Serial Serum Creatine Phosphokinase MB 
Isoenzyme Activity after Myocardial Infarction

Studies in the Baboon and Man

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SUMMARY Serum CPK-MB isoenzyme activity was measured serially after mercury embolization of the left circumflex coronary artery in five baboons and after clinical acute myocardial infarction (AMI) in 20 patients. The calculated amount of enzyme released into the baboons’ circulation (CPK-MBₐ) correlated well (r = 0.991) with the amount of MB isoenzyme depleted from the myocardium (CPK-MB₂) when a previously determined decay constant (Kd) was used, but not when Kd was calculated from individual curves or when CPK-MM values were used. In clinical AMI, CPK-MMₐ averaged 97% (0 to 350%) greater than CPK-MM₂, probably because of release of MM isoenzyme from nonmyocardial sources. The mean Kd for CPK-MB in patients (0.0012 min⁻¹) was significantly (P < 0.01) lower than that obtained in the baboon following bolus injections (0.0018 min⁻¹), probably reflecting delayed myocardial release of enzyme. Therefore, in both experimental and clinical AMI, serial samples for CPK-MB activity, but not total or CPK-MM activity, could provide an accurate index of myocardial enzyme depletion.

THE CPK ACTIVITY in serial serum samples after myocardial infarction has been used by a number of investigators to estimate myocardial CPK depletion and thereby to quantitate acute myocardial damage.¹⁻⁵ However, considerable controversy exists regarding the accuracy of the equations utilized to calculate myocardial CPK depletion from serum enzyme activity.⁶⁻¹⁰ One possible cause of inaccuracy is that most previous studies have utilized analysis of total CPK, which includes enzyme released from nonmyocardial tissue. Whereas CPK-MB isoenzyme, which is more specific for myocardium, represents approximately 20% of the total CPK in human myocardium, its low concentration in dog myocardium (2 to 3%)¹¹,¹² makes this animal unsatisfactory for evaluating the use of the MB isoenzyme to quantitate enzyme loss from the heart.

In the present study, the baboon was chosen as the experimental animal because it is genetically close to man, its coronary circulation is similar to that of man, and approximately 20% of its myocardial CPK activity is contributed by the MB isoenzyme.¹¹,¹² In previous work from this laboratory,¹³ bolus injections of CPK-MB were given to seven baboons in order to calculate the distribution volume (DV), which averaged 5.22 ± 1.06 (SD)% and the decay constant (Kd), which averaged 0.00175 ± 0.00019 min⁻¹. These constants were used in the present study to estimate the total activity of CPK-MB released in the circulation after coronary embolization in comparison to the directly measured myocardial depletion of CPK-MB.

Enzyme release also was estimated from the CPK-MB and CPK-MM activities of blood drawn serially from 20 patients with acute myocardial infarction. Relative release of the two CPK isoenzymes could therefore be compared and Kd values of CPK-MB in man after acute infarction could be contrasted to that following bolus injection in the baboon.

Materials and Methods

Studies in the Baboon

Production of Myocardial Infarct

Five baboons ranging in weight from 10 to 20 kg were anesthetized with phencyclidine HCl (1 mg/kg) and sodium barbital (3 mg/kg). A catheter was placed in the femoral artery and positioned under fluoroscopy in the left circumflex coronary artery. Lidocaine (100 mg) was administered intravenously, after which the left circumflex coronary artery was embolized with 0.1 ml of elemental mercury.¹⁴

About 3 to 5 ml aliquots of venous blood were taken immediately before and after infarction, and thereafter at two hour intervals for the first 24 hours, four hour intervals for the following 12 hours, and 12 hour intervals for the following 5 to 10 days. After each sampling, the volume of blood that was removed was replaced by an equal volume of heparinized physiological saline. After recovery from anesthesia, the animals were kept mildly sedated with ketamine HCl to make them manageable for blood sampling. Serum samples were separated from the blood and frozen at −20° C until assayed.

Preparation of Tissue Extracts

After the last sampling of blood, the animals were anesthetized with sodium barbital (3 mg/kg). The chest was opened and the heart immediately excised and the pericardium removed. The atria and fat were dissected from the right and left ventricles, which were then washed rapidly in ice cold water, dried with gauze and weighed. For the estimation of CPK-MB depleted from myocardium, two samples of

*Myocardial CPK depletion was utilized by Shell et al.¹ as synonymous with infarct size. However, this measurement would correlate with the histologically defined area of infarction only if the following prerequisites were met: (1) enzyme loss occurs to a similar and measurable extent in all infarcted muscle; (2) enzyme is retained at normal concentrations in all noninfarcted muscle; (3) changes in mass of normal and infarcted tissue do not alter the relationship among enzyme depletion, concentration and mass. Because these prerequisites have not yet been proved, we have avoided use of the term infarct size and have instead calculated enzyme depletion from the myocardium and enzyme release into peripheral blood.

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left ventricular tissue (about 1–2 g each) were cut out and weighed: one sample from the center of the infarct and another distant from the infarct representing normal myocardium. The remaining tissue and the normal and infarcted pieces were then separately homogenized in 20 volumes of homogenizing medium and the cellular debris was removed by centrifugation as previously described.\textsuperscript{11, 12}

**Separation and Assay of CPK Isoenzymes**

CPK isoenzymes in serum or tissue extract were separated by discontinuous elution from micro-columns of DEAE-Sephadex A-50 and the eluates were assayed at 37° C by the method of Rosalki, as previously described.\textsuperscript{13}

**Calculation of Myocardial CPK-MB Depletion (CPK-MB\textsubscript{D})**

The amount of enzyme depleted from the heart was measured by subtracting the total CPK-MB activity of the infarcted heart from the product of heart weight and CPK-MB of 1 g of normal myocardium.

\[ \text{CPK-MB}_D = W \times DV \int_0^t \left[ \frac{dE}{dt} - Kd \, E \right] \, dt \]

where \( W \) is the weight of the baboon in kg, \( DV \) and \( Kd \) the fractional distribution volume (0.052) and decay constant (0.0018 min\(^{-1}\)) obtained following bolus injection of the isoenzyme in the baboon,\textsuperscript{14} \( t \) the time in minutes at which the blood samples were removed and \( E \) the serum CPK-MB activity in units/liter at any time after infarction. In practice, the integral function \( \int_0^t \left[ \frac{dE}{dt} - Kd \, E \right] \, dt \) represents the total activity of CPK-MB released in one liter of serum and was calculated as the summation \( \Sigma \left[ \frac{\Delta E}{\Delta t} + 0.0018 \, E \right] \)

\( \Delta t \), where \( \Delta t \) is the interval in minutes between any two consecutive serum CPK-MB activities and \( \Delta E \) and \( E \) the corresponding difference and average, respectively, of the two activities.

**Calculation of CPK-MM Released into Serum (CPK-MM\textsubscript{R})**

Since no data are available on the Kd for CPK-MM isoenzyme in the baboon or man, the Kd for total CPK in patients with uncomplicated myocardial infarctions may represent the closest available approximation. CPK-MM\textsubscript{R} was therefore calculated from the above formula using values for CPK-MM activity and a Kd of 0.001 min\(^{-1}\), which is close to the mean values reported in humans for total CPK by Roberts et al.\textsuperscript{15} and Norris et al.\textsuperscript{8}

**Studies in Man**

Serial venous blood samples were obtained from 20 patients admitted with chest pain and electrocardiographic changes consistent with acute myocardial infarction. Blood samples were drawn as soon as possible after admission, at 8 hour intervals for the first 48 hours, and then at 72 and 96 hours after the initial sample. No attempt was made to select uncomplicated infarcts. Although many had a benign course, some patients developed moderate to severe left ventricular failure and in others the course was complicated by arrhythmias and recurrent chest pain.

Enzyme release was measured from both CPK-MB and CPK-MM serum activity. Kd and DV values used in the calculation were the same as described above for the baboon, since these figures are very close to those previously observed in dogs and in man.\textsuperscript{1, 6, 16}

**Results**

**Myocardial CPK-MB Depletion**

Total CPK-MB isoenzyme depletion from the ventricular myocardium of the five baboons ranged from 1656 to 5994 units (U) (table 1). Since normal myocardium had a CPK-MB concentration ranging from 87 to 126 U/g, this depletion was equivalent to the total loss of enzyme from 14.9 to 53.0 gm of myocardium, or partial loss of enzyme from a larger mass of myocardium.

Tissue obtained from the infarcted zone exhibited CPK-MB enzyme activity ranging from 4 to 74 U/mg. Enzyme depletion in the area of infarction therefore ranged from 34 to 97% of normal CPK-MB activity. The wide variability in enzyme depletion appeared to be related to nonhomogeneity of the infarct, which on visual inspection was particularly patchy in the hearts whose infarcted zone had less enzyme depletion.

**Serum Enzyme Profiles**

The pattern of CPK-MB activity varied in the five baboons. In baboons 1 and 2 the pattern was typical of a single episode of infarction as shown in figure 1 for baboon 1. CPK-MB activity rose to above the upper limit of normal (4

<table>
<thead>
<tr>
<th>Baboon</th>
<th>Heart weight (g)</th>
<th>Myocardial CPK-MB (U/g)</th>
<th>CPK-MB\textsubscript{D} (U)</th>
<th>CPK-MB\textsubscript{R} (U)</th>
<th>CPK-MM\textsubscript{R} (U)</th>
<th>CPK-MM\textsubscript{R}/CPK-MB\textsubscript{D}</th>
<th>CPK-MM\textsubscript{R}/CPK-MM\textsubscript{R}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.6</td>
<td>126</td>
<td>5665</td>
<td>736</td>
<td>0.13</td>
<td>16,385</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>110.0</td>
<td>113</td>
<td>5994</td>
<td>904</td>
<td>0.15</td>
<td>8,999</td>
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</tr>
<tr>
<td>3</td>
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<td>87</td>
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<td>223</td>
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</tr>
<tr>
<td>4</td>
<td>58.1</td>
<td>112</td>
<td>1872</td>
<td>251</td>
<td>0.13</td>
<td>10,562</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>57.5</td>
<td>123</td>
<td>1850</td>
<td>256</td>
<td>0.14</td>
<td>15,562</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Myocardial CPK-MB = isoenzyme activity in normal myocardium; CPK-MB\textsubscript{D} = isoenzyme depleted from myocardium; CPK-MB\textsubscript{R} = calculated CPK-MB isoenzyme released into circulation; CPK-MM\textsubscript{R}/CPK-MB\textsubscript{D} = fraction of depleted isoenzyme released into circulation; CPK-MM\textsubscript{R} = calculated CPK-MM isoenzyme released into circulation.
U/liter) two hours after infarction, reached peak value at 16 to 24 hours and subsequently decreased monoeXponentially until about 90% of the peak activity was cleared. The Kd for this rate was 0.0015 and 0.0016 min⁻¹ for baboons 1 and 2, respectively, which is close to that (0.0018 min⁻¹) previously obtained by bolus injection of CPK-MB.⁴ After this initial rapid phase, CPK-MB activity in serum decreased at a much slower rate reaching the normal range in 5 to 10 days. A somewhat similar pattern was obtained for baboon 3, except that the initial rapid phase of decay in this animal was much slower (Kd = 0.0007 min⁻¹), suggesting that the infarct may have evolved gradually with continued release of enzyme over a relatively long period of time.

In baboons 4 and 5, the CPK-MB patterns were different. As shown in figure 2, baboon 4 had a second episode of apparent enzyme release about 24 hours after the initial infarct. Baboon 5 showed essentially the same pattern except that the animal died during the second period of enzyme release, 32 hours after the initial infarct was induced.

The patterns of CPK-MM release into the circulation varied appreciably from those of CPK-MB. Serum CPK-MM activities showed a wide scatter and the presence of more than one peak in all five baboons. CPK-BB isoenzyme appeared in the serum of all five baboons after infarction. In four baboons the serum activity peaked at levels from 6–22 U/liter six hours after infarction and then declined. In the fifth baboon CPK-BB activity rose progressively until death 32 hours after infarction.

CPK Release in the Baboon

Table 1 shows the values for CPK-MB₉ and CPK-MB₁₀ obtained from the serum and myocardial CPK-MB activities. Data in baboon 5, who died prematurely, are based on enzyme released up until the time of death. In all five baboons, the released CPK-MB represented 13 to 15% of that depleted from the myocardium. These data are in close agreement with the report by Roberts et al.¹⁸ that the mean activities of CPK-MM and CPK-MB released in the circulation following experimental infarction of the dog (N = 5) are 14 and 13%, respectively, of the corresponding activities which are depleted from the myocardium. Simple regression analysis of the results in table 2 yielded the equation CPK-MB₉(U) = −14.84 + 0.144 CPK-MB₁₀(U) (r = 0.991).

When myocardial enzyme depletion was calculated from serum CPK-MB values in these five baboons using their individual Kd values, only in baboons 1 and 2 were values close to those obtained using the optimized Kd (0.00175 min⁻¹). In the other three baboons Kds were either much lower or were distorted by secondary peaks which made the correlation between CPK-MB₉ and CPK-MB₁₀ poor.

Enzyme release calculated from CPK-MM values was preposterously high, thus strongly indicating the release of MM isoenzyme into the circulation from nonmyocardial tissue (table 1).

CPK Release in Man

A typical pattern of serum CPK-MM and CPK-MB activity in a patient following acute myocardial infarction is illustrated in figure 3. In this case both the MM and MB activities peaked at about 16 hours and showed a monoeXponential rate of decay reaching essentially normal values in about 72 and 48 hours, respectively. The Kd value for CPK-MB was 0.00144 min⁻¹ and for CPK-MM, 0.00109 min⁻¹.

Table 2 lists the Kd values calculated from the serum CPK-MM and CPK-MB activities of 20 patients following
AMI. The Kd for CPK-MM and CPK-MB ranged from 0.00057 to 0.00109 min⁻¹ (mean = 0.00079; SD = 0.00014) and 0.00086 to 0.00149 min⁻¹ (mean = 0.00119; SD = 0.00020), respectively.

Table 2 also lists the enzyme release into blood calculated from both CPK-MM and CPK-MB serum activity. In seven of the 20 patients serum samples could not be obtained early enough after infarction to calculate total enzyme release; the CPK-MBₐ and CPK-MMₐ are considered partial in these patients and are designated as P in the table. The CPK-MM release averaged 97% larger than the CPK-MB release. In only one patient did the two values agree and in only five patients was the CPK-MMₐ less than 30% greater than the CPK-MBₐ. In eight patients MM release was more than twice the MB release.

To determine whether the greater CPK-MM release was due to enzyme appearing in blood from nonmyocardial sources, the MM/MB enzyme release ratio for each patient was plotted as a function of the percentage of total CPK represented by MB isoenzyme in serum at peak value. As shown in figure 4, the patients with the greatest serum % CPK-MB activity (8-13%) show the best correlation of MM vs MB enzyme release whereas those with the least (4-8%) show the poorest.

**Discussion**

Since CPK is widely distributed in tissues other than myocardium, especially skeletal muscle, a rise in serum enzyme activity is not specific for acute myocardial infarction, and the serum level of CPK activity after infarction may come from both myocardial and nonmyocardial sources. Therefore, use of total CPK activity in serum as an index of myocardial loss of enzyme may be grossly inaccurate.

The isolation of an isoenzyme of CPK relatively specific for myocardium (CPK-MB) has now made it possible to assay serum activity of enzyme released selectively from the myocardium. Although moderate amounts of MB isoenzyme are present in the intestine and small amounts in other tissues, little if any exists in skeletal muscle, which is the major source of total serum CPK activity and most likely to distort the myocardial enzyme release pattern after myocardial infarction. Recent modifications of the original assay
method have made quantitative measurement of CPK-MB practical.11, 16-19

Using the baboon, whose myocardial CPK-MB concentration is similar to that of man,14 we have been able to test whether, as suggested by Shell and associates,1 the depletion of this enzyme from the heart can be reliably assessed from peripheral serum enzyme activity. In the five animals subjected to myocardial infarction, calculated CPK-MB release correlated very well with CPK-MB myocardial depletion. The ratio of CPK released to CPK depleted was quite constant in these animals, but since this ratio averaged only .147 a minor alteration in the ratio could lead to a considerable effect on calculated enzyme depletion. Constancy of this ratio in a larger series of animals under varying experimental conditions would need to be demonstrated before CPK-MB release could be accepted as a reliable guide to myocardial enzyme depletion.

No attempt was made in these animals to quantitate the infarct histologically, since the entire heart was utilized for enzyme analysis. In baboons 3–5 enzyme depletion was equivalent to loss of enzyme activity from 15–19 g of myocardium, which represents an average of only 30% of the combined weight of the right and left ventricles. In baboons 1 and 2, however, the enzyme depletion was equivalent to loss from 45 and 53 g of myocardium, respectively. Either enzyme depletion overestimated the area of infarction, or these animals survived infarcts involving 54% and 48% of their total ventricular weight.

The method for producing infarcts in this study required only minor surgery, but anesthesia with low dose barbiturate (3 mg/kg) and phencyclidine was necessary to allow coronary catheterization, and ketamine was given repeatedly over the ensuing 5 to 10 days to sedate the baboons for blood sampling. Although barbiturates in much larger doses may depress CPK Kd,18 the regimen used in the present studies apparently did not have much effect, since Kd values in two of the experimental animals were similar to those observed after bolus injection of CPK-MB in baboons sedated only with ketamine,18 and these values are very similar to the maximum Kd observed after myocardial infarction in man.5, 18

Despite the sedation, considerable muscle restraint often was necessary in order to obtain blood samples from these baboons. The high levels of CPK-MM after infarction in some of these animals are likely the result of this skeletal muscle trauma. Although two baboons exhibited patterns of CPK-MB release reminiscent of that following a bolus injection with fairly rapid exponential disappearance, in other baboons the pattern suggested slow and sometimes repeated episodes of release. The low Kd in these latter animals is indicative of either delayed infarction of myocardium or delayed appearance of enzyme in blood because of variable perfusion of the infarcted tissue. Since our previous studies demonstrated a remarkably uniform Kd after bolus injection of CPK-MB in baboons,18 it is unlikely that variability in enzyme clearance from the blood could account for the reduced Kd in these animals. If this individual variation in Kd after infarction represents primarily a variation in enzyme release, and enzyme clearance is relatively constant, then a serious error would be introduced into the calculation of enzyme release if the Kd were calculated for individual animals from their own disappearance slopes. Therefore, calculations of enzyme release in these studies were made using the previously established Kd and DV in the baboon. Indeed, myocardial enzyme depletion correlated poorly with CPK-MB release when Kd was individualized on the basis of disappearance slopes in each animal. It is possible that the delayed enzyme release in some of these baboons was related particularly to the mercury injection technique, which may produce an arterial and myocardial inflammatory reaction as well as coronary obstruction and infarction.

The variability in CPK-MB isoenzyme patterns in clinical myocardial infarction was similar to observations in the baboon. The Kd of CPK-MB in man usually was considerably lower than that after bolus injection in the baboon. Although it is possible that this discrepancy results from a species difference, it is more likely that human infarction often represents a continuously evolving process in which enzyme is released over a long period of time.30 If this is the case, calculation of enzyme depletion in man as well as in the baboon should be based on an ideal Kd rather than on the Kd calculated from serum values obtained after infarction in individual subjects, as recently advocated by Norris et al.9

Considerable variation in the MB fraction of serum CPK activity also was apparent in these clinical studies. Since the MB fraction of enzyme released from myocardium should be constant, the low fraction must represent the appearance of MM isoenzyme from nonmyocardial sources. Calculations using total CPK or the CPK-MM isoenzyme often result in an overestimation of enzyme release, as demonstrated in 15 of the 20 patients studied in the present series. This discrepancy accounts for the unreasonably high values for calculated infarct size previously reported in some clinical studies5** and reaffirms the importance of using the MB isoenzyme if enzyme depletion from the heart is to be assessed.

References

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The Effect of Exercise Training on Heart Rate during Coitus in the Post Myocardial Infarction Patient

RICHARD A. STEIN, M.D.

SUMMARY The effect of interval exercise training on the peak coital heart rate in post myocardial infarction patients was studied. Sixteen men (ages 46–54) underwent a 16-week bicycle ergometer training program 12 to 15 weeks following their first myocardial infarction. Portable ECG tape recorders were used to record the ECG during coitus twice before and twice after the training program. The maximum minute oxygen consumption (VO$_{2}$max) was measured in each subject during bicycle ergometer ECG examinations before and after the training program. A control group of six post myocardial infarction patients who were not trained was evaluated in the same manner.

The exercise-trained group had an average increase in VO$_{2}$max of 11.5% (2.7 to 3.9 L/min) and an average decrease in peak coital heart rate of 5.5% (127/min to 120/min). The control group demonstrated a 2% increase in VO$_{2}$max and no significant change in peak coital heart rate.

The increase in aerobic capacity (VO$_{2}$max) and the consequent reduction in peak coital heart rate in our trained group suggests the potential value of exercise training in improving sexual function in the patient with angina during coitus.

SEXUAL DYSFUNCTION following a myocardial infarction is a commonly noted phenomenon.$^1$ The basis of this dysfunction is frequently the patient’s fear that the stress of coitus will precipitate a myocardial infarction.$^2$

For the majority of patients whose exercise capacity safely exceeds the demands of coitus, these concerns are not physiologically sound. Hellerstein and Friedman have found that the peak coital heart rate among middle-aged married men ranged from 101 to 121 beats per minute. The mean peak coital heart rate was 117 beats per minute. In fact, their subjects’ maximal occupational heart rate frequently exceeded the peak coital heart rate.$^3$

For those patients whose exercise capacity safely exceeds the demands of coitus, explicit sexual counseling has been shown to be effective in encouraging the resumption of pre-infarction sexual patterns. This author has reported the value of having the spouse observe the patient’s multistage exercise ECG examination and thus receive a tangible demonstration of the patient’s exercise capacity.$^4$

For a segment of the population of post myocardial infarction men, sexual dysfunction is not solely psychogenic, but is based on a limited tolerance which is exceeded during coitus. Such individuals may suffer coital or post-coital angina, arrhythmias, or exceptional fatigue. Pre-coital nitrates frequently allow asymptomatic coitus in these patients and are the treatment of choice at present.

Exercise training has become a commonly employed component in the treatment of the post myocardial infarction patient. The rationale for this therapy is the improvement in cardiac efficiency and the ability to perform specific work tasks at reduced heart rate–blood pressure products and cardiac oxygen requirements. In the Hellerstein and Friedman study, two-thirds of the trained subjects reported fewer symptoms during sexual activity after training as compared to before training.$^5$ The subject of this study is the physiological basis of exercise training as a specific form of therapy for sexual dysfunction in the post myocardial infarction patient.

Method

Patient Selection

Twenty-two men ages 46 to 54 were evaluated 12 to 15 weeks following an initial myocardial infarction. The subjects had no complaints of post infarction angina, were on no drug therapy, and were all sexually active with the same marital partners for more than seven years. None of the subjects had clinical manifestations of congestive heart failure. Each of the subjects had resumed sexual relations six to ten weeks prior to beginning the study protocol.

Coital ECG Recording

Each subject and his spouse received a comprehensive...
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