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Elevated Serum Creatine Kinase MB and Creatine Kinase BB-isoenzyme Fractions after Ultra-marathon Running

T. D. Noakes, G. Kotzenberg*, P. S. McArthur, and J. Dykman*

Metropolitan Sport Science Centre, Department of Physiology, University of Cape Town Medical School, Observatory, 7925, South Africa

Summary. To determine the incidence and range of serum creatine kinase MB (CK-MB) isoenzyme activity after ultra-marathon running, a popular test kit was used to measure total serum creatine kinase (CK) and CK MB-activity in 75 athletes immediately after they had completed an 88-km running race. Total serum CK activity was markedly elevated after the race (mean value: 637 $U \cdot l^{-1}$) and 45 (60%) runners showed abnormal CK-MB isoenzyme activities (greater than 4% of total CK activity – range 1–19%).

Electrophoresis of 31 sera with either CK-MB to total CK activity greater than 4% or with total CK activity greater than 854 $U \cdot l^{-1}$ showed that 31 (100%) had visible CK-MM bands, 21 (68%) had visible CK-BB bands, but only 14 (44%) had visible CK-MB bands.

We conclude that prolonged exercise increases the serum activity of all three CK-isoenzymes, and that the CK-MB test kit used in this study identified a greater number of sera with elevated post-race CK-MB isoenzyme activity than did electrophoresis. This discrepancy could result either from cross-reaction of elevated CK-BB activity with the test kit, or from relative insensitivity of the electrophoresis.

The tissue source and long-term significance of the elevated serum CK-MB and CK-BB isoenzyme activity induced by ultra-marathon running are uncertain. Until these issues are resolved, these biochemical findings in ultra-marathon runners must be interpreted with the appropriate caution.

Key words: Serum creatine kinase – Creatine kinase MB and BB isoenzymes – Ultra-marathon running

Offprint requests to: T. D. Noakes at the above address
* Dr. Kotzenberg and Mr. Dykman are attached to the Department of Chemical Pathology, University of Stellenbosch Medical School, Tygerberg, South Africa

Introduction

Although serum creatine kinase (CK) activity is elevated even after quite mild exercise (Berg and Haralambie 1978; Griffiths 1966; La Porte et al. 1978; Sanders and Bloor 1975; Vejjajiva and Teasdale 1965) gross elevations have been reported in marine recruits during the first 6 days of basic training (Olerud et al. 1976), in standard 42-km marathon runners (Berg et al. 1978; Riley et al. 1975; Siegel et al. 1980), and in walkers, runners, and skiers completing distances of between 85 and 160 km (Berg et al. 1978; Griffiths 1966; Kielblock et al. 1979; Noakes and Carter 1976; Olivier et al. 1978; Refsum et al. 1971; Schiff et al. 1978). Several authors have pointed out that appropriate caution must, therefore, be used when clinical judgements are made on the basis of serum enzyme levels in physically-active subjects (Griffiths 1966; La Porte et al. 1978; Nuttall and Jones 1968; Sanders and Bloor 1975; Vejjajiva and Teasdale 1965), yet errors continue to be made (Bunch 1980; Solomon 1979).

More recent interest has been shown in the finding that the Marsh-Bender isoenzyme of CK (CK-MB) may be found in the sera of apparently healthy runners during their daily training (Brunch 1980; Apple 1981) and after completing running races of 26 km (Schnohr et al. 1980), 42 km (Phillips et al. 1982; Siegel et al. 1981; Stansbie et al. 1982) 50 km (Diamond et al. 1983), 90 km (Olivier et al. 1978), and 160 km (Kielblock et al. 1979). Although elevation of this isoenzyme is not considered indicative of cardiac damage under these conditions (Bunch 1980; Kielblock et al. 1979; Olivier et al. 1978; Siegel et al. 1981), nevertheless cases of unrecognised myocardial infarction occurring during races of 42 and 90 km (Noakes et al. 1977) have been described. If an athlete were to develop the symptoms of myocardial infarction during exercise, it is probable that testing his serum for its CK-MB isoenzyme

activity would be considered an important diagnostic aid. But interpretation of the results is complicated as there are few studies of the "normal" serum CK-MB isoenzyme response to ultra-marathon running (Diamond et al. 1983; Standsbie et al. 1982).

We therefore considered it necessary to document the incidence and extent of CK-MB isoenzyme elevation in a large group of runners of quite different athletic abilities competing in an 88-km running race. This race has previously been shown to cause elevated serum CK-MB isoenzyme activity (Olivier et al. 1978). A second objective was to evaluate the specificity of a commercially-available CK-MB test kit under these conditions because of the possibility of cross reactivity between the CK-MB and CK-BB isoenzymes (Kaste and Sherman 1982).

Materials and Methods

Blood was drawn from 75 (2%) of 3,665 athletes completing the 1981 88-km Comrades Marathon, a race that is run annually between Durban and Pietermaritzburg, South Africa, in times ranging between 5 h 37 min (the race winner) to 10 h 15 min (the 2,877th finisher). All the runner had been informed of the study before the race, and the distribution of the finishing times of those who participated in the study was the same as was that of all the race finishers, indicating a representative sample.

Immediately the blood samples had been drawn, they were placed on ice for 30 min before being centrifuged twice for 5 and 10 min, 30 min apart. The supernatant was then pipetted into fresh tubes and transported frozen to Cape Town where it was stored at −20° C until analysis.

Creatine kinase was assayed by a kinetic UV method monitored at 340 nm with a Centrifichem 500 analyser using CK NAC-activated test kit (Monotest: Boehringer Mannheim, FRG). CK-MB isoenzyme was assayed by a similar technique using the CK-MB NAC-activated test kit (Monotest: Boehringer Mannheim, FRG). This test utilizes an immuno-inhibition technique which inhibits the B-sub-units of the CK enzyme. Subtraction of the value measured with this kit, from that for the total CK, gives the CK-MB value. The accuracy of this value depends on there being little or no serum CK-BB isoenzyme activity. Control samples were run simultaneously with the sera.

To determine whether the CK-BB fraction was present, electrophoresis on a cellulose acetate membrane was performed on selected sera. The method of application of sera to the membrane was as follows: If the total CK value was less then 250 U · l^{-1}, four applications, each of 0.25 µl serum, were made. If the total CK was between 250 and 500 U · l^{-1} three applications of 0.25 µl were made. For total CK values between 500 and 750 U · l^{-1}, two applications were made, and if total CK was more than 750 U · l^{-1}, only one application was made. Electrophoresis was carried out for 20 min at 240 V.

A second membrane was soaked with CK substrate, superimposed on the electrophoresed membrane, blotted and then incubated for 10 min at 37° C. The membranes were then inspected under ultraviolet light. No efforts were made to quantify the amount of each isoenzyme present. If the isoenzyme band was visible, it was documented as positive (+).

Table 1. Total serum creatine kinase (CK), CK-MB isoenzyme, and % CK-MB/total CK measured with a test-kit in 75 runners after an 88-km race

	Mean	SD	SE	Range
Total CK U · l^{-1}	634	728	84	61–4,224
CK-MB U · l^{-1}	22,5	17,3	20	3–111
CK-MB Total CK (%)	4.8	2.6	0.3	1–19

The following values are considered abnormal: total CK activity greater than 160 U · l^{-1}; CK-MB greater than 10 U · l^{-1}, and % CK-MB/total CK greater than 6%

Experimental Results

The test kit measured values of total serum CK, CK-MB and % CK-MB were all elevated about normally-accepted control levels after the race (Table 1). The mean post race total CK value (637 U · l^{-1}) was quite similar to the value of 730 U · l^{-1} measured by Olivier et al. (1978), after the Comrades Marathon. The highest serum CK activity (4,224 U · l^{-1}) was measured in a physician who had run only 363 km in the 12 months before the race. We have previously reported that the highest immediate post-race serum CK activities are found in the least trained runners (Noakes and Carter 1982).

Forty-five runners (60%) had levels of CK-MB which constituted more than 4% of total serum CK activity, a value which is generally considered to be strongly suggestive of myocardial injury (Lott and Stand 1980). Electrophoresis of 21 sera in which % CK-MB activity measured with the test kit was greater than 4% showed that all had CK-MM bands, 19 (91%) had CK-BB isoenzyme bands, but only 10 (48%) had visible CK-MB bands (Samples 1–21, Table 2). Electrophoresis of a further 10 sera with high total CK activity also showed that all had CK-MM bands, two had CK-BB bands and four had CK-MB bands (Samples 22–31, Table 2).

Thus, of the 31 sera analyzed, all had CK-MM bands, 21 (68%) had CK-BB and 14 (44%) had CK-MB bands. CK-BB bands were present in 17 of 18 sera with % CK-MB activity greater than 5% and in six of 12 sera with total CK greater than 837 U · l^{-1} · CK-MB bands were present in eight of 18 (44%) sera with % CK-MB greater than 5%, and in six of 13 (46%) with total CK activity greater than 837 U · l^{-1}. Mean total CK activity of sera with and without the CK-MB band was not significantly different, (1,183 ± 1,013 U · l^{-1} vs 764 ± 992; mean ± SD, $P = 0.3$) but sera which recorded "false positive" CK-MB activity (i.e., % CK-MB greater than 4% on the test kit but without visible electrophoretic CK-MB bands –

Table 2. Electrophoretic CK-isoenzyme patterns of 31 sera

Sample no.	Total CK $U \cdot l^{-1}$	CK-MB $U \cdot l^{-1}$	$\frac{\text{CK-MB}}{\text{CK-MM}}$ %	MM	MB	BB	Running time Hours : minute : second
1	193	21	11	+		+	10 : 13 : 21
2	156	15	9.6	+		+	06 : 44 : 35[a]
3	148	14	9	+		+	08 : 38 : 36
4	148	12	8	+		+	05 : 57 : 07
5	144	9	8	+		+	06 : 21 : 53
6	119	9	7.5	+		+	06 : 34 : 53
7	297	22	7.4	+	+	+	06 : 54 : 50
8	309	22	7	+	+	+	08 : 47 : 41
9	293	21	7	+		+	07 : 28 : 51
10	268	19	7	+		+	09 : 20 : 41
11	173	12	7	+			07 : 01 : 06
12	375	24	6.4	+	+	+	08 : 03 : 54
13	383	24	6.3	+	+	+	06 : 35 : 47
14	437	27	6.1	+	+	+	06 : 33 : 56
15	420	24	5.7	+		+	06 : 40 : 26
16	2,154	111	5	+	+	+	06 : 18 : 08
17	847	43	5	+	+	+	07 : 20 : 31
18	470	24	5	+	+	+	06 : 15 : 07
19	1,023	43	4	+	+	+	06 : 37 : 24
20	837	34	4	+	+	+	09 : 29 : 29
21	767	29	4	+			09 : 58 : 11
22	4,224	38	1	+			10 : 08 : 55
23	3,680	101	3	+	+		07 : 19 : 20
24	2,820	58	2	+	+	+	07 : 28 : 23
25	1,799	41	2	+			10 : 12 : 04
26	1,758	48	3	+	+		05 : 37 : 28[b]
27	1,415	26	2	+			07 : 32 : 32
28	1,176	34	3	+	+	+	07 : 40 : 02
29	982	26	3	+			08 : 49 : 20
30	891	27	3	+			07 : 21 : 23
31	854	14	1.6	+			08 : 33 : 14

+ Indicates the presence of an isoenzyme band on electrophoresis
[a] First Lady finisher
[b] First Male finisher

Table 3. Incidence of positive CK-MB bands for different values of total CK

Total CK value $U \cdot l^{-1}$							
0–250	250–500	500–750	750–1,000	1,000–2,000	2,000–3,000	3,000–4,000	> 4,000
Volume serum added to electrophoretic membrane (µl)							
1.0	0.75	0.5	0.25	0.25	0.25	0.25	0.25
No of samples							
7	9	0	6	5	2	1	1
No. (%) with electrophoretic CK-MB bands							
0 (0%)	6 (67%)	0	2 (33%)	3 (60%)	2 (100%)	1 (100%)	0

samples 1–6, 9–11, 15) were more likely to have % CK-MB greater than 7%.

To determine whether the method by which sera were plated on the electrophoretic membrane (i.e., less sera added as total CK activity increased) influenced the results, the percentage of sera which had visible CK-MB bands was calculated for eight different total CK activities (0–250, 250–500, 750–1,000, 1,000–2,000, 2,000–3,000, 3,000–4,000, > 4,000 $U \cdot l^{-1}$ (Table 3).

The results showed an apparently random distribution of visible CK-MB electrophoretic bands for the different total CK activities, suggesting that the plating technique did not influence the detection of visible CK-MB bands.

Finally it is of interest that the mean (± SD) running time of athletes who had CK-MB bands present in their sera after the race was significantly less than that of runners without this band (433 ± 60 vs 493 ± 88 min; $P = 0{,}04$).

Discussion

This study shows that, when tested with a commonly-used kit, the serum %CK-MB isoenzyme activity apparently exceeds 4% in 60% of ultra-marathon runners. This level of CK-MB activity might be considered to indicate possible myocardial injury (Lott and Stand 1980).

However, electrophoresis of 31 sera with either %CK-MB activity greater than 4% or total CK activity greater than 854 $U \cdot l^{-1}$ showed that all had visible CK-MM bands, 21 (68%) had visible CK-BB bands, but only 14 (44%) had visible CK-MB bands. Thus the CK-MB test kit used in the study identified a greater number of sera with apparently elevated CK-MB isoenzyme activity than did electrophoresis. This discrepancy could result either from cross-reaction of elevated CK-BB activity with the test kit, or from relative insensitivity of the electrophoresis. These possibilities deserve further study (Kaste and Sherman 1982) and should be considered when studies of this nature using either electrophoretic (Siegel et al. 1980, 1981) or radioimmunoassay techniques (Diamond et al. 1983; Stansbie et al. 1982; Phillips et al. 1982) are compared. In particular, the possibility of cross-reactivity make the interpretation of the findings of the latter three studies, difficult. In this regard it should be noted that in our study, (i) a greater percentage of runners had visible CK-BB than CK-MB bands, and (ii) the CK-BB electrophoretic band was more likely to be present in those sera which also had high % CK-MB activity. This suggest that at least some of the elevated CK-MB activity measured on the test kit in our study, and possibly in those of the other workers (Diamond et al. 1983; Stansbie et al. 1982; Phillips et al. 1982) may be due to cross-reactivity with the CK-BB isoenzyme.

Nevertheless this study provides further firm evidence that serum CK-MB isoenzyme activity is commonly elevated after very prolonged exercise. Olivier et al. (1978) found this electrophoretic pattern in 11 (50%) of 22 Comrades Marathon runners, a value similar to own (44%), whilst Kieblock et al. (1979) found the band in 13 (65%) of 20 160-km runners. Similarly, Siegel et al. (1981) have recently reported a mean serum CK-MB isoenzyme activity of 1301 $U \cdot l^{-1}$ (26 times the upper limit of normal) in 35 runners, 24 h after a standard 42-km marathon race. Paradoxically, elevated serum CK-MB activities have not been found in patients recovering from acute myocardial infarction who underwent a maximum, symptom-limited treadmill test (Grande et al. 1980).

The origin and meaning of the elevated serum CK-MB activity remains uncertain, however, and no one has yet provided substantive evidence that the isoenzyme either originates from the myocardium or that it indicates myocardial damage (Siegel et al. 1981). Siegel et al. (1981) have concluded that the elevated CK-MB isoenzyme activity probably represented a skeletal muscular origin. That elevated post-race CK-MB isoenzyme activity does not seem to adversely affect future running performance is suggested by our finding that in this study, the race winner had a visible CK-MB band (sample no. 26 – Table 2) after the race but has subsequently won both the 1981 and 1982 London to Brighton 84-km race and the 1982 and 1983 Comrades Marathons. Indeed, it was of interest that the mean running time of athletes whose sera had visible CK-MB bands was significantly less than was that of runners whose sera did not have this visible isoenzyme band.

Three previous workers (Diamond et al. 1983; Kielblock et al. 1979; Phillips et al. 1982) have noted elevated serum CK-BB activity after prolonged exercise, but only Phillips et al. (1982) have discussed the significance of this finding. They reported CK-BB levels equivalent to those found in patients with severe concussion, in half of a group of runners after a 42-km marathon, and expressed concern that the elevated serum CK-BB activity might have originated from cerebral tissue. However, as pointed out by Kaste and Sherman (1982), cross-reaction of the CK-BB radioimmunoassay with the elevated CK-MB levels also measured in those runners, could not be excluded in that study. Furthermore, the CK-BB fraction exists not only in brain, lung, thyroid, small intestine, and bladder muscle (Wong and Swallen 1975), but also in skeletal muscle (Armstrong et al. 1977), and it is at least equally plausible that the CK-BB fraction in these runners arises either from skeletal muscle or from other extra-cerebral sources (Kaste and Sherman 1982).

The clinical relevance of this study is to indicate that the interpretation of elevated serum CK-MB or serum CK-BB isoenzyme activities in marathon and ultra-marathon runners must be approached with appropriate caution. Additional studies are required to explain the discrepant CK-MB results measured with either the test kit or with electrophoresis as well

as the source and long-term significance of the changes in serum CK-MB and CK-BB activities after ultra-marathon races.

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