Change in Concentrations of Myogenic Components of Serum during 93 h of Strenuous Physical Exercise

Hiroshi Kosano,1 Tokuyasu Kinoshita,1 Naokazu Nagata,1 Osamu Takatani,1 Mitsuaki Isobe,2 and Yoshio Yazaki2

Effects of 93 h of long, strenuous ranger training on activities of creatine kinase (CK) and lactate dehydrogenase (LD), along with their isoenzymes, and on concentration of myosin light chain were examined in sera of young soldiers. Total CK activity in serum was measured before, during, and after the training. Throughout, total CK activity in serum increased steadily. At the end of the training, activity of CK-MB was increased but its activity ratio to total CK remained unchanged; the activity ratio of LD1/LD2 also was not increased, although total LD activity was increased. Myosin light chain was increased by about fourfold at the end of the training and remained high for three days thereafter. However, its concentration was much lower than in myopathies such as polymyositis and Duchenne muscle dystrophy. The increased activities in serum of total CK and CK-MB isoenzyme on strenuous physical exercise evidently were of non-cardiac origin. Although CK activity was comparable with that seen in myopathies accompanied by disintegration of skeletal muscle, the relatively low concentration of myosin light chain in serum suggests minimal skeletal muscle damage.

Additional Keyphrases: cardiac vs skeletal-muscle damage - myopathies compared - creatine kinase - lactate dehydrogenase - isoenzymes - myosin light chain in serum - military medicine

Increased activity of creatine kinase (CK, EC 2.7.3.2) MB isoenzyme activity in serum has been used diagnostically as a specific indicator of acute myocardial infarction (1). Recently, however, much attention has been given to CK-MB activities in sera of highly trained athletes (2), especially long-distance marathon runners (3-9). An increase in the isoenzyme after a marathon race is a constant finding and raises a difficult problem in diagnosing the myocardial damage that may occur during strenuous physical exercise: it is not clear whether the increased activity indicates structural damage or simply a change in the cell membrane permeability of muscle or both.

Addressing these questions, we examined the effects of long and strenuous exercise (93 h of ranger training) on activities of CK, lactate dehydrogenase (LD, EC 1.1.1.27) and their isoenzymes and, concurrently, the concentration of myosin light chain in serum from young soldiers. We conclude that changes in cell membrane permeability of skeletal muscle probably mainly contribute to the increased concentrations of such myogenic components in serum.

Materials and Methods

All subjects were healthy, well-trained male soldiers, ages 25–27 years (n = 11 to 23). Blood was sampled from the cubital vein or ear lobe, allowed to clot at room temperature for 30–40 min, and centrifuged. The serum was preserved at −80 °C until analysis. There was no evidence of hemolysis in any of the samples. Such samples were taken at the start and end of the 93-h training period and also on the third day after it ended. Samples were collected from the ear lobe at intervals of several hours during the training.

Activities of CK, CK isoenzyme (10), and LD (11) were measured spectrophotometrically at 30 °C. We fractionated CK isoenzymes by ion-exchange chromatography according to Takahashi et al. (12), LD isoenzymes by agarose electrophoresis (13). Myosin light chain in serum was measured by

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1. The Third Department of Medicine, National Defense Medical College, 3-2, Namiki, Tokorozawa, Saitama, 359 Japan.
2. The Third Department of Medicine, Tokyo University Medical School, Tokyo, Japan.

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radioimmunoassay (14, 15), with use of an antibody raised against cardiac myosin light chain I, which cross reacts by 10.2% with myosin light chain from skeletal muscle.

The ranger training was practiced for 93 h with continuous strenuous physical exercise. These trainees moved about 50 km through a secluded place among the mountains, carrying 30–40 kg of equipment. Sleep was limited to about 3 h per day, meals to about 600 kcal each.

All subjects were examined by a physician three days after the end of the training, including electrocardiography. Statistical significances of differences were assessed by Student's t-test.

**Results**

We tested the validity of the specimen from the ear puncture for use in CK assay by comparing the activities of sera sampled concurrently by ear-lobe puncture and from the cubital vein three days after the end of the training. The respective mean values (and SDs) were 147.1 (45.1) and 144.5 (41.8) U/L, not significantly different.

The activity of total CK (Figure 1) increased steadily after the start of the training, reaching its maximum by the end of the training. It decreased almost linearly thereafter, returning to the pre-training value within 72 h.

The activity of CK-MB was significantly greater at the end of the training, but had returned to the pre-training value by three days. However, the ratio of the isoenzyme to total CK activity remained unchanged throughout (Table 1).

Total LD also was increased at the end of the training, but the LD₁/LD₂ activity ratio was decreased significantly (p <0.01)—and total LD activity also returned to pre-training value within three days (Table 1).

Table 1 also shows the concentration of myosin light chain in serum. At the end of the training, the value was about four times that before the start of the training. It was still significantly above normal on the third day after the end of it. For serum obtained at the end of the training, total CK activity was correlated with myosin light chain, but no such correlation was found for the other specimens (Figure 2).

The hematocrit did not change during the period of training, but it did decrease significantly on the third day after the end of it (45.2 ± 1.4% vs 43.6 ± 2.9%, p <0.05).

No subject showed either any sign or symptom or electrocardiographic change indicative of cardiac distress.

**Discussion**

The increase in the activities of CK and its CK-MB isoenzyme in serum after strenuous physical activity has attracted much attention recently (2–9), but the serial CK profile during the exercise has not been examined. The present study shows that the almost continuous and strenuous ranger training was accompanied by a steady increase in serum CK activity throughout the exercise, with the value being greatest at the end of the training. The time course of the change after the end of the training was very similar to that reported for a 22-h march (16), but differed from the result of marathon running (3, 4, 7), in which the highest value for CK was observed 24 h after the race. Such a difference may result from differences in the severity and (or) duration of the exercises.

At the end of the training, the increase of total CK activity was accompanied by an increase of CK-MB activity, but the ratio of the isoenzyme to total CK remained unchanged. Moreover, although LD activity was increased after the training, the LD₁/LD₂ ratio was rather decreased. LD₁ and LD₂ are cardiac-specific isoenzymes (18), and an increase in the ratio of LD₁/LD₂ in serum is supposed to indicate myocardial damage (4). Thus, these isoenzyme patterns, considered together with previous studies (6, 8), may indicate that the source of CK and LD is not myocardium but some other tissue, probably skeletal muscle.

In an attempt to better ascertain whether the increase of CK and CK-MB resulted from a disintegration of skeletal muscle or only from increased permeability of muscle cell membrane, we measured myosin light chain in serum. The assay system we used was initially developed to measure cardiac myosin light chain I, but the cross reactivity of the antibody was exploited to measure skeletal muscle myosin light chain in serum. Myosin is considered to be a component of structural protein of muscle cell, and release of it therefore must reflect a disintegration of muscle rather than a change in cell membrane permeability. In contrast, CK is present in the cytosolic fraction and it supposedly is released even when there is a change in cell membrane permeability. As compared with the pre-training value, the concentration of myosin light chain was increased by more than threefold, paralleling total CK activity, at the end of

![Figure 1](image-url). Changes in serum CK activity during the ranger training. CK activity was measured on blood sampled either from ear lobe or vein. Each mean (± SD) shown is for 11 subjects.

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<thead>
<tr>
<th>Table 1. Effects of Strenuous, Prolonged Training on CK, LD, Their Isoenzymes, and Myosin Light Chain (Mean ± SD)</th>
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<tr>
<td><strong>Total CK activity, U/L</strong></td>
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<td>143.5 ± 90.2</td>
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<tr>
<td><strong>CK-MB activity, U/L</strong></td>
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<td>(% of total CK)</td>
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<td><strong>Total LD activity, U/L</strong></td>
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<td><strong>LD₁/LD₂</strong></td>
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<td><strong>Myosin light chain, µg/L</strong></td>
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*p <0.001, **p <0.01, and ***p <0.05, for the level of significance as compared with the value before the training. *n = 23; **n = 11; *n = 12.
training, and it still was significantly above normal three days later. The biological half-life of serum myosin light chain is estimated to be about 4.5 h (14), so the sustained increase in myosin light chain may indicate a continuous outflow from the affected muscle, even after the end of the training.

By the same assay, the concentration of myosin light chain is 12 to 25 μg/L in active polymyositis and 20 to 520 μg/L (mean 174, SD 86 μg/L) in Duchenne muscle dystrophy, correlating linearly with serum total CK activity in serum. This is lower than what we observed in the ranger training (845 (SD 342) U/L vs 462 (SD 238) U/L in Duchenne muscle dystrophy; unpublished data). The increase in myosin light chain in serum is disproportionately low in relation to CK activity as compared with the relation found in a disease that is characterized by the disintegration of the skeletal muscle; this suggests that the origin of the marked increase we saw in serum CK and CK-MB is mainly the result of an increased permeability rather than of the breakdown of the muscle cells. Newly synthesized myosin light chain may be present in the free form in the cytosol and possibly released when there is a change in cell membrane permeability (19). If so, the structural damage to skeletal muscle during the ranger training must be much less than that deduced from the observed concentration of myosin light chain in serum. Recently, Warhol et al. (20) studied specimens of muscle biopsy from marathon runners and suggested that enzyme efflux may take place from a reversibly injured portion of the cell and may not imply irreversible cell injury or cell death.

We conclude that the increased activities of CK and CK-MB as well as of LD during long, exhausting physical exercise are of non-cardiac origin. Although the CK activity may increase up to values comparable with those seen in myopathies such as polymyositis or Duchenne type muscle dystrophy, the relatively low values for myosin light chain suggest that there is minimal structural disintegration of the skeletal muscle.

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References