The dilemma of the creatine kinase cardiospecific iso-enzyme (CK-MB) in marathon runners

T. H. DIAMOND, R. SMITH, A. P. GOLDMAN, D. P. MYBURGH, J. M. BLOCH, F. VISSER

Summary

Serum creatine kinase iso-enzyme (CK-MB) levels were measured in 51 marathon runners before and after a 50 km marathon event. Ninety-five per cent of the runners were found to have pathologically elevated values, i.e. CK-MB concentrations were elevated to the range normally considered indicative of myocardial necrosis. Results indicate that a rise in the CK-MB level is common after marathon running. We therefore believe that cardiac enzymes are an unreliable indicator of myocardial infarction in patients who experience chest pain following strenuous muscular exercise.

S Afr Med J 1983; **63**: 37-41.

The presence of the cardiospecific creatine kinase (CK) iso-enzyme, viz. CK-MB, has been widely accepted as the most sensitive and specific indicator of myocardial necrosis.[1-5] The importance of this enzyme fraction has continuously been stressed as being the diagnostic cornerstone of acute myocardial infarction (AMI) in patients complaining of chest pain, especially in those not exhibiting early electrocardiographic features of ischaemia.[1] Recently, however, several reports have questioned the cardiospecificity of this iso-enzyme because of the discovery that certain conditions result in falsely positive elevated values (Table I).

Studies performed since the 1960s have shown elevation of CK and lactate dehydrogenase (LD) plasma enzymes with strenuous physical exertion,[17] in particular with marathon running.[18-20] In addition, several reports have documented the presence of and increments in the CK-MB fraction, both qualitatively and quantitatively,[21,22] after long-distance marathons. The occurrence of CK-MB fractions after running has however been disputed in other studies.[23,24]

In view of these conflicting reports and in order to clarify the origin and the role of the CK-MB fraction in marathon runners, a detailed enzymological analysis was performed on 51 athletes engaged in a 50 km marathon event in Johannesburg.

Institute for Aviation Medicine, Verwoerdburg, Tvl
T. H. DIAMOND, M.B. B.CH.
R. SMITH, M.B. B.CH.
A. P. GOLDMAN, M.B. B.CH., F.C.P. (S.A.)
D. P. MYBURGH, *SM.*, M.B. CH.B., F.A.C.C.
J. M. BLOCH, B.SC. HONS, N.D.P. (CHEM.PATH.)
F. VISSER, DIP.MED.TECH. (CHEM.PATH.)

Date received: 22 April 1982.

TABLE I. DISEASES CONTRIBUTING TO AN ELEVATION OF THE CK-MB VALUE

Heart disease	
Myocardial infarction/ischaemia[1-4]	Cardiac cell necrosis
Pericarditis (associated epicarditis)[6]	Increased permeability of the cells
Myocarditis[6,7]	
Muscle disease	
Muscular dystrophies[1,8-10]	Associated cardiac disease or abnormal skeletal muscle fibres
Viral myositis/ischaemic rhabdomyolysis[11,12]	
Alcoholic cardiomyopathy[11]	
Traumatic 'crush' syndrome[13]	Abnormal, atypical CK-MB fractions
Brain disease (confused because of an elevation in CK-BB fraction)	
Malignant hyperpyrexia[14]	
Other	
Hypothyroidism,[15] hypoparathyroidism[15]	Cardiac involvement
Carcinoma, e.g. prostatic[16]	Elevation of CK-BB fraction

Subjects and methods

The ages of the runners ranged from 19 to 72 years (mean 35,6 years). Blood samples from a randomly selected group of 42 participants were collected both before and after the race. In addition, the winning 6 and the last 3 competitors in the race were selected to have blood samples drawn on completion of the race only.

In order to prevent denaturation of the enzymes all the blood specimens were kept at 4°C and separated within 1 hour of collection. The separated sera were then immediately subjected to the following biochemical analysis:

Quantitative measurement of CK, aspartate aminotransferase (AST) and LD. These enzyme activities were assayed at 30°C on the Gemini centrifugal analyser using Boehringer test kits.[25] This method comprised both kinetic and spectrophotometric assays. The results obtained were documented, but specimens with elevated values, i.e. values above the normal range for the specific enzymes (CK > 110 IU/l; AST > 23 IU/l; LD > 320 IU/l) were rechecked. Serum samples were diluted out, using a pooled serum containing low specific enzyme activities, for enzyme assay.

Quantitative CK-MB analysis. All specimens with total CK values above normal (> 110 IU/l) were analysed for CK-MB iso-enzyme fractions, using the standard immuno-inhibition method as described for the Boehringer test kit.[26] In order to prevent any false-positive results due to laboratory error, certain precautions were taken:

1. A specially prepared pooled serum with a specific low total CK activity was made up. This was used to dilute any specimens

with high CK values, which might have affected the total immuno-inhibition in the abovementioned test.[26,27]

2. The test kit for CK-MB (Boehringer) contained a specially prepared adenosine monophosphate/adenosine phosphate 50 buffer mixture which was used to minimize the effects of the high serum adenylate kinase (AK) activity.[26,27] The latter results from muscle breakdown after strenuous exercise. AK from liver and muscle increased both total CK and subsequently CK-MB fractions in the serum.

3. A comparative CK-MB assay was also performed at a different laboratory using a similar method, viz. the CK-MB immuno-inhibition technique described for the Abbott test kits.[28] Results with this method were reported as standard CK-MB values in the appropriate units, plus results from diluted serum samples (1:10), again to minimize the effects of AK activity.

Qualitative analysis of the CK-MB fractions by electrophoresis. All specimens with elevated total CK values (> 110 IU/l) and those with positive quantitatively measurable CK-MB fractions were subjected to electrophoresis on cellulose acetate plates at room temperature. Four samples were applied to each plate and were allowed to run for 20 minutes at room temperature. The running buffer employed was a 50 mM barbitol buffer (pH 8,8). Following this, the plates were overlaid with a CK substrate (CK-NAC; Boehringer) and incubated for 15 minutes at 37°C. The plates were then removed and allowed to dry for 10 minutes. Thereafter they were analysed under ultraviolet light to distinguish specific iso-enzyme bands. The bands were then identified, the most anodic being the rapid CK-BB fraction, followed by the CK-MB and the last, most cathodic fraction being the CK-MM. These plates were then photographed for documentation and compared with the quantitated CK-MB fractions.

Qualitative analysis of LD iso-enzyme fractions by electrophoresis. All specimens assayed for LD enzyme activity were also subjected to electrophoresis on cellulose acetate plates, to determine the specific LD iso-enzyme bands. The procedure performed was a modified Helena procedure.[29] In this analysis, 8 samples were applied to each plate and electrophoresis was carried out for 30 minutes at room temperature using a 50 mM barbitol buffer (pH 8,3) as a running buffer. The plates were then incubated in a specially prepared colour reagent at 37°C for 15 minutes. After the incubation period the 5 iso-enzyme bands were scanned by the Helena Quick-Scan and identified for abnormal LD 1 and LD 2 bands, i.e. the iso-enzyme LD 1:2 'flip'.

A questionnaire had to be completed by all runners studied and oral temperatures were measured immediately after the race.

Results

Clinical findings

Of the 42 runners selected prior to the marathon, 39 completed the event. These were then subjected to a second series of enzyme analyses. The questionnaire eliminated any medical reasons which could have interfered with the CK-MB values. There were no complaints of chest pain experienced during the race. All the runners denied having any illness which could have resulted in falsely elevated CK-MB values (Table I). All oral temperatures after the race were below 38°C.[14] No runners had consumed any phenothiazines, antihistamines or diuretics for at least 1 week before the event.[14] Serum urea and creatinine estimations, performed before and after the race, eliminated the possibility of renal failure.[30]

Enzymology

The pre- and post-marathon values for the total serum CK, the percentage CK-MB and the CK/AST ratio are depicted in Table II.

Total serum CK values. The mean pre-race CK value was 109,58 IU/l (range 38,23-180,93 IU/l), compared with a mean value after the race of 437,32 IU/l (range 140,51-734,13 IU/l), a fourfold increase. No precise trend could be related to the fitness of the runners and the degree of rhabdomyolysis, but runners who had trained well and had not overexerted themselves exhibited only minor changes. The most striking increase, in an undertrained athlete, was a post-race CK value of 1 306 IU/l, i.e. 17,6 times the pre-race value. This is in contrast to the least change, exhibited by a fit runner with a post-race CK value of 117 IU/l, i.e. only 17 IU/l higher than his pre-race value. The undertrained athlete completed the marathon event in 5,5 hours (mean 4,8 hours), compared with the fit runner, who completed the event in 3,01 hours.

CK-MB values. Four of the pre-race (25%) and 46 of the post-race (96%) absolute CK-MB values were pathologically elevated (> 10 IU/l).[26] The mean post-race CK-MB value was 18,24 IU/l (range 10,6-25,88 IU/l), i.e. 1,8 times the upper limit of normal according to the CK-MB kit. In order to establish that the measured CK-MB fractions were truly CK-MB iso-enzymes, and not CK-BB, the original CK electrophoresis bands were examined. Serum electrophoresis revealed strongly positive CK-MM bands (pronounced after the event) with distinct CK-MB bands wedged in between, and faint CK-BB bands. One individual exhibited strongly positive CK-BB bands, both in the pre-race and in the post-race specimens. This accounted for an elevation in both the pre-race and post-race CK-MB values, viz. 37,5 and 53 IU/l respectively. Only 12 specimens (30%) showed elevated post-race CK-MB percentage values (i.e. > 6%);[26] the mean CK-MB% value was 5,1%. There was no statistically significant difference in the CK-MB values obtained by using the Boehringer, the Abbott and the Abbott 1:10 dilution method ($P > 0,05$).

LD values. All the LD values were elevated after the event. However, these increases were not regarded as significant, owing to the influence of both haemolysis and rhabdomyolysis. Their importance lay in the changes in the iso-enzymes. All post-race electrophoretic plates revealed an elevation in all the LD bands (mild form of the 'crush' syndrome,[13,20]) while 22 specimens revealed larger increments in the LD 2 and LD 4 bands. In addition, 1 runner had an abnormally elevated LD 3 band after the race. It was striking, however, that there were no 'flipped' LD patterns visible in the post-race specimens. The abnormally strong LD bands were further proved to be due to extensive rhabdomyolysis after measurement of the serum aldolase levels, which were raised.

CK/AST ratio. This ratio was determined in all the runners who completed the event. The mean value was 18,07 (range 9,16-26,98), within the documented normal range for subjects with rhabdomyolysis.[26] Only 4 values were within the ominous range (1,9-9,1) quoted by Szasz *et al.*[31] as suggesting myocardial necrosis. However, these subjects had normal CK-MB and LD values.

Discussion

An increase in serum CK activity has long been accepted as indicating diseases involving both cardiac and skeletal muscle.[1-4] Recently, however, more detailed enzymological methods have been developed to construct a 'cardiac profile' which would increase both the specificity and sensitivity of these tests in the diagnosis of AMI (Table III).

The CK-MB iso-enzyme has for a long time been considered diagnostic of cardiac cell necrosis.[1-5] However, it was later also found to occur in conditions resulting in an increase in cardiac cell permeability.[6,7,17,19,34] Furthermore, several reports have

TABLE II. PRE- AND POST-RACE ENZYME VALUES*

Runner No.	CK		CK-MB (%)		CK/AST ratio
	Before	After	Before	After	
1	62	482		35,0 (7,3)	482/21 = 23,0
2	73	265		18,0 (6,8)	265/17 = 15,6
3	75				
4	67	573		19,0 (3,3)	573/29 = 19,8
5	74	1 306		48,0 (3,7)	1 306/37 = 35,3
6	125	354	5,0 (4,0)	16,0 (4,5)	354/21 = 16,9
7	126	253	6,0 (4,8)	21,0 (8,7)	253/19 = 13,3
8	103	315		27,0 (8,6)	315/19 = 16,6
9	147		7,0 (4,8)		
10	210	1 105	9,0 (4,3)	26,5 (4,3)	1 105/34 = 32,5
11	257		15,0 (5,8)		
12	101	492		12,0 (2,4)	492/28 = 17,6
13	125	444	37,5 (30,0)	53,0 (11,9)	444/18 = 24,7
14	68	983		27,0 (2,7)	983/27 = 36,4
15	92	1 198		23,0 (1,9)	1 198/28 = 42,8
16	105	360		24,0 (6,7)	360/28 = 12,8
17	463	693	13,0 (2,8)	15,0 (2,2)	693/33 = 21,0
18	67	132		11,5 (8,7)	132/17 = 7,8
19	66	453		22,0 (4,9)	453/21 = 21,6
20	111	870,5		16,0 (1,8)	870/25 = 34,8
21	67	156		19,0 (12)	156/28 = 5,6
22	58	165		12,0 (4,5)	165/15 = 17,7
23	61	357		16,0 (4,5)	357/15 = 23,8
24	100	276		16,0 (5,8)	276/24 = 11,5
25	51	173		15,0 (5,5)	273/20 = 13,7
26	90	240		15,0 (6,3)	240/23 = 10,4
27	115	265	5,0 (4,3)	13,0 (4,9)	265/21 = 12,6
28	100	117		8,0 (6,8)	117/13 = 9,0
29	118	336	8,0 (6,8)	17,0 (5,1)	336/26 = 12,9
30	59	306		13,0 (4,2)	306/20 = 15,3
31	142	606	9,0 (6,3)	14,0 (2,3)	606/19 = 31,9
32	164	366	12,0 (7,3)	24,0 (6,6)	366/26 = 14,1
33	112	282	5,0 (4,5)	14,0 (5,0)	181/19 = 14,8
34	84	375		15,0 (4,0)	375/25 = 15,0
35	47	204		14,0 (6,9)	204/19 = 10,7
36	174	297	9,0 (5,2)	14,0 (4,7)	297/24 = 12,4
37	216	339	9,0 (4,2)	16,0 (4,7)	339/24 = 14,1
38	126	228	8,0 (6,3)	10,0 (4,4)	228/20 = 11,4
39	86	360		13,0 (3,6)	360/22 = 16,4
40	121	681	8,0 (6,6)	27,0 (4,0)	681/30 = 22,7
41	84	171		12,0 (4,0)	171/26 = 6,6
42	76	273		14,0 (5,1)	273/17 = 16,1
43		414		24,0 (5,8)	414/22 = 18,8
44		285		16,0 (5,6)	285/25 = 14,3
45		694		40,0 (5,8)	694/35 = 19,8
46		624		34,0 (5,4)	624/41 = 15,2
47		282		19,0 (6,7)	282/26 = 10,8
48		564		23,0 (4,0)	564/24 = 23,5
49		560		27,5 (4,9)	560/36 = 15,6
50		379		14,0 (3,7)	379/25 = 15,2
51		299		15,0 (5,0)	299/22 = 13,6
Mean	109,58	437,32	8,53 (5,2)	18,24 (5,12)	18,07
SD	71,35	296,81	2,95 (1,26)	7,64 (2,23)	8,91
Range	38,23-180,93	140,51-734,13	5,58-11,42	10,6-25,88	9,16-26,98

*Values in parentheses represent the CK-MB percentage fraction, figures preceding the parentheses being the absolute CK-MB measurements in mmol/l.

TABLE III. CARDIAC PROFILE SUGGESTIVE OF AMI[1,2,6,31-33]

1. **Total serum CK value > 110 IU/l.**
2. **Serum CK-MB value > 10 IU/l.**
3. **Serum CK-MB value (electrophoresis) for documentation and densitometric scanning.**
4. **Serum CK-MB percentage > 6% (30° C).**
5. **CK/AST ratio with a mean value of 5,2 (range 1,9 - 9,1).**
6. **Serum LD electrophoresis documenting LD1:LD2, i.e. 'flipped' LD pattern.**
7. **Serum myoglobin value (not routinely available).**

shown that CK-MB is present in 3,1% (0-8,2%) of skeletal muscle fibres[13,22,35] (type II fibres) and in up to 30% of regenerating striated muscle.[22,36]

The present study has demonstrated the release of large quantities of CK enzymes following rhabdomyolysis after a marathon event. Further analysis showed that, besides an increase in the CK-MM iso-enzyme, there were also pathological increases in the CK-MB iso-enzyme values into the range normally considered to be diagnostic of myocardial necrosis. The specificity of the latter was verified by electrophoresis, and by the elimination of conditions which might have caused falsely high CK-MB values (once again, the effect of the AK enzyme must be stressed).

The presence of the CK-BB iso-enzyme, known to cross-react on the CK-MB immuno-inhibition test kit, was not considered to be a major factor in the elevation of the absolute CK-MB values obtained in the runners both before and after exercise. This assumption was made because of the strongly positive CK-MB bands and the very faint CK-BB bands found on specific electrophoresis. An exception was runner No. 13, in whom the high CK-MB value was due to the high CK-BB value which was documented on electrophoresis.

It must be emphasized that only 1 (2,6%) of the absolute CK-MB values found after the race fell within the normal range. In contrast to these pathological CK-MB values, normal CK-MB percentage values (< 6%)[25,26] were obtained in 27 (71%) runners. (Runner No. 13, who had elevated CK-BB values for unknown reasons and runners with no control values were not included.) The severity of the rhabdomyolysis also resulted in large increments in the serum LD and aldolase levels. The absence of a 'flipped' LD pattern on electrophoresis lent further weight to the interpretation of the CK-MB percentage value as normal. This is contrary to the reports of Kaman *et al.*,[33] who demonstrated 'flipped' LD patterns on electrophoresis in athletes 8 hours after exercise. However, they thought that these changes were of no diagnostic significance, since they occurred 40 hours sooner than the expected maximal LD activity seen in AMI.

The data in Table II thus clearly indicate a so-called 'normal exercise profile' with normal CK-MB percentage values, a normal CK/AST ratio and the absence of 'flipped' LD patterns. However, the absolute CK-MB values are pathologically high. This is contrary to the findings in many previous studies performed on athletes and deserves emphasis.[1-4,23,24,33]

This pathological elevation of CK-MB values after stress remains a problem. Adornato and Engel[36] stressed the importance of an increase in CK-MB in regenerating skeletal muscle. They showed that experimentally regenerated muscle has an increased CK-MB fraction, similar to that in normal embryonic skeletal muscle. This was also the explanation they put forward for the high CK-MB values found in patients with muscular dystrophies. Further evidence for the role of skeletal muscle regeneration may be deduced from the article by Siegel *et al.*,[37] who found that endurance runners with high CK-MB values had normal myocardial scintigrams. Although this has not been proved, histologically or by biochemical and histochemical assays, marathon running may represent a continuous turnover of skeletal muscle catabolism and anabolism, and this cycle may result in synthesis of newly regenerating muscle fibres with increased levels of skeletal CK-MB iso-enzyme.

In addition, we cannot totally exclude the possibility that cardiac CK-MB iso-enzyme contributed to the elevated CK-MB iso-enzyme levels determined after the marathon event. Schmidt and Schmidt[34] stressed the importance of the biochemical and physiological effects of marathon running on the cardiovascular system (e.g. the persistent sustained tachycardia[38]). These factors may all be responsible for increasing cardiac cell permeability, and hence for the elevated CK-MB iso-enzyme release.

The above reasons may be sufficient to account for the elevated CK-MB values, but histological studies and myoglobin assays will be required to verify the hypothesis. The importance of the present study was to assess the significance of these rises in enzyme levels in a so-called 'healthy' population with minimal risk factors for ischaemic heart disease[39-42] and who present an otherwise normal exercise 'cardiac profile'. Surely this elevation in the CK-MB values must be interpreted as of no pathological significance!

In conclusion, it is important to stress that a so-called 'cardiac profile' may be only of limited value in the diagnosis of AMI. The CK-MB percentage value and not the absolute CK-MB value should be of diagnostic importance after stress. Furthermore, patients who present with exercise-induced symptoms suggestive of AMI should be carefully screened, since enzyme assay is of limited value only. Objective diagnosis of AMI in these cases should be based on serial electrocardiographic and enzymatic changes, if necessary in conjunction with thallium or technetium pyrophosphate myocardial scintigraphy.

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