Temporal relation between leukocyte accumulation in muscles and halted recovery 10–20 h after strength exercise

Truls Raastad,1 Bjørn Audun Risøy,1 Haakon Breien Benestad,2 Jan Gunnar Fjeld,3 and Jostein Hallén1

1Norwegian University of Sport and Physical Education, 2Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, and 3Section of Nuclear Medicine, The National Hospital, N-0806 Oslo, Norway

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Raastad, Truls, Bjørn Audun Risøy, Haakon Breien Benestad, Jan Gunnar Fjeld, and Jostein Hallén. Temporal relation between leukocyte accumulation in muscles and halted recovery 10–20 h after strength exercise. J Appl Physiol 95: 2503–2509, 2003. First published June 27, 2003; 10.1152/japplphysiol.01064.2002.—Effects of normal strength exercise on leukocyte accumulation were examined in 10 well-trained male subjects (27.2 ± 2.7 yr). The workout, consisting of five maximal sets of three repetitions of leg press exercise and five maximal sets of six repetitions of knee extension exercise, was performed with the dominant leg, and the other leg served as control. Repeated maximal isokinetic knee extensions at 60°/s were performed to evaluate neuromuscular fatigue and recovery after the workout. Accumulation of leukocytes was assessed with 99mTc-labeled cells, and repeated images of the thighs were taken 1–24 h after the workout. Maximal force-generating capacity in the exercised leg was reduced by 17 ± 2% (P < 0.01) after the workout. The course of recovery followed a biphasic pattern characterized by halted recovery 10–23 h after exercise. The presence of leukocytes was ~10% higher in the exercised than in the control thigh 10 h after exercise (P < 0.05). This difference increased to ~15% at 20 h after exercise (P < 0.05). The retarded recovery of maximal force-generating capacity 10–20 h after exercise, together with a significant infiltration of leukocytes in exercised muscle during the same time interval, shows a temporal relation between leukocyte infiltration and impaired recovery.

A biphasic recovery of maximal force-generating capacity has been observed after normal strength exercise, as well as after strenuous eccentric exercise, in humans (12, 23). After strength exercise, recovery was characterized by rapid recovery during the first 5–10 h, a leveling off or a drop in performance 10–24 h after exercise, and then recovery toward preexercise levels (23). With the eccentric exercise protocol, the second drop in performance is characterized by the fact that phagocytic activity not only removes damaged structures but also harms some intact structures, exacerbating the initial damage.

In a study where muscle damage was induced by eccentric contractions in mice, increased rates of protein degradation correlated in time with infiltration of phagocytes in exercised muscles (10). Moreover, Morozov et al. (16) reported increased proteolytic activity in exercised rat muscles, with infiltration of neutrophil granulocytes. Proteolytic activity was abolished when the leukocyte infiltration was inhibited. This suggests that phagocytic activity can cause delayed muscle damage and reduced muscle function hours after the initial damage during exercise (2). However, increased protein degradation is not always correlated with changes in force production (10). Leukocytes have been identified in areas with muscle damage in human biopsies and correlated to the extent of damage (4). Measurements of infiltrated leukocytes in serial biopsies have been criticized because of the possible influence on the inflammatory response initiated by the surgery of the past biopsies (14, 15). However, this problem can be avoided by the use of a noninvasive method with 99mTc-labeled leukocytes (12).

There are several differences between normal strength exercise and more severe muscle-damaging eccentric protocols. The acute muscle fatigue caused by our strength exercise protocols is normally seen as a 10–20% reduction in maximal force-generating capacity, and recovery seems to be completed within 48 h (22, 23). In addition, our subjects report little or no muscle soreness. In contrast, the muscle fatigue caused by heavy eccentric protocols normally ranges from 30 to 50%, and recovery may take >1 wk (5, 12, 17). The large force deficit is accompanied by severe muscle damage, muscle soreness peaking 1–2 days after exercise, and large increases in creatine kinase (CK) plasma concentrations. Therefore, the use of heavy eccentric protocols to explore recovery mechanisms in athletes subjected to normal strength exercise does have limitations.

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Address for reprint requests and other correspondence: T. Raastad, Norwegian University of Sport and Physical Education, PO Box 4014 US, N-0806 Oslo, Norway (E-mail: truls.raastad@nih.no).
The use of $^{99m}$Tc-labeled leukocytes as a marker of inflammation allows repetitive noninvasive investigation of leukocyte distribution during the first 24 h after injection. Our working hypothesis was that the biphasic course of recovery of maximal force-generating capacity could be explained by the inflammatory response in the exercised muscles. Therefore, the purpose of the present study was to compare the time course of leukocyte infiltration in exercised muscle with recovery of maximal force-generating capacity after normal strength exercise in well-trained subjects.

MATERIALS AND METHODS

Subjects. Ten male students gave informed consent to participate in the study. All subjects had performed strength training for $\geq 2$ yr and were able to lift more than twice their own body weight in the squat exercise. In addition, all subjects performed strength training on leg extensors twice a week during the last months before they entered the study. Their age, body weight, and height were as follows (mean $\pm$ SD): 27.2 $\pm$ 2.7 yr, 84.1 $\pm$ 7.8 kg, and 182 $\pm$ 5 cm, respectively. The experimental protocol complied with current national laws and was approved by the Regional Ethics Committee of Norway.

Experimental design. Recovery from neuromuscular fatigue and distribution of $^{99m}$Tc-labeled leukocytes were monitored for 47 and 24 h, respectively, after a single bout of strength exercise. Blood (50 ml) was taken by venipuncture at 7:30 AM on day 1 for the radionuclide-labeling procedure (Fig. 1). The $^{99m}$Tc-labeled leukocytes were reintroduced intravenously 2 h later, and a bout of one-legged strength exercise was performed from 10:12 to 11:00 AM. Recovery from fatigue was measured with repeated tests of maximal isokinetic knee extensions: two tests were performed before the workout to establish baseline values (at 8:00 and 9:30 AM), and the third test started 5 min after the end of the strength workout. The tests were repeated 3, 9, 23, 28, and 47 h after the workout. Radionuclide images of both thighs (anterior and lateral views) were taken 1, 4, 8, 21, and 24 h after exercise. Blood samples for CK measurements followed by three maximal contractions. Peak torque and total work were used in the data treatment (coefficient of variation $<5\%$).

Radionuclide investigation of leukocytes. Fifty milliliters of whole blood were drawn from each of the subjects. After they were isolated, leukocytes were incubated for 15 min with 500 MBq of $^{99m}$Tc-hexamethylpropylene amine oxime solution, which preferentially tagged the granulocytes (3, 19). Non-cell-bound $^{99m}$Tc-hexamethylpropylene amine oxime and $^{99m}$Tc-pertechnetate were washed off with cell free plasma. The activity injected ranged from 220 to 291 MBq. The half-life of the $^{99m}$Tc is only 6 h. Therefore, the leukocyte migration was followed for $\leq 26$ h after injection.

Labeled leukocytes in the thighs were detected by gamma camera scintigraphy. Images ($256 \times 256$ matrix) were acquired (15-min acquisition time) 1, 4, and 8 h after exercise and on the next day at 20 and 24 h after exercise (30-min acquisition time). To quantify accumulation of labeled leukocytes in different parts of the thighs, a grid system containing 45 equal squares was placed over each thigh on the scintigrams (Fig. 2). In anterior view images, the lower edge of the

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**Fig. 1.** Experimental design. Knee extension (exten) strength was measured by voluntary isokinetic knee extensions. WBC, leukocytes.

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**Table 1.** Radionuclide investigation of leukocytes. WBC, white blood cells; CK, creatine kinase.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Radionuclide images</th>
<th>Blood samples</th>
<th>Knee exten. strength</th>
<th>Muscle soreness</th>
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<tr>
<td>07:30</td>
<td>08:30</td>
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**Table 2.** Time of day (hours). WBC, white blood cells.

| Time (hours after exercise) | 07:30 | 08:30 | 09:30 | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | 15:00 | 16:00 | 17:00 | 18:00 | 19:00 | 20:00 | 21:00 | 22:00 | 23:00 | 24:00 | 25:00 | 00:00 |
|-----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Breakfast                    |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| Workout                      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| Lunch                        |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| Dinner                       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| Time of day (hours)          |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |

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One-legged strength workout. The strength workout consisted of one-legged leg press and one-legged knee extension performed with the subject’s dominant leg. In both exercises, each repetition consisted of an eccentric and a concentric contraction phase. The subjects completed five sets of three repetitions with a load that could be lifted for a maximum of three repetitions (100% of 3 repetitions maximum (RM)) in the leg press exercise before they continued with five sets of six repetitions with a load that could be lifted for a maximum of six repetitions (100% of 6 RM) in the knee extension exercise. The load was adjusted in the next set if the subject was unable to complete all repetitions without help from one of the investigators. For each exercise, the subject warmed up by gradually increasing loads during four sets of leg press and three sets of knee extension. Rest between sets and exercises was 3 min. The workout lasted 48 min.
grid system was placed at the upper edge of the patella (position marked with a radioactive pen), and the grid was extended vertically upward to the lower edge of the ischium, roughly indicated by increased radiation from bone marrow (Fig. 2, left). The width of the grid system was adjusted to the thickest part of the thigh 1 h after exercise. On the lateral view images, the vertical length of the grid system was from the upper edge of the patella (marked with a radioactive pen) to the lower edge of the ischium (Fig. 2, right). Again, the width of the grid system represented the thickest part of the thigh 1 h after exercise. Once a grid system was constructed, it was kept constant for that subject throughout the consecutive image analysis. The area of each square in the grid varied from 11 to 15 cm², depending on the size of each subject’s thigh.

In the anterior view images, four areas of the thighs, in which we had a special interest to determine the average counts per pixel, were chosen. These four areas are meant to represent the rectus femoris muscle, the muscle-tendon junction, the vastus medialis muscle, and the vastus lateralis muscle (Fig. 2, left). In the lateral view images, regions over the ventral (quadriceps) and dorsal (hamstrings) parts of the femur were chosen (Fig. 2, right).

Blood analysis. Blood was drawn from an antecubital vein into 5-ml heparin-treated Vacutainer tubes. The tubes were set on ice and centrifuged within 30 min at 1,500 g for 10 min at 4°C. Plasma was stored at −20°C until analysis. CK was recorded using a Reflotron (Bohering Mannheim, Mannheim, Germany; coefficient of variation <5%). All samples from each subject were analyzed on the same day.

Muscle soreness. Muscle soreness was subjectively evaluated with a 10-cm visual analog scale, where 0 represented no soreness and 10 cm represented extreme soreness. Subjects rated muscle soreness of the distal, medial, lateral, and central regions of the thigh, corresponding to the areas defined in the radionuclide images (Fig. 2).

Statistics. Repeated-measures ANOVA with Dunnett’s post hoc test was performed to identify changes in exercise and control leg differences from baseline and to identify differences from baseline values in both legs. This test was also performed to identify significant differences between selected postexercise tests to indicate phases of recovery. A paired t-test was used to identify differences between values obtained 8 and 20 h after exercise in the radionuclide images.
mean difference between the exercised and the control thigh was observed 8–21 h after exercise in the ventral part (from 10±100 to 2±17%), but this was not statistically significant (P=0.10).

Muscle soreness. The subjects reported statistically significantly increased soreness in the exercised thigh during all registrations after exercise (Fig. 6). Soreness ratings were, however, low, and there was no sign of delayed-onset muscle soreness (DOMS). There were no apparent differences between the four regions of the thigh in regard to soreness rating. The individual values for labeled leukocytes in the exercised leg correlated significantly with the individual soreness rating only in the distal region of the thigh (r=0.31–0.90 at 3–23 h after exercise).

CK. Plasma CK concentration peaked 9–23 h after exercise (Fig. 7). There were large interindividual differences in blood CK accumulation after exercise (44–555% increase above baseline), but no correlation was observed between individual peak CK concentrations and muscle fatigue. Nor was any correlation observed between individual peak CK concentrations and accumulation of labeled leukocytes at any time point.

DISCUSSION

The number of $^{99m}$Tc-labeled autologous leukocytes was higher in the exercised than in the control leg 1–24 h after normal strength exercise in well-trained sub-

jects. Moreover, the number of labeled leukocytes in the exercised leg increased from 8 to 21 h, when a delayed recovery of maximal force-generating capacity was also recorded. This finding shows a temporal relation between leukocyte infiltration in exercised muscles and the biphasic course of recovery observed in this and previous studies (22, 23).

Traditionally, the relation between muscle fatigue, leukocyte infiltration, soreness, and indicators of muscle damage has been studied with exercise protocols utilizing eccentric contractions, which cause severe muscle damage (11–13). For example, MacIntyre et al. (12) reported a biphasic course of recovery after 300 maximal eccentric knee extensions in untrained subjects and a time relation between the second drop in maximal force-generating capacity and the increased number of $^{99m}$Tc-labeled leukocytes in exercised muscles. This extreme exercise protocol may, however, not be relevant to the actual training program for athletes. We recently reported a biphasic course of recovery of maximal force-generating capacity, even after normal strength exercise in well-trained strength athletes, which possibly indicates some kind of delayed muscle-damaging mechanism here also (23). Interestingly, our finding is reminiscent of that reported by MacIntyre et al.
Although the use of $^{99m}$Tc-labeled leukocytes does not allow detailed information about leukocyte localization, it is possible to make some interpretations. Because radioactivity was higher in all parts of the exercised thigh than in the control thigh, increased presence of labeled leukocytes in bone marrow and in the largest blood vessels can be excluded as the only reasons for the observed differences. The region dominated by bone marrow, the central region, showed the smallest relative difference in radioactivity between the exercised and the control leg. Furthermore, MacIntyre et al. (11) did not observe any differences between the exercised and the control leg in regard to blood flow 2–4 h after exercise, as assessed with $^{99m}$Tc-labeled red blood cells. With the assumption of no blood flow differences hours after our exercise as well, it seems...
reasonable to conclude that the increased presence of leukocytes was due to increased vascular adherence and migration into muscle tissue.

The long recovery time makes us believe that structural changes in proteins important for force production caused the reduced maximal force-generating capacity (23). Such damage has often been reported after eccentric exercise (for review see Ref. 2) and also after normal strength exercise (5). It has therefore been hypothesized that initial structural damage caused by mechanical and/or metabolic stress initiates an inflammatory response, including attraction of leukocytes to the sites of damage (2). Leukocytes, mainly neutrophil granulocytes and, later, monocytes (i.e., the professional phagocytes), have been identified in areas with muscle damage after eccentric exercise in humans (4, 26) and animals (9, 10, 21). Although the findings of leukocytes in muscle tissue to some extent might be biased by repeated biopsies in humans (15), the use of noninvasive methods, as in the present study, has demonstrated an exercise-induced attraction of leukocytes per se (11, 12). Because of the short physical half-life of $^{99m}$Tc, we could not obtain reliable images for $\geq 24$ h after exercise. The further course of leukocyte infiltration is therefore unknown, and this limits the interpretation of our data to the first 24 h after exercise.

Increased leukocyte accumulation from 8 to 21 h after exercise was evident in the lateral, medial, and central parts of the quadriceps but not in the distal part. In contrast to the observations by MacIntyre et al. (12), accumulation was not greater in the distal part of the quadriceps than in the other parts during the first hours after exercise. The greatest accumulation was observed in the lateral part of the quadriceps, but the relatively small differences in leukocyte accumulation between regions indicate that all parts of the quadriceps were equally affected. In light of our observations, it seems that biopsies from the vastus lateralis, the most common method for studying leukocyte accumulation in the quadriceps, are fairly representative of the whole quadriceps after this kind of exercise.

The presence of leukocytes in muscle tissue was associated with increased protein degradation 48 h after exercise in mice (10). Although the degradation of damaged structures seemed to be quite well regulated (10), it is likely that some intact structures also are affected by the scavenger phagocytes, and the repair process per se may therefore cause some delayed and additive muscle damage. It is known that prevention of neutrophil granulocyte accumulation in exercised muscle abolishes the increase in proteolytic activity observed 24 h after intense exercise in rats (16). However, results from studies in which nonsteroidal anti-inflammatory drugs were administered to humans are inconsistent in regard to direct and indirect measures of delayed muscle damage in the recovery period after eccentric exercise (1, 6, 7, 18, 20). Although we suggest that infiltrated leukocytes cause delayed and additive damage in exercised muscle, we do not think that blocking infiltration will enhance recovery, because infiltrating leukocytes seem to be a major trigger of tissue repair, at least in terms of regeneration and satellite cell activation (8).

The decrease in maximal force-generating capacity during the night in the control leg corresponded in time to the second drop in performance in the exercised leg. Therefore, this diurnal variation can apparently at least in part explain the biphasic course of recovery. However, when the recovery course of maximal force-generating capacity was corrected for this normal diurnal variation, recovery still came to a standstill when the number of labeled leukocytes reached peak levels in the exercised leg. Because of the short half-life of $^{99m}$Tc, we did not include preexercise comparisons between the exercised and the control leg in the radionuclide investigations. Therefore, it is possible that there were some differences between the dominant and the nondominant leg before exercise. However, in a later study, we exercised the nondominant leg as well, and the result shows a marked increase of labeled leukocytes in the exercised leg whether the dominant or the nondominant leg was exercised (unpublished results).

Weak correlations between the number of labeled leukocytes and subjective soreness in the exercised muscle do not support causal relations, nor do similar ratings of soreness in all regions of the thigh, despite differences in the number of leukocytes in these regions. MacIntyre et al. (13) suggested a relation between inflammation and soreness, but this suggestion was based on a correlation between IL-6 concentrations in blood and DOMS. In contrast to findings in other studies, we found that the sensation of muscle soreness was present during the first hours after exercise, and no significant increase in soreness was observed thereafter. No real DOMS was reported by any of our well-trained subjects. A modest amount of thigh swelling was, nevertheless, observed after exercise, with a 1–2% increase in distal and medial circumference of the exercised thigh during the 1st h after exercise (data not shown). This, together with the leukocyte accumulation, indicates an inflammatory response, even from normally strength-trained muscles.
It is difficult to interpret the large interindividual variation in CK response. As in a previous study (23), we did not observe any correlation between individual CK responses and other indexes of muscle damage, such as force deficit or number of infiltrating leukocytes. Furthermore, Vincent and Vincent (27) reported no association between the magnitude of the CK response and indirect signs of muscle damage after strength exercise. These results support the skepticism expressed by Sorichter et al. (25) about the suitability of judging the magnitude of muscle damage from elevations in CK blood concentrations.

In conclusion, the halted recovery of maximal force-generating capacity 10–20 h after strength exercise, taken together with a significant infiltration of leukocytes in exercised muscle during the same time interval, shows a temporal relation between leukocyte infiltration and impaired recovery. The normal strength exercise performed by well-trained strength athletes did not lead to appreciable DOMS, and the early, modest muscle soreness bore no apparent relation to the leukocyte infiltration.

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REFERENCES