

# Serum CPK isoenzymes after cardiac catheterization<sup>1</sup>

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*Exclusion of acute myocardial infarction preoperatively, particularly in patients undergoing cardiac catheterization, is an important requirement for optimal results following coronary revascularization. Unfortunately, activity of conventionally measured serum enzymes (AST, LDH, total CPK) is frequently raised because of enzyme released from non-cardiac sources during the catheterization procedure.*

*However, serum activity of the MB CPK isoenzyme, an isoenzyme found primarily in heart muscle, appears to be more specific. Accordingly, in the present study, total CPK and MB CPK activities were determined in serum samples from 53 patients undergoing diagnostic catheterization, immediately before study and serially for 24 hours afterwards. A comprehensive range of catheterization procedures included selective coronary arteriography in 39 patients by brachial (17) or femoral (22) artery approaches. Myocardial infarction was excluded by clinical and electrocardiographic criteria in all patients before and after the procedure. MB CPK isoenzyme activity was also measured in serum samples from 50 patients with acute myocardial infarction documented electrocardiographically, and in 20 controls admitted to hospital but without cardiovascular disease.*

*In patients with acute myocardial infarction, both total CPK and MB CPK isoenzyme levels were significantly raised ( $0.78 \pm 0.087$  and  $0.086 \pm 0.037$  IU/ml, respectively), exceeding the upper limit of normal in all cases. MB CPK activity remained within normal limits ( $< 0.004$  IU/ml) in all 20 subjects without cardiovascular disease. Peak total serum CPK activity exceeded control levels in all patients undergoing catheterization ( $0.260 \pm 0.033$ ). However, in each case, MB CPK isoenzyme activity remained within normal limits ( $< .004$ ). Thus, in contrast to an increase of activity of conventionally used serum enzymes, increased MB CPK isoenzyme activity is a reliable indicator of myocardial infarction, even in patients undergoing cardiac catheterization.*

The use of coronary arterial by-pass grafting for patients with angina pectoris has increased the importance of cardiac catheterization and coronary arteriography in patients with coronary artery disease. Sometimes surgery is contemplated on an urgent basis, particularly in patients with 'pre-infarction' coronary insufficiency (Favaloro *et al.*, 1971). It appears likely that correct selection of patients for coronary bypass surgery requires identification and perhaps exclusion of evolving infarction, since operation may lead to extension of the infarct, or the result of the operation may be unsatisfactory (Roberts and Sobel, 1974; Dawson *et al.*, 1974). During catheterization, patients with severe coronary insufficiency sometimes develop chest pain or electrocardiographic abnormalities compatible with myocardial damage (Ross and Gorlin, 1968).

Furthermore, despite improved techniques of cardiac catheterization, myocardial infarction, albeit infrequent, remains a possible complication, particularly in patients with severe proximal coronary obstruction (Lavine *et al.*, 1972) or advanced left ventricular dysfunction (Takaro *et al.*, 1973). There is therefore a need for a means for the objective detection of myocardial injury in relation to these investigations.

Conventional criteria of myocardial injury may be unreliable in patients undergoing cardiac catheterization. Often, electrocardiographic abnormalities related to previous myocardial infarction or conduction abnormalities are present and may cloud the issue. Conventionally measured serum enzymes, including serum aspartate transferase, lactic acid dehydrogenase (LDH), LDH<sub>5</sub> isoenzyme, and total serum creatine phosphokinase (CPK), may be raised after catheterization (Adrouny *et al.*, 1963; Burckhardt *et al.*, 1968), in part because of non-

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cardiac soft tissue injury (Wolfe, Ruttenberg, and Moss, 1970), and thus cannot be used as reliable indices of myocardial infarction (Marpole *et al.*, 1968; Michie *et al.*, 1970). Recently, we and others have shown that raised serum MB CPK activity, an isoenzyme found primarily in myocardium, is not only a sensitive (Roe *et al.*, 1972; Kontinen and Somer, 1972) but also a remarkably specific index of myocardial injury (Roberts *et al.*, 1975). Three serum CPK isoenzymes have been recognized and referred to as MM, BB, and MB on the basis of subunit composition. Only four human tissues are rich in CPK activity: skeletal muscle, myocardium, brain, and the gastrointestinal tract (Roberts *et al.*, 1975). We have recently surveyed surgically obtained human tissues and found that myocardium is the only one containing more than trace amounts of MB isoenzyme under standardized assay conditions. We previously demonstrated that raised serum CPK activity after intramuscular injections is not associated with increased MB CPK activity (Klein, Shell, and Sobel, 1973), nor is non-cardiothoracic, abdominal, genitourinary, or orthopaedic surgery (Gowda, Roberts, and Sobel, 1974). Accordingly, the present study was designed to determine whether raised serum CPK activity after cardiac catheterization is caused by release of enzyme from the heart, and whether increased MB CPK remains a reliable index of myocardial ischaemic injury in this setting.

Until recently, it has not been possible to measure serum CPK isoenzyme activity accurately because of insensitivity or imprecision in assay procedures (Roberts and Sobel, 1973). The recent availability of a fluorometric kinetic assay system permits precise quantification of individual serum CPK isoenzyme activity even in samples from normal subjects with low activity (Roberts *et al.*, 1974). In the present study, this technique was used; total CPK and CPK isoenzyme activity was determined in serial samples from 53 patients after cardiac catheterization. Results were compared with those ob-

TABLE 2 *Electrocardiographic findings before catheterization*

<i>Abnormalities</i>	<i>No. of patients</i>
Transmural infarction	5
Nonspecific ST-T changes	16
Ventricular hypertrophy	
LVH	8
RVH	3
Conduction disturbances	5
None	16

tained in 50 patients with documented myocardial infarction and in 20 patients in hospital without cardiovascular disease.

### Subjects and methods

The patients undergoing cardiac catheterization comprised 33 men and 20 women, age range 17 to 67 (mean 44.3 years). Clinical assessments (Table 1) included suspected coronary insufficiency and chest pain (31 patients), valvular heart disease (15), and congenital cardiac defects (7). Two patients were investigated for prosthetic valve dysfunction and 2 were studied to determine graft patency 3 months after coronary arterial bypass grafting. Catheterization was performed on an emergency basis in 5 patients in whom recent exacerbation of angina pectoris was considered to represent 'pre-infarction'. Remote previous infarction was demonstrated by the electrocardiograms in 5 (Table 2). Clinical evidence of congestive heart failure was present in 3 patients, 1 with tricuspid atresia and 2 with mixed aortic and mitral valve disease. Neither prolonged chest pain nor major arrhythmia requiring electrical cardioversion occurred during catheterization. Frequent ventricular premature contractions were observed in 4 patients with coronary artery disease, but were readily controlled by intravenous lignocaine ('xylocaine'). No patient sustained a major complication (Braunwald and Swan, 1968). Specifically, no clinical or electrocardiographic signs of myocardial infarction were observed. Minor complications were limited to superficial groin haematomas in 4 patients undergoing percutaneous femoral artery procedures. All patients were premedicated 30 minutes before the procedure with 5 to 10 mg of diazepam ('valium') and 100 mg pentobarbitone ('nembutal') intramuscularly. Sites for percutaneous puncture and brachial cutdown were anaesthetized by infiltration of 20 ml of 2 per cent lignocaine. Systemic anticoagulation with heparin, 100 units per kilogram body weight, was employed in all cases. Right and left heart catheterization with angiography was performed in 36 patients, and left heart catheterization alone with angiography in 17. Selective coronary angiography was performed in 39 patients, using the brachial artery approach (Sones and Shirey, 1962) in 17, and the per-

TABLE 1 *Clinical groups and catheterization findings*

<i>Indications</i>	<i>No.</i>	<i>Vessels with major occlusions</i>			<i>Depressed left ventricular function</i>
		<i>1</i>	<i>2</i>	<i>3</i>	
Chest pain	31	5	3	11	12
Valvular heart disease	15	1		1	7
Congenital heart disease	7				6

cutaneous femoral artery technique (Judkins, 1967) in 22. Adequate non-selective coronary opacification was obtained in the remaining 14 cases during root cineangiography. Cardiac output was determined in all cases, by use of the Fick principle, indocyanine green dilution, or thermodilution techniques. Hydrogen studies were performed for the detection of intracardiac shunts in all 7 patients with suspected congenital heart disease. The haemodynamic response to exercise performed on a bicycle ergometer was evaluated in 4 patients with mitral valve disease. His bundle electrography with atrial pacing was performed as part of the investigation in 3 cases. In 9 patients, temporary right ventricular endocardial pacing was performed during selective coronary arteriography. Angiocardiography was performed with pressure injection of 35–50 ml of 75 per cent Hypaque M (sodium diatrizoate). Manual injections of 6 to 12 ml Renografin 76 (meglumine diatrizoate and sodium diatrizoate) were employed for selective coronary arteriography.

The criterion for the diagnosis of significant coronary artery disease was 70 per cent or greater obstruction visualized by multiple projection arteriography. Left ventricular function was assessed by visual inspection of left ventriculograms filmed in one or two planes, ventricular haemodynamics, and ejection phase indices of performance, including ejection fraction, mean circumferential fibre shortening rate (Karlner *et al.*, 1971), and normalized mean systolic ejection rate (Peterson *et al.*, 1974).

Blood samples for total CPK and CPK isoenzyme activity determinations were obtained immediately before catheterization and every 2 hours thereafter for a total of 24 hours. Blood samples were collected in 0.005 moles neutralized EGTA, centrifuged at 2000 *g* for 10 minutes, decanted, and protected with 0.010 moles mercaptoethanol (final concentration). Samples were assayed immediately for CPK isoenzyme activity or fast frozen, stored at  $-20^{\circ}\text{C}$ , and assayed within 4 weeks. Samples stored under these conditions do not show loss of activity for at least 6 weeks (Roberts *et al.*, 1974).

The results of the CPK isoenzyme determinations in patients undergoing catheterization were compared to those obtained from 50 patients admitted to the cardiac care unit who sustained electrocardiographically documented transmural myocardial infarction, and from 20 controls in hospital but without significant cardiovascular disease. In patients in the cardiac care unit, blood samples were collected every 2 hours through a peripheral venous heparin lock for a total of 48 hours and assayed for total CPK and CPK isoenzyme activity exactly as in patients who were catheterized.

#### Total CPK and CPK isoenzyme determinations

Total CPK activity was determined spectrophotometrically (Rosalki, 1967) in serum samples diluted with Tris, 0.01 moles, pH 7.4 containing 0.2 per cent bovine serum albumin such that CPK activity was within the range of linearity of the assay (Bresnahan *et al.*, 1974). CPK activity was assayed in 50  $\mu\text{l}$  aliquots in a final volume of

1.050 ml at  $30^{\circ}\text{C}$ . Results were expressed in international units/ml (IU/ml). The upper limits of normal in our laboratory are 0.040 IU/ml and 0.050 IU/ml for women and men, respectively. Assays were performed with and without creatine phosphate as substrate to exclude spurious contributions to apparent CPK activity from myokinase and other moieties.

Activity of CPK isoenzymes was determined qualitatively by cellulose acetate electrophoresis as previously described (Klein *et al.*, 1973) and quantitatively with a fluorometric, kinetic assay recently developed in our laboratory (Roberts *et al.*, 1974). For qualitative analysis of serum CPK isoenzymes, 10  $\mu\text{l}$  aliquots of samples diluted to contain 0.1 IU/ml were separated by cellulose acetate electrophoresis at a constant voltage of 250 volts for 60 minutes at  $4^{\circ}\text{C}$  in 0.05 moles Tris barbitone, pH 8.8 containing 0.001 moles EGTA. Isoenzyme bands were visualized by applying cellulose acetate strips previously soaked in NADPH generating medium. This technique permits detection of isoenzyme activity exceeding 0.010 IU/ml (Kontinen and Somer, 1972). Quantitative determinations of activity of individual CPK isoenzymes were performed with a kinetic fluorometric procedure as recently described (Roberts *et al.*, 1974). Results with this method are linear with respect to time and enzyme activity and reproducible within 3 per cent. Its sensitivity is less than 0.002 IU/ml and it avoids several difficulties associated with quantification of isoenzyme activity by fluorescence scanning of electrophoretic media. Serum from normal subjects assayed under these conditions contains less than 0.005 IU/ml of MB CPK (Roberts *et al.*, 1974).

#### Results

Peak total serum CPK activity was raised above control levels in all patients undergoing catheterization (mean peak  $0.260 \pm 0.063$  IU/ml) (range 0.036–0.570) (Fig. 1). In 41 patients, peak levels exceeded the upper limits of normal for this laboratory; in the

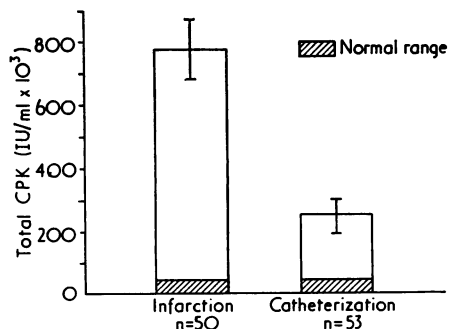


FIG. 1 Mean peak serum total CPK activity after myocardial infarction (left bar graph) and after catheterization (right bar graph). Although the bars begin at the abscissa, the range of patient values was 180–1360 (left bar) and 36–570 (right bar). The vertical line represents  $\pm 1$  SD.

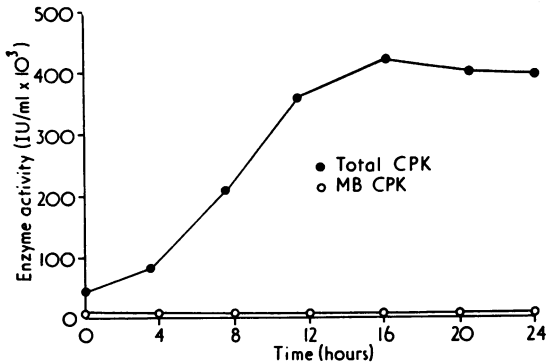


FIG. 2 Total serum CPK and MB CPK activity after catheterization in a representative patient.

other 12, peak levels were raised above control values, but remained within the normal range. Levels rose progressively after catheterization, generally reaching peak values 12 to 20 hours after the procedure (Fig. 2). No significant difference in peak levels was observed in patients requiring cut-down compared to those studied percutaneously, nor in those undergoing selective, compared to non-selective coronary arteriography. Peak total CPK activity was not disproportionately raised in patients exercising on the bicycle ergometer or undergoing atrial or ventricular pacing, intracardiac electrography, investigation of prosthetic valve function, or selective coronary graft arteriography.

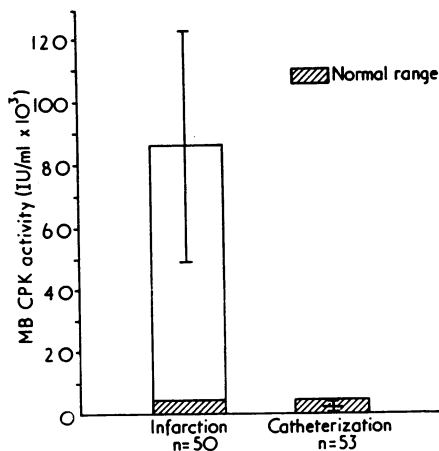


FIG. 3 Mean peak serum MB CPK activity after myocardial infarction (left bar graph) and after catheterization (right bar graph). Although the bars begin at the abscissa, the range of patient values was 23-96 (left bar) and 1-5 (right bar). The vertical line represents  $\pm 1$  SD.

In spite of the obvious increase in total serum CPK activity generally observed in these patients, MB CPK isoenzyme activity determined quantitatively remained within the normal range in all cases after catheterization, with no value exceeding 0.004 IU/ml (Fig. 3). Results of the qualitative assay showed no MB CPK in any of the cases, with MM CPK as the only isoenzyme visualized. As we have previously shown, the quantitative procedure used detects some MB CPK in serum from normal subjects though qualitative assay of the same sample fails to do so. No patient undergoing catheterization developed clinical or electrocardiographic evidence of myocardial infarction, arrhythmia, or haemodynamic decompensation. Thus, cardiac catheterization was not associated with increased MB CPK despite substantial rise in total serum CPK activity.

In 50 patients with documented acute myocardial infarction, total peak CPK averaged  $0.780 \pm 0.087$  (SD), (range 180-1360) (Fig. 1), exceeding the upper limit of normal in all cases. Furthermore, in contrast to patients undergoing catheterization, MB CPK isoenzyme activity was significantly raised in all cases after infarction with peak MB CPK averaging  $0.086 \pm 0.037$  (SD) (range 23-96) (Fig. 3).

As can be seen in Fig. 3, MB CPK activity in samples from normal subjects was indistinguishable from activity in samples from patients undergoing catheterization. However, tenfold increases in MB CPK were evident in patients with infarction.

## Discussion

The interpretation of serum enzyme increases after cardiac catheterization and coronary arteriography has been difficult. Significant rise in serum aspartate transferase (AST) has been reported in up to 55 per cent of cases (Adrouny *et al.*, 1963), while other investigators have observed only trivial changes (Harrison, Matloff, and Wexler, 1972). Raised lactic dehydrogenase has been observed (Burckhardt *et al.*, 1968) but not consistently (Chahine, Eber, and Kattus, 1974; Michie *et al.*, 1970). Significant rises in total serum CPK activity after cardiac catheterization have been reported frequently (Chahine *et al.*, 1974; Harrison *et al.*, 1972) and it is, therefore, apparent that detection of myocardial infarction after cardiac catheterization cannot be based on total serum CPK rises (Marpole *et al.*, 1968). Some imply that raised serum CPK activity after coronary arteriography reflects release of enzyme from the heart (Sitzman and Gutheil, 1966), though contributions from intramuscular injections (Harrison *et al.*, 1972; Meltzer, Mrozak, and Boyer, 1970) or

other soft tissue trauma have been recognized (Wolfe *et al.*, 1970). Since patients with 'pre-infarction angina' commonly receive parenteral medications before being admitted to hospital, enzyme increases may be particularly difficult to interpret in this group. Accurate detection of myocardial infarction in the immediate post-catheterization period in these patients is of paramount importance, since the success of coronary revascularization may be compromised in the presence of evolving or established infarction (Roberts and Sobel, 1974).

In the present study, serum CPK isoenzyme activity was determined in serial samples from 53 patients after cardiac catheterization. All patients received intramuscular premedication. In keeping with results of other studies (Marpole *et al.*, 1968; Michie *et al.*, 1970), total serum CPK activity was significantly raised in 41 patients and minimally increased in the remainder. However, MB CPK activity remained within the normal range in all cases, indicating that myocardial damage did not occur and that increased serum CPK activity did not reflect release of enzyme from the heart. This interpretation was supported by the lack of clinical and electrocardiographic evidence of myocardial injury.

Results were quite different in 50 patients with proven myocardial infarction. All patients with confirmed myocardial infarction had a significant increase in serum MB CPK within 6 to 8 hours of the onset of chest pain. Thus, it appears likely that a rise in MB CPK within 6 to 8 hours after cardiac catheterization would occur in patients with coronary artery disease who have sustained myocardial infarction before, or even precipitated by, the procedure. Detection of infarction on the basis of CPK isoenzymes in this context may be particularly critical since the electrocardiogram may be misleading or difficult to interpret because of previous infarction, conduction defect, ventricular hypertrophy, arrhythmia, tachycardia, or non-specific changes. Thus, determination of MB CPK activity is particularly useful in the selection of patients for coronary revascularization, particularly those with 'pre-infarction angina' and those at high risk with left main coronary disease or conditions such as calcific aortic stenosis for whom prompt surgery is frequently contemplated.

Our results indicate that in contrast to an increase in activity of conventionally measured serum enzymes, increased MB CPK isoenzyme activity is a sensitive and useful criterion of myocardial infarction in patients undergoing cardiac catheterization.

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