% for CD34<sup>+</sup> cells measured in the present study). Further studies are required to examine the changes in subpopulations of CD34 cells and other progenitor cells following the same eccentric exercise or other types of resistance exercise, using a more sensitive method if available, and with multiple baseline measures.

## 5. Conclusion

Eccentric exercise-induced muscle damage to the elbow flexors does not influence the number of circulating CD34<sup>+</sup> cells. These results suggest that muscle damage is not the main factor for the changes in CD34<sup>+</sup> cells after eccentric exercise. However, changes in circulating CD34<sup>+</sup> cells in response to muscle damage cannot be completely denied based on the results from the present study, since the eccentric exercise in the present study did not appear to induce extensive muscle fiber damage as indicated by the mild increase in plasma CK activity, and the volume of muscle affected by the exercise was not large. Further studies are necessary to investigate the role of circulating progenitor cells in muscle regeneration after exercise.

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