Profile of Creatine Kinase Isoenzymes in Skeletal Muscles of Marathon Runners

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The proportion of creatine kinase (CK; EC 2.7.3.2) isoenzyme MB activity was increased in skeletal muscle biopsies obtained from five long-distance runners, both 2 h before (mean 7.7%, SD 2.4%) and 30 min after (mean 7.2%, SD 1.2%) a marathon race, as compared with that in biopsies from five nonrunners (controls ≤ 1.0%). Further, mitochondrial CK and CK-BB isoenzymes were present in homogenates of the runners' skeletal muscle samples but not in those of the nonrunners. However, there were no substantial differences in the mean total CK activities per gram (wet wt.) of muscle tissue among premarathon samples, postmarathon samples, and nonrunners' samples (3148, 3365, and 3049 U/g, respectively). We conclude that the metabolically active gastrocnemius muscle of long-distance runners is qualitatively similar to the heart muscle in its CK isoenzyme composition.

Additional Keyphrases: gastrocnemius-heart muscle comparison • myocardial infarction • source of circulating isoenzymes • sports medicine • effects of exercise, training

Creatine kinase (CK; EC 2.7.3.2) isoenzymes have been intensively investigated to evaluate myocardial and skeletal muscle damage (1, 2). The release and clearance of CK-MB from the heart after acute myocardial infarction has also been well studied (3, 4). Increases in activities of serum total CK and CK-MB and the temporal relationship of total CK and CK-MB are highly sensitive and specific for acute injury to the heart (5, 6). However the specificity of serum CK-MB for heart muscle is not absolute; as early as 1965 Rosalki (7) showed that skeletal muscle enriched in red (slow twitch) fibers contained more CK-MB than did white (fast twitch) fibers. The recent popularity of marathon running has increased interest in measuring CK-MB activities in the sera of these runners (8-10). In the runners' blood, both during training for and after a marathon race, CK-MB percentages (1 to 10% of total CK activity) are in the range indicative of acute myocardial damage. In addition, the pattern of increase and decrease of total CK and CK-MB in serum after a marathon race (42.2 km) reportedly is similar to that after an acute myocardial infarction (11).

We undertook the studies reported here to: (a) determine the CK isoenzyme pattern in skeletal muscle biopsies obtained from long-distance runners; (b) compare these patterns with those for nonrunning controls; and (c) determine the relationship, if any, between the percentage of CK-MB isoenzyme and the percentage of slow-twitch muscle fibers.

Materials and Methods

Subjects

Five male long-distance marathon runners (ages 21-28; mean 25.4 years), none with cardiac risk factors (hypertension, smoking, hyperlipidemia), and five male nonrunning controls volunteered for the study after being informed of the purpose, methods, and possible complications of the procedures. Written and verbal informed consent was obtained for the study, which had been reviewed and approved by the appropriate institutional review committees. During the 10 weeks of training before the marathon race, each of the runners averaged approximately 65 miles per week. None of the subjects experienced clinical symptoms characteristic of acute myocardial infarction before, during, or after the marathon race (12).

Muscle biopsies, obtained by the needle biopsy technique of Bergstrom (13), were taken from the lateral portion of the gastrocnemius muscle described previously (14), 2 h before and within 30 min after the race for the runners and at random times from the nonrunners. Muscle fibers were identified as slow twitch (ST) or fast twitch (FT) on the basis of myosin ATPase (EC 3.6.1.8) activity, as described by Mabuchi and Streeter (15).

Tissue Preparation

The tissue samples were stored at −70°C in tightly sealed glass tubes. At the time of isoenzyme analysis we homogenized with 40 strokes at 4°C a precisely weighed amount (3-5 mg) of tissue in a 15-mL Dounce glass homogenizer containing 3 mL of ice-cold pH 7.2 buffer (per liter: 50 mmol of Tris HCl, 250 mmol of sucrose, 10 mmol of EDTA, and 5 mmol of 2-mercaptoethanol). Cell fragments, including mitochondria, were removed by centrifugation (Servall SS-34, 15 000 rpm, 20 min). We kept the supernates on ice and analyzed them the same day for total CK activity and CK isoenzymes before storing them at −70°C.

Determination of Enzyme Activity

We measured total CK activities at 30°C with a kinetic enzyme analyzer (CentrifibChem 400; Baker, Pleasantville, NY) using N-acetyl-cysteine activated reagent (Calbiochem-Behring, La Jolla, CA) and a modification of the Rosalki procedure (16). To separate CK isoenzymes, we used electrophoresis on agarose (Corning Medical, Medfield, MA) as described by the manufacturer. We electrophoresed samples undiluted if the total CK activities were less than 350 U/L, or we diluted samples (Tris buffer, 50 mmol/L, pH 7.5) to give a total CK activity of 300 ± 20 U/L, because the upper

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limit of linearity for our system for total CK was 450 U/L. In addition, for purified CK-MM or CK-MB the relation between densitometer reading and amount of isoenzyme was linear to 400 U/L. After electrophoresis, we located the isoenzymes in the presence of substrate by NADPH fluorescence, the lower limit of sensitivity being 3 U/L. To rule out non-creatine kinase artifacts, we electrophoresed samples with and without substrate (creatine phosphate). Results are reported as the mean of three separate electrophoreograms of each specimen. For standards, we used purified CK-MB from human heart muscle. The within-run precision (CV) for CK-MB was 8.3% (n = 23), compared with 8.2% between runs (n = 28). To identify isoenzyme migration we used a human CK isoenzyme control (MM, MB, BB; Beckman Instruments, Brea, CA), and we quantified the isoenzymes by scanning densitometry (Beckman Model CDS 200). The increased percentages of muscle CK-MB were verified by ion-exchange chromatography (17).

Results

Figure 1 shows a typical pattern for separation of the CK isoenzymes in muscle homogenate. Table 1 lists findings for homogenates of skeletal muscle biopsied from runners 2 h before the race. The mean CK-MB percentage was 7.7 (SD 2.4%), the range 5.7 to 10.9%. Two runners had traces of CK-BB (less than 1%), while all runners exhibited traces of a mitochondrial CK, migrating cathodally to CK-MM. The CK-MB content (in percent) correlated well with the percentage of slow-twitch muscle fibers \( r = 0.84, \%\ ST = (4.85 \times \%\ MB) + 27.6 \). The CK isoenzyme composition of biopsies from the same runners, taken within 1 h after the race, are also shown in Table 1. The CK-MB percentage was 7.2% (SD 1.2%; range 5.2 to 8.0%), not significantly different from the pre-marathon samples. However, runner 4 exhibited a 6% CK-BB fraction while only having a trace of CK-BB in the pre-marathon sample. All post-marathon biopsies contained trace amounts of mitochondrial CK. CK-MB content and the percentage of slow-twitch muscle fibers in the biopsies obtained after the race correlated poorly \( r = -0.12, \%\ ST = (1.36 \times \%\ MB) + 74.8 \). The CK isoenzyme composition of biopsies from the nonrunning controls exhibited 99–100% CK-MM, with less than 1% CK-MB. There was no evidence of either CK-BB or mitochondrial CK. While there was a qualitative CK isoenzyme difference between skeletal muscles of marathon runners and controls, there were no differences in total CK activities. The total CK activity per gram of tissue was 3148 (SD 404) U/g in pre-marathon samples, 3365 (SD 293) U/g in post-marathon samples, and 3049 (SD 181) U/g in control samples (Table 1).

Discussion

The CK in human heart is 15 to 25% CK-MB and 75 to 85% CK-MM, with trace amounts (<1%) of mitochondrial CK and CK-BB (2). We show that the gastrocnemius in the long-distance runners resembles heart muscle in both its mitochondrial and cytoplasmic CK isoenzyme composition. The proportion of CK-MB, mitochondrial CK, and CK-BB activities are increased in comparison with non-running controls. The percentages of CK-MB in pre- and post-marathon biopsies were comparable: 7.7% and 7.2%, respectively.

Most reports on the CK isoenzyme composition of skeletal muscle describe only trace amounts to 3% of CK-MB present in cytoplasmic preparations (17-20). Even when Kettunen et al. (21) analyzed quadriceps muscles obtained before and after a hard training session from junior ice-hockey players, CK-MB constituted less than 2% of the total CK-MB. Our post-marathon muscle CK-MB percentages (5–11%) are comparable to those recently reported (8–9%) by Siegel et al. (22), even though their muscle samples were not collected immediately after the marathon race. In addition, correlations between the CK-MB percentage and the percentage of slow-twitch fiber in post-race samples were poor in both studies \( r = -0.12 \) and 0.085, respectively). But, our post-marathon CK-MB percentages correlated well with the percentages of slow-twitch fibers \( r = 0.84 \). Possibly CK-MB was selectively released from selective fiber types into the circulation from the stressed/injured skeletal muscle after the race, but the explanation of the differences among reports is still unclear.

Explanations for the increase of CK-MB in the gastrocnemius muscle itself include the following: First, the slow-twitch muscle fibers may contain a greater proportion of CK-MB, or have a greater propensity to synthesize this molecule than fast-twitch fibers, or both. Thus, skeletal muscle from highly trained marathon runners contains a greater proportion of slow-twitch muscle fibers than that from shorter-distance runners or nonrunning controls (23). We substantiated this finding: the long-distance runners had 65.0 (SD 13.9)% ST fibers as compared to 46.6 (SD 14.5)% ST fibers in nonrunners. Further, the excellent correlation between the percentages of CK-MB and of ST fibers before the stress of the race support this contention. Second, when damaged skeletal muscle begins its regenerative process, the CK isoenzyme composition could progress through the fetal mix of isoenzymes, which includes increased CK-MB (24). Hikida et al. (14) observed ultrastructural changes by electron microscopy of gastrocnemius obtained from the same runners we investigated. Their findings suggest that exercise-induced muscular necrosis occurs during training (chronic stress) and after a race (acute stress), reminiscent of rhabdomyolysis. Thus repetitive or continuous damage to and regeneration of muscle fibers could cause them to contain an increased proportion of CK-MB. Third, the stress induced by training or racing might resect optimal state from the "B"-subunit gene to initiate and increase synthesis of CK-MB or CK-BB, or both.

Mitochondrial CK has been found in human heart (25), liver (25), and brain (26), but no other report describes

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**Fig. 1. Separation of CK isoenzymes from skeletal muscle tissue by agarose electrophoresis**

A, control; B, representative skeletal muscle homogenate, electrophoresed undiluted (total CK = 4520 U/L); C, homogenate B diluted to 310 U/L (CK-MB was 5% of total CK activity); 1, CK-BB; 2, CK-MB; 3, CK-MM; 4, mitochondrial CK. Cathode (−) at bottom; anode (+) at top.
mitochondrial CK in human gastrocnemius muscle (Table 1 and Figure 1), nor have substantial amounts of CK-BB been reported in skeletal muscle. These new findings, along with reports that other oxidative and mitochondrial muscle enzymes are increased in long-distance runners (27), support our hypothesis that the metabolically active gastrocnemius tends qualitatively to resemble heart muscle in CK isoenzyme composition. Further, the lack of significant differences in total CK activities per gram of tissue in pre-marathon, post-marathon, and nonrunning control samples is in agreement with previous reports (22, 28).

When skeletal muscle enriched in CK-MB (up to 11% in our sampling) is damaged, as may occur in marathon runners, it should follow that increased serum CK-MB activities can be ascribed, wholly or partly, to release of CK-MB from skeletal muscle. We recently reported significantly different half-lives of circulating CK-MB during the six days after a marathon race and during the six days after acute myocardial infarction—6 h and 12 h, respectively (11)—and we postulated that CK-MB was released from damaged skeletal muscle into the circulation substantially slower (days) than the corresponding release of CK-MB after acute myocardial infarction (hours), thus accounting for the difference in clearance. However, it cannot be ruled out that the stress of marathon running causes simultaneous release of CK-MB from both heart and skeletal muscle.

In summary: we report increased CK-MB, CK-BB, and mitochondrial CK activities in biopsies of gastrocnemius muscle obtained from long-distance runners, both before and after a marathon race, as compared with nonrunning controls. We suggest that the metabolically active gastrocnemius muscle in long-distance runners adapts its CK isoenzyme composition to become qualitatively similar to that of cardiac muscle. Our findings also suggest that the higher CK-MB activity found in the serum of long-distance runners is ascribable to its release from skeletal muscle. Thus clinicians should recognize the potential for false biochemical evidence (i.e., false-positive results) for acute myocardial infarction when interpreting patterns of CK-MB isoenzyme in serum from long-distance runners.

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References

**Table 1. Total CK Activity and Proportions of Isoenzymes in Skeletal Muscle from Five Marathon Runners**

<table>
<thead>
<tr>
<th>Slow-twitch fiber</th>
<th>Total CK activity, U/g wet wt.</th>
<th>CK-MM</th>
<th>CK-MB</th>
<th>CK-BB</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Control</td>
<td>Pre</td>
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<td>Slow-twitch</td>
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*Biopsies obtained from runners' gastrocnemius muscle 2 h before and 30 min after marathon. All samples contained trace amounts of mitochondrial CK. Biopsies from nonrunning controls contained <99% CK-MM, <1% CK-MB. No CK-BB or mitochondrial CK was detected. TR, trace (present but <1%); ND, not detected.


