Ratio of Creatine Kinase 2 Mass Concentration to Total Creatine Kinase Activity Not Altered by Heavy Physical Exercise

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Serum creatine kinase isoenzyme 2 concentrations (CK 2 mass) were measured in marathon runners during training and 1 and 2 days after a race and compared with values from 36 acute myocardial infarction (AMI) patients whose total CK and (or) CK 2 activities were similar to those of runners in the basal state. During training, runners had CK and CK 2 activities 53% and 43% above reference values, respectively, and 36% had CK 2 activity >5% of total CK. Nine runners (26%) showed CK 2 mass values >6 µg/L but ≤10 µg/L; 35 of the AMI subjects, despite having CK activities similar to those of runners, had values >10 µg/L. The ratio of CK 2 mass to total CK activity was significantly (P < 0.0002) different between sexes for runners. At 1 and 2 days after racing, 100% of CK and CK 2 activities and 71% and 57% of the percentages of CK 2 activity, respectively, were abnormally high; 57% and 43% of CK 2 mass values were >10 µg/L, being comparable with those observed for the AMI group. Basal CK 2 mass values of the runners appeared only slightly higher than that for sedentary subjects, but after exercise half the subjects presented increased values similar to those observed for AMI subjects. The ratio of CK 2 mass to total CK activity appeared unaltered by exercise in all but one of the samples assayed, indicating its utility in evaluating CK 2 mass increases originating in skeletal muscle.

Additional Keyphrases: myocardial infarction · isoenzymes · immunochemiluminometric assay · marathon runners

Supranormal increases of creatine kinase 2 (CK; EC 2.7.3.2) isoenzyme activity in serum can be observed after both myocardial and skeletal muscle damage, which makes the biochemical characterization of the tissue of origin difficult. Because myocardium contains the highest tissue proportion of CK 2 of all human tissues (1, 2), reaching values >20% of total CK activity, it has been assumed that myocardial damage is the most likely source for serum CK 2 activity increases. However, some facts argue against this assumption. Some myocardial infarction patients have small increases of CK 2 activity (3), indicating that myocardial infarction can occur with normal or only slightly increased serum CK 2 activity. Skeletal muscle of aerobically trained subjects shows increases in CK 2 activity content in response to exercise (4, 5); after heavy exercise, these subjects can have serum CK 2 activity above normal values. On the basis of these findings, skeletal muscle damage could be postulated to be the most frequent cause of the serum CK 2 increases observed in many well-trained subjects; however, myocardial damage must also be excluded. Therefore, a biochemical measure discriminating between skeletal and myocardial origin of CK 2 is needed for evaluating this special group of subjects.

Immunochemistry-based methods have permitted the measurement of CK 2 mass concentration instead of CK 2 catalytic activity. These methods allow better differentiation between myocardial and skeletal muscle damage than does measurement of CK 2 activity, especially when CK 2 concentration is expressed as a ratio of CK 2 concentration to total CK activity (6). Since an immunoenzymometric method for measuring CK 2 concentration was first described (7), many other assays have been developed whose utility in myocardial damage detection has been studied (8, 9). However, there has been no mention of CK 2 mass concentrations in aerobically trained subjects nor about the possibility of basal or postexercise values being indistinguishable from those observed in myocardial infarction.

Here we measured the mass concentrations of CK 2, using an immunochemiluminometric assay, in a group of highly trained marathon runners to analyze the effect of heavy physical work on CK 2 concentrations. We compare the results obtained with those from a group of acute myocardial infarction (AMI) patients presenting with total CK and (or) CK 2 enzymatic activities similar to those of runners in basal state.

Materials and Methods

Subjects

Marathon runners. The group of runners consisted of 34 subjects, 21 men and 13 women, ages 25–42 years. All were active national and international marathon runners (best times for a marathon race were between 2 h 13 min and 2 h 30 min for men and between 2 h 35 min and 2 h 59 min for women), training between 60 and 190 km/week. Blood samples were obtained during routine medical examinations, which included a physical exam and an electrocardiogram. Samples were drawn ≥2 h after the last exercise period; samples obtained after weight-lifting training or downhill running were excluded. In a subgroup of seven subjects, additional samples were obtained 24 and 48 h after finishing a marathon race.

AMI patients. A group of 26 clinically and electrocardiographically confirmed AMI subjects (4 women, 22 men) was studied. Samples from these subjects were

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obtained on admission to the hospital; the interval between the onset of symptoms and blood collection was <12 h. Patients were included if their initial blood sample had total CK and (or) CK 2 enzymatic activities similar to those of runners in the baseline state. One subject presented with normal total CK activity and seven with normal CK 2 activity; thus 30.8% of patients showed no enzymatic evidence of AMI. These selection criteria, designed to study CK 2 mass in subjects with similar enzymatic values but different health status, excluded AMI patients presenting with total CK and CK 2 activities lower than those of runners. Consequently, results obtained apply only to these specific groups of AMI patients and runners.

Methods

Serum was separated immediately by centrifugation at 4 °C. Samples for CK 2 mass measure were frozen at −20 °C for ≤5 days. Total CK and CK 2 catalytic activities were assayed immediately after serum separation.

Total CK catalytic activity was determined by an N-acetylcytistine-activated assay at 37 °C (Boehringer Mannheim, Mannheim, FRG). Upper reference limits for this assay obtained with sedentary healthy individuals are 180 U/L for men and 150 U/L for women. Catalytic activity of CK 2 was measured by an immunoinhibition method (Boehringer Mannheim), and residual CK activities were measured as above. Adenylate kinase activity was assayed in all samples by omitting the substrate and results were subtracted from the residual CK values. The upper reference limit for CK 2 activities obtained with this correction is 14 U/L for sedentary healthy men and women.

Mass concentration of CK 2 was determined by an immunochimiluminometric assay, with acridium ester as the luminescent label and monoclonal anti-CK 2 antibodies (Magic Lite; Ciba-Corning Diagnostic Corp., Medfield, MA). Samples were assayed in duplicate according to manufacturer’s recommendations. The upper reference limit obtained in our laboratory for healthy, sedentary people is 6 μg/L. A relative index of CK 2 mass concentration to total catalytic activity of CK was calculated by dividing mass concentration in μg/L by total activity in U/L and multiplying by 100. The upper reference value of 4.0 used in the study was derived from the literature (6).

Results and Discussion

The results obtained for all 34 athletes during training and in a subgroup of 7 subjects 24 and 48 h after a marathon race are summarized in Table 1, where values obtained in AMI patients are also shown. The proportions of runners with values greater than reference values or similar to those for AMI patients are described in Figure 1.

In samples obtained during training, catalytic activities of total CK and CK 2 isoenzyme were increased in 53% and 43%, respectively, the total CK being significantly higher (P <0.003) for men than for women. This sex-related difference in CK activity was reported for reference individuals (10) and for trained people basally and after exercise (11); greater muscle mass or greater CK content in skeletal muscles in men and a protective effect of estrogen over exercise-induced muscle damage in women have been given as possible explanations for the lower CK values observed in women (11). During training, 36% of subjects had ratios of CK 2 activity to total CK that were above the upper reference limit. Using agarose-gel electrophoresis to determine CK 2 activity, Apple et al. (12) noted no abnormal values for the percentage of CK 2 activity in seven marathon runners. Haibach and Hoeler (13), measuring CK 2 by column chromatography, reported that 4 of 21 marathon runners had higher than normal percentages of CK 2 activity. The studies included different-duration rest periods before a marathon race, which, along with methodological differences, could account for the differences in the number of subjects with altered percentages of CK 2 activity.

The increase in total CK, absolute CK 2 activity, and percentage of CK 2 activity in these trained subjects occurred without electrocardiographic modifications. This finding is consistent with our previous report (14) and that of others (15), showing that no electrocardiographic or scintigraphic signs of myocardial infarction are seen after exhaustive exercise. This indicates a nonmyocardial origin for the increased CK 2 activity.

No sex-related differences were observed for CK 2 mass concentration, in accord with other published results (16, 17). CK 2 concentrations showed relatively few abnormal values (26%) over the reference limit of sedentary people (6 μg/L), but no subject presented a value >10 μg/L during baseline training. Recent studies involving radial partition fluorescence or chemilumimetric immunoassays derived upper cutoff limits for CK 2 concentration as high as 14 μg/L, 10 μg/L being the upper reference limit usually found in healthy subjects (8, 9, 18). According to these limits, no athlete had abnormal values of CK 2 concentrations during training. The difference observed between the upper limits of

Table 1. CK Measures in Marathon Runners during Training (n = 34) and 24 and 48 h after a Marathon Race (n = 7) and in AMI Patients (n = 26)

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>24 h</th>
<th>48 h</th>
<th>AMI patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Runners</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CK, U/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men [180]</td>
<td>111-440</td>
<td>274-2857</td>
<td>398-1492</td>
<td>66-2096</td>
</tr>
<tr>
<td>Women [150]</td>
<td>70-5565</td>
<td>306-1169</td>
<td>208-7565</td>
<td></td>
</tr>
<tr>
<td>CK 2 acty, U/L</td>
<td>ND-48</td>
<td>20-193</td>
<td>21-64</td>
<td>1-87</td>
</tr>
<tr>
<td>ND-11.8</td>
<td>4-2.1-113</td>
<td>2-17.5</td>
<td>1.2-11.0</td>
<td></td>
</tr>
<tr>
<td>CK 2 mass, μg/L</td>
<td>2-10</td>
<td>6-131</td>
<td>5-27</td>
<td>7.8-118</td>
</tr>
<tr>
<td>CK 2 mass/total CK</td>
<td>4.5-14.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men [4.0]</td>
<td>1.2-2.8</td>
<td>2.1-4.9</td>
<td>1.4-2.2</td>
<td></td>
</tr>
<tr>
<td>Women [4.0]</td>
<td>1.6-4.5</td>
<td>2.2-2.6</td>
<td>1.8-3.0</td>
<td></td>
</tr>
</tbody>
</table>

* Upper cutoff value given in brackets. ND, not detectable. Significantly different from values for men: *P <0.003, **P <0.001, ***P <0.002.
the range of \( \text{CK} \) concentrations of athletes and sedentary patients suggests that cutoff points used to differentiate AMI patients from normal people need to be extended to the maximum value seen in well-trained individuals.

In our particular group of AMI subjects, with basal enzymatic activities similar to those of runners, 25 of 26 (96.1%) individuals had values of \( \text{CK} \) concentrations >10 \( \mu \text{g/L} \), whereas only 1 patient had a value of 7.8 \( \mu \text{g/L} \). Indeed, published differences for the upper reference limit of \( \text{CK} \) concentrations (between 6 and 10 \( \mu \text{g/L} \)) could be due not only to methodological differences but also to the reference groups comprising varied percentages of sedentary and trained individuals. Thus, reference values for \( \text{CK} \) mass concentrations should be obtained in sets of subjects with known amounts of physical activity. Finally, the ratio of \( \text{CK} \) 2 mass to total \( \text{CK} \) activity showed only 6% of results (2 of 34) >4.0, being the measurement studied that was least affected by exercise practices of the subjects. Women showed significantly higher (\( P <0.0002 \)) values than did men. This difference in the ratio of \( \text{CK} \) 2 mass to total \( \text{CK} \), attributable to lower values of total \( \text{CK} \) activity observed in female athletes, has not been previously described and indicates that different values for men and women must be used for establishing reference ranges. No runner had a value >4.5, the lowest value observed in our group of AMI patients.

All subjects presented higher than normal values of total \( \text{CK} \) and \( \text{CK} \) 2 catalytic activities 24 and 48 h after the marathon race. These results agree with published values (4, 19); total \( \text{CK} \) values also showed the same sex-related difference observed in the basal samples. Of the runners, 71% and 57% had higher than normal percentages of \( \text{CK} \) 2 activity 24 and 48 h after the race, respectively. Similar percentages of subjects (57% and 43%, respectively) presented values >10 \( \mu \text{g/L} \) of \( \text{CK} \) 2 mass concentration 24 and 48 h postexercise, whereas only 1 (7%) of the 14 postrace samples showed values <6 \( \mu \text{g/L} \). \( \text{CK} \) 2 of 131 \( \mu \text{g/L} \) was observed for one female runner. These results suggest that, after heavy exercise, aerobically well-trained subjects can have activities not only higher than reference \( \text{CK} \) 2 activities, as already described (4, 5), but also \( \text{CK} \) 2 concentrations similar to those observed in myocardial infarction. However, the ratio of \( \text{CK} \) 2 mass to total \( \text{CK} \) activity was abnormally high in only one sample drawn 24 h after running, this sample being the only one with a value in the range obtained for AMI subjects. Although there is no association described between \( \text{CK} \) 2 mass concentration and \( \text{CK} \) 2 catalytic activity in skeletal muscles of marathon runners, one explanation for normal serum values for the ratio of \( \text{CK} \) 2 mass to total \( \text{CK} \) activity could be that this ratio for runners is not different from that for sedentary individuals.

Our results, obtained from marathon runners after strenuous exercise (men completed their runs in <145 min, women in <195 min), demonstrate the utility of the ratio of \( \text{CK} \) 2 concentration to total \( \text{CK} \) in evaluating high \( \text{CK} \) 2 concentrations related to skeletal muscle damage.

In conclusion, \( \text{CK} \) 2 concentrations of aerobically well-trained athletes appear to be only slightly higher than those of sedentary subjects. This fact should be taken in account when establishing cutoff limits for evaluating high \( \text{CK} \) 2 concentrations. After heavy exercise, a high proportion of athletes have \( \text{CK} \) 2 concentrations that are greater than reference values and indistinguishable from values observed in some AMI patients. Both basally and postrace, the ratio of \( \text{CK} \) 2 concentration to total \( \text{CK} \) was within reference values in most samples, indicating its utility for evaluating \( \text{CK} \) 2 increases that originate in skeletal muscle. However, the sex-related difference observed for the ratio requires the use of separate reference ranges for men and women.
women. The discrepancy between results of the ratios of CK 2 activity and CK 2 mass to total CK needs further investigation but suggests that skeletal muscle of marathon runners contains a ratio of CK 2 mass to total CK that may not be different from that of sedentary subjects.

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