Reversible myocardial ischemic injury is not associated with increased creatine kinase activity in plasma

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Creatine kinase (CK) isoenzymes MM, MB, and BB are located primarily in the cell cytosol, and increased CKMB in plasma is the hallmark of myocardial infarction. However, whether CK is released with reversible ischemic injury remains controversial. Here, we assessed plasma CK activity—cytosolic and mitochondrial CK—in serial samples (every 10 min for 60 min, then hourly or every 4 h for 48 h) from 46 conscious dogs after transient or sustained coronary occlusion. Four dogs were sham-operated (controls); four underwent sustained coronary occlusion (96 h); and 38 underwent transient coronary occlusion (10–40 min) followed by 48 h of reperfusion. In postmortem histological examination of the dogs’ hearts by light and electron microscopy, we looked for ischemia or necrosis. The presence of cell swelling and glycogen depletion was indicative of ischemia, whereas the added presence of cell disruption indicated necrosis. Coronary occlusion for ≥20 min consistently increased plasma mitochondrial and total CK activity and produced histologically evident myocardial necrosis. In contrast, after 10 to 15 min of coronary occlusion, 12 of 14 animals, despite extensive severe reversible ischemia, showed no increase in plasma CK; the remaining 2, which had increased plasma CK, had subendocardial necrosis. Thus, cytosolic or mitochondrial CK is released from the heart only when there has been irreversible myocardial injury—a finding with significant diagnostic and therapeutic implications.

INDEXING TERMS: mitochondrial enzymes • coronary occlusion • dogs • heart damage • radioimmunoassay

The creatine kinase (CK; EC 2.7.3.2) isoenzymes MM, MB, and BB, each with a molecular mass of 82 kDa, are located primarily in the cell cytosol. The CKMB isoenzyme offers cardiac specificity and has for some time been considered the diagnostic hallmark for assessing myocardial infarction [1–3]. Increased plasma CK activity in the setting of chest pain and electrocardiographic (EKG) changes has been interpreted to reflect irreversible myocardial injury (necrosis) rather than reversible injury, e.g., ischemia of unstable angina; however, this interpretation is controversial, not standard [4, 5]. The diagnostic distinction between myocardial infarction and ischemia—which is usually not possible from clinical or EKG findings alone—has taken on significant therapeutic and economic implications, given the development of new therapies. For example, thrombolytic therapy [6, 7], which markedly reduces mortality in patients with acute myocardial infarction, can increase the incidence of death and infarction in patients with unstable angina [8]. The recent utilization of CKMB subforms to detect infarction within 3–6 h from onset and to triage patients for specific management is also based on the assumption that release of CKMB reflects necrosis [3]. Accordingly, we decided to test the hypothesis that plasma CK activity does not increase with transient reversible myocardial ischemia.

In this study, we induced transient coronary occlusion of increasing duration in conscious dogs and serially measured their plasma concentrations of cytosolic and mitochondrial CK. Cardiac mitochondrial CK, derived from a gene separate from that of CK M or B [9], is located on the inner aspect of the outer membrane of the mitochondria. Thus, release of mitochondrial CK into the plasma would most likely reflect irreversible cellular injury because it would imply disruption of the mitochondria as well as the sarcolemma membrane. Utilizing an
antibody specific for canine cardiac mitochondrial CK [10], we developed an RIA to detect mitochondrial CK in plasma as a potential in vivo marker of cell death. We also examined the dogs’ hearts postmortem by light and electron microscopy for morphological evidence of ischemia, necrosis, or both.

**Materials and Methods**

**ANIMAL STUDIES**

All studies were performed after approval of the protocols by the Animal Protocol Review Committee for Baylor College of Medicine and Washington University. Studies were performed in 46 male mongrel dogs weighing 16–20 kg. The instrumentation for coronary occlusion required performing a left thoracotomy with sterile surgical technique in an anesthetized dog, induced with pentobarbital (30 mg/kg); ventilation was maintained with room air by a Harvard pump. The thoracotomy was performed via an incision in the fourth intercostal space; the pericardial sac was opened, and an inflatable balloon-cuff was placed loosely around the left anterior descending coronary artery immediately proximal to the first major branch, as previously described [11]. The coronary cuff was secured with 0–0 silk to the wall of the left ventricle. The proximal end of the polyethylene tubing continuous with the balloon cuff was exteriorized and secured to the skin. A 12-lead EKG was obtained before and after coronary occlusion, immediately after deflating the balloon, and in all animals before death. An indwelling catheter was placed in the jugular vein for collection of blood samples. Five to 10 days later, when the animals had recovered completely and plasma CK activity had returned to normal, coronary occlusion was induced in conscious animals by infusing the balloon with saline. Only the animals exhibiting ST segment elevation ≥1 mm were selected for the study. In the animals selected for transient ischemia, myocardial perfusion was restored by deflating the balloon after selected intervals of coronary occlusion.

The dogs were divided into four groups. In Group I, four dogs underwent sustained coronary occlusion for 96 h to determine whether mitochondrial CK is released after infarction and to characterize its temporal release profile without reperfusion. In Group II, 35 dogs underwent transient coronary occlusion for either 10, 15, 20, 25, or 40 min, followed by subsequent reperfusion for 48 h; 7 animals were studied at each interval. In Group III, three dogs underwent coronary occlusion for 15 min, followed by reperfusion for 24 h, and then underwent a second episode of coronary occlusion for 40 min, followed by another 24 h of reperfusion. Thus, we could compare the plasma concentrations of mitochondrial and cytosolic CK during brief and prolonged intervals of ischemia in the same animal. In Group IV, four animals (sham-operated) underwent a thoracotomy and a catheter was placed in the jugular vein and a balloon catheter around the coronary artery, but the artery was not occluded. Plasma samples, obtained as in the other groups, were used to determine the variation in plasma CK without coronary artery occlusion.

In all four groups, the heart rate and rhythm were monitored for arrhythmias and evidence of ischemia. Lidocaine, in 20-mg boluses, was administered intravenously immediately before ischemia and throughout the remaining interval as necessary to control arrhythmias. The animals in each group were killed with an overdose of pentobarbital. The left anterior descending coronary artery was examined to determine the degree of obstruction in the group undergoing sustained occlusion and for the degree of patency in the animals that underwent transient occlusion. The heart was removed for examination by light and electron microscopy.

Blood samples for CK analysis were obtained before coronary occlusion, immediately before reperfusion, and every 10 min during the first 60 min after the reperfusion, then every hour for the subsequent 23 h, and every 6 h thereafter until the animal was killed. Samples were collected in EGTA (5 μmol/L, pH 7.4), and plasma was separated from cellular components by centrifugation (2000 g for 10 min for 4 °C); after which mercaptoethanol (5 mmol/L) was added [11]. Samples were analyzed without further delay. The primary endpoint to determine the presence of ischemia or infarction was based on histological analysis.

**MICROSCOPIC ANALYSIS**

Hearts from all animals undergoing coronary occlusion were removed and sliced into 1.0-cm transverse slices in a plane parallel to the atrioventricular groove. The average number of slices per heart was six (mongrel dogs, 15 to 25 kg). Each transverse slice was sectioned throughout its circumference into transmural sections ~5 mm thick, and at least one sample from each section was analyzed by light and electron microscopy. Thus, the subendocardium, the area most vulnerable to ischemia, was sampled throughout the left ventricle and analyzed by light and electron microscopy as was the mid- and epicardial regions. The total number of sections analyzed by light and electron microscopy was ~220 per heart. We carefully examined the anterior papillary muscles, which are the end-organs for the coronary flow from the left anterior descending coronary artery and thus are the most vulnerable for ischemia.

In preparation for light-microscopic analysis, transmural tissue slices were fixed in buffered formalin (final concentration of formalin 100 mL/L), embedded in paraffin, and cut into 5-μm-thick sections. Sections were stained with hematoxylin–eosin and phosphotungstic acid–hematoxylin. Myocardial samples for electron microscopic examination included samples from the anterior and posterior papillary muscle, respectively. Tissue was minced into blocks (~1 mm per side), fixed in glutaraldehyde (30 mL/L in 0.1 mol/L sodium cacodylate buffer,
mitochondrial CK with 125I, using the Bolton–Hunter method, as previously described. Immunized rabbits were used for the preparation of specific antisera to mitochondrial CK, which was purified from canine brain and heart. Mitochondrial CK was isolated from fresh canine myocardium as previously described [10] and had a specific activity of 320 kU/g.

RIA for mitochondrial CK. Specific antisera to mitochondrial CK were obtained from immunized rabbits as previously described [10]. To develop an RIA, we labeled the mitochondrial CK with 125I, using the Bolton–Hunter method, as previously described [13]. All determinations were carried out in triplicate in 12 × 75 mL siliconized glass tubes. For each batch of samples analyzed, we prepared a calibration curve for assessing inhibition of enzyme activity, assaying in triplicate samples of unlabeled mitochondrial CK, 0.2 to 10 ng. A reference sample containing no inhibitor (unlabeled mitochondrial CK) and a blank containing no antiserum were assayed for maximum binding and background radioactivity, respectively. To confirm the specificity of the assay, we analyzed samples before and after the addition of 0.2 to 1000 ng of purified CKBB and CKMM. To reproduce reproducibility and accuracy, we repeatedly assayed (10 times) serial dilutions of a plasma sample containing mitochondrial CK.

Total CK activity. Plasma samples were analyzed spectrophotometrically at 30 °C for total CK activity according to the method of Rosalki; results are reported in U/L [13]. The lack of an antibody specific for canine CK MB prohibited quantification of CK MB mass.

RESULTS

EKG ANALYSIS

Only animals showing ST segment elevation after coronary artery occlusion were selected for further analysis. This was to ensure the presence of severe ischemia involving a large area of myocardium (extent) to facilitate the likelihood for extensive release of CK and ease of detection; ischemia that involved only a very small amount of myocardium that was minimally severe would be more difficult to detect. Secondly, dogs have extensive coronary collateral flow and are known to frequently manifest ST segment depression with coronary occlusion because of adequate blood flow from collaterals; also, ST segment depression may also be nonspecific and occur for reasons other than cardiac ischemia. Lastly, ST segment depression may occur when an artery is not completely occluded by the balloon and thus is less likely to be associated with severe, extensive myocardial involvement. The animals that underwent 20 min or less of coronary occlusion exhibited a normal EKG 48 h later. All of the animals that underwent sustained coronary occlusion and those that had transient coronary occlusion for 40 min exhibited the development of Q-waves on the EKG obtained before death.

RIA SPECIFICITY AND REPRODUCIBILITY

A representative inhibition curve for mitochondrial CK is shown in Fig. 1 (top), indicating a detection limit of 0.5 ng. The specificity is indicated by the complete lack of inhibition of CK MM or CK BB (Fig. 1, bottom). The reproducibility of the assay (CV) was ±5%, as indicated by the results of the repeated analyses of samples with mitochondrial CK concentrations ranging from 5 to 50 µg/L. Results of analysis of mitochondrial CK by the RIA in samples assayed before and after storage at −70 °C for 2 months showed values differing on average by <9%.

PLASMA CK ACTIVITY

After sustained coronary occlusion. In animals with sustained coronary occlusion (Group I), plasma CK activity was greatly increased after 4.2 ± 1.4 h, reaching peak activity at 11.5 ± 1.3 h and returning to baseline at ~48 h. A typical time–activity curve for total plasma CK in an animal with sustained coronary occlusion is shown in Fig. 2 (top). The time to initial detection of mitochondrial CK in plasma and to peak concentrations averaged 8 h and 22.5 h after occlusion, respectively. The mean peak value was 113.0 ± 47.8 µg/L and returned to baseline by 72 h after occlusion. A representative plasma mitochondrial CK curve after sustained occlusion is also shown in Fig. 2 (top).

After transient coronary occlusion. The total plasma CK activity was increased in all animals after coronary occlusion of 20 min or more followed by reperfusion (Group II), peaking only 4.5 ± 2.4 h after the occlusion, compared with 11.5 ± 1.3 h in groups with sustained occlusion. This
presumably results from restoration of coronary flow and more rapid washout of the isoenzymes. Mitochondrial CK was not detected in plasma of Group II animals before occlusion but was detected in all of them after occlusion, peaking 4.7 ± 2.1 h after reperfusion to 47.0 ± 22.0 μg/L (range 32.0–78.0 μg/L). Mitochondrial CK was increased in the plasma much earlier than was observed in animals after sustained occlusion, peaking after only 4.7 h, compared with 22.5 h in animals after sustained occlusion (Fig. 2, middle).

In the animals that underwent 10 min of coronary occlusion, mitochondrial CK was not detected in any of the plasma samples, and plasma total CK activity did not increase significantly over that of baseline values (Fig. 2, bottom). The baseline values for total plasma CK activity in the animals undergoing 10 min of coronary occlusion ranged from 52 to 96 U/L, the same as the baseline values for the sham-operated animals. The maximum increase in plasma total CK ranged from 5% to 14%, very similar to the increases of 7–11% seen in the sham-operated animals.
Also, no mitochondrial CK was detected in any of the sham-operated animals.

Mitochondrial CK was not detected in any of the seven animals undergoing 15 min of coronary occlusion. Also, plasma total CK activity was not increased in five of the animals, but two animals (nos. 5 and 7) showed minor but significant \( (P < 0.01) \) increases in total plasma CK activity over baseline, compared with the values seen in sham-operated animals (Fig. 3). Both animals showed small focal areas of subendocardial necrosis (see next section), unlike the other animals in this group, which showed no evidence of necrosis. All Group III animals had no increase in cytosolic or mitochondrial CK in plasma after 15 min of coronary occlusion followed by 24 h of reperfusion. However, after a second coronary occlusion for 40 min and 24 h of reperfusion, plasma mitochondrial and cytosolic CK concentrations were significantly \( (P = 0.001) \) increased, the temporal plasma time sequence of the increase being similar to that observed previously in Group II animals after 20–25 min of coronary occlusion.

**MICROSCOPIC ANALYSIS**

On visual inspection, subendocardial hemorrhagic infarction was observed in all of the hearts exposed to 20 min or more of coronary occlusion even when followed by reperfusion. By light microscopy, the infarction was restricted to the subendocardium in the animals undergoing 20–25 min of occlusion but extended from the endocardial surface to at least the mid-portion of the anterior wall in the animals with 40 min of occlusion; in some sections, the infarction was focally transmural. The necrotic tissue was edematous, diffusely hemorrhagic, and characterized by widespread contraction-band necrosis of myocytes. A mixed inflammatory infiltrate was well developed at 48 h after 20 min of coronary occlusion. Similar findings were observed on light microscopy in Group III animals.

These findings were in marked contrast to the microscopic appearance of the ischemic myocardium obtained from the anterior wall of the left ventricle and anterior papillary muscle of animals with only 10 or 15 min of coronary occlusion. The myocardia, except for glycogen depletion and cell swelling (15 min of coronary occlusion), were indistinguishable from that observed in sections from the nonischemic posterior ventricular wall and posterior papillary muscles of the same animals. However, in two of the seven dogs (nos. 5 and 7) from Group II with 15 min of coronary occlusion, scattered microscopic foci of infarction were observed in the subendocardium. The size of these microinfarctions never exceeded 3 mm and thus would constitute a minute portion of the total left ventricular mass. Both animals displayed a significant increase in plasma total CK but no increase in mitochondrial CK.

The electron microscopic appearance of mitochondria from nonischemic posterior papillary muscles was compared with that of the ischemic anterior papillary muscle in the animals that had undergone only 10 or 15 min of coronary occlusion. In the unaffected tissues, glycogen was present, there was no tissue swelling, and the mitochondria had an intact double membrane, compact orderly cristae, and a homogeneous dense matrix (Fig. 4). The appearance of the mitochondria in the ischemic anterior papillary muscle was similar to that of normal tissue (Fig. 5); namely, the mitochondria were intact, homogeneous, and lacked the ultrastructural features of irreversible organellar damage [15]. The only difference observed in the ischemic tissue vs normal tissue was complete depletion of glycogen and the presence of cell swelling—the only ultrastructural features that distinguished ischemic from control tissue in these animals.

![](image_url1)

Fig. 3. Plasma CK time–activity curves in two animals (nos. 5 and 7) after 15 min of coronary occlusion, showing minor but significant increases in total plasma CK activity over baseline compared with values in sham-operated animals.

Both animals showed subendocardial microinfarction. In animal no. 5, peak CK activity increased from (baseline) 58 U/L to 83 U/L, a 44% increase, and returned to baseline after 36 h. In animal no. 7 plasma CK increased 30%, from 86 to 110 U/L.

![](image_url2)

Fig. 4. Electron micrograph of perinuclear mitochondria (M) from nonischemic control tissue (normal myocardium). The mitochondria are intact and have orderly cristae and a dense matrix. Glycogen (G) is abundant. Bar = 1.0 \( \mu \)m.
the animals that underwent 10 min of coronary occlusion, the ischemia observed, even in the anterior papillary muscle, showed partial or complete depletion of glycogen but no tissue swelling. In contrast, ischemic tissue from animals that underwent 20 min or more of coronary occlusion exhibited (in addition to glycogen depletion and cell swelling) the ultrastructural hallmarks of irreversible cell injury in every animal; namely, mitochondria were swollen, the cristae were distended and disorganized, and the mitochondrial matrix lacked its normal homogeneous appearance. Virtually all mitochondria contained one or more dense bodies (Fig. 6), and disrupted and fragmented mitochondrial remnants were numerous (Fig. 7). A similar degree of damage was observed in the myocyte sarcolemma.

In each animal with 20 min or more of coronary occlusion followed by reperfusion and in each animal with sustained coronary occlusion, the myocardium exhibited unequivocal morphological evidence of irreversible injury consistently associated with release of cytosolic and mitochondrial CK. In contrast, plasma concentrations of neither mitochondrial nor cytosolic CK were increased in animals with 15 min or less of coronary occlusion despite extensive EKG and morphological evidence of ischemic reversible injury. Two animals that had only 15 min of coronary occlusion demonstrated minute areas of infarction and their plasma total CK was slightly but significantly increased \( (P = 0.01) \) over that in the sham-operated controls. Thus, irreversible cardiac cell damage was present in all animals in whom we observed increased plasma activities of total or mitochondrial CK.

**Discussion**

In this study severe, extensive myocardial ischemia was induced by complete occlusion of the proximal left anterior descending coronary artery for 10–15 min in conscious dogs. Ischemia was manifested in EKG by ST segment elevation and histologically by myocardial tissue swelling and glycogen depletion. Despite this, plasma CK activity increased over controls in only two animals, both of which had small foci of subendocardial necrosis in addition to ischemia. The remaining 12 animals so treated exhibited no increase in plasma total or mitochondrial CK activity. In contrast, all animals undergoing 20 min or more of coronary occlusion exhibited increased plasma activities of total and mitochondrial CK and showed histological evidence of myocardial necrosis. Thus, these results strongly indicate that increased CK activity in the plasma does not occur with reversible myocardial injury as induced by ischemia, but rather occurs only when myocardial injury is irreversible.

The canine heart, like that of humans, is prone to develop collateral vessels and thus exhibits great variability in coronary flow. We circumvented the problem of variability in coronary flow and the need to measure...
coronary flow by using direct histological indices of ischemia or necrosis. Thus, our conclusion that CK is not released with ischemia but is released with necrosis was not influenced by the potential variability in coronary flow induced by the collaterals or the extent of myocardial injury. Furthermore, this conclusion is strengthened by measures taken to induce severe and extensive myocardial ischemia to optimize conditions to detect even a very small release of CK into the plasma, which may occur with even minimal reversible injury. These measures were as follows: (a) selecting animals showing ST segment elevation; (b) inducing an extensive area of myocardium by proximally occluding the left anterior descending coronary artery, which perfuses 25–40% of the ventricular myocardium; (c) restoring coronary flow for more rapid and complete washout of CK; (d) sampling blood frequently for CK analysis; (e) using sensitive assays to detect mitochondrial and cytosolic CK; (f) assaying for mitochondrial CK independent of enzyme activity; (g) keeping the animal alive for 48 h after occlusion to optimize detection of manifestations of irreversible injury by light and electron microscopic analysis.

Sommers and Jennings [16], studying anesthetized dogs, showed that coronary occlusion for 10–15 min induced severe reversible ischemia in most animals, whereas occlusion for 20 min or more of ischemia was associated with cell death. Within 30 s of ischemia, lactic acid accumulates, the pH decreases, and ATP concentrations decrease; within 5–10 min, myocardial glycogen in the ischemic area is depleted [15, 16] and tissue swelling occurs. We observed similar findings in our animals undergoing 10–15 min of coronary occlusion, namely, depletion of glycogen and mild cell swelling, but the mitochondria remained intact and there was no evidence of irreversible injury. Thus, the ischemia was severe and the duration of occlusion for 15 min in the conscious animal probably represents the limit of cell viability, in that two of the seven animals did show microinfarction. Given the sensitivity of our assays for CK, the lack of dependence of the mitochondrial assay on enzyme activity, and the frequent sampling over the 48-h intervals, our results indicate strongly that severe reversible ischemic cardiac injury, despite involvement of a large portion of the myocardium, does not increase plasma activities of total or mitochondrial CK.

Results of data on CK release in isolated hearts and intact animals have been inconclusive. Sakai et al. [17], studying the isolated guinea pig heart, showed that after 20 min of ischemia the heart exhibited good hemodynamic recovery despite enzyme release; they concluded that enzyme release reflects reversible injury. However, mechanical recovery does not exclude the presence of subendocardial infarction, which could account for the enzyme release. In a similar study, Hearse et al. [18], using isolated rat heart, reached the opposite conclusion that enzyme release reflects irreversible injury. Conrad et al. [19], using the perfused septum preparation and loss of radioactive potassium as an indicator of irreversible injury, concluded that release of CK reflected irreversible injury. Histological detection of irreversible injury usually requires 24 to 48 h after coronary occlusion, which is not possible in the isolated heart; thus, studies in isolated hearts, while compelling, remain somewhat presumptive without correlative morphological studies. In a previous study in dogs, we showed by light microscopy that the release of cytosolic CK into plasma correlated with irreversible cardiac injury [20]. Studies by Hirzel et al. [21] in dogs after sustained coronary occlusion showed a depletion of myocardial CK correlated with morphological evidence of necrosis, and others have shown that CK depletion from the myocardium correlates quantitatively with the extent of myocardial necrosis [1, 22]. Heyndrickx et al. [23] reported that coronary occlusion of only 5–15 min resulted in transient impairment of regional mechanical function and minimal increase in plasma CK activity. The investigators discussed the possibility of microinfarction but, because no histological studies of the myocardium were performed, reached no definitive conclusion.

In a previous study in the dog [24] with sampling from the thoracic duct, we showed that CK appeared in the lymph upon reperfusion after 10 and 15 min of coronary occlusion. In these studies, plasma CK activity was not determined. Because only one transmural slice was examined for histology, we could not exclude the possibility of microinfarction. Furthermore, during coronary occlusion, CK could have concentrated in the lymph during occlusion and upon reperfusion could have been associated with increased CK activity independent of any CK released from the myocardium.

It has been suggested [25, 26] that CKMB may be released from the heart in association with chest pain without conventional EKG evidence of infarction. However, EKG changes of ischemia are indistinguishable from those of subendocardial infarction [27]. Frequent sampling in patients with unstable angina showed no increase in plasma CKMB [28]. Exercise-induced myocardial ischemia confirmed by a reversible perfusion defect detected by thallium scintigraphy also showed no increase in total plasma CKMB activity and no change in the ratio of the CKMB subforms [29].

The differentiation of ischemia from myocardial infarction has significant therapeutic and economic implications. In a National Heart, Lung, and Blood Institute-sponsored prospective study of patients exhibiting nondiagnostic EKG changes with unstable angina and subsequently shown not to have increased plasma CKMB, thrombolytic therapy was associated with increased incidence of infarction and death [8]. Patients presenting with unstable angina exhibiting increased CKMB (non-Q-wave infarction) treated with thrombolytic therapy, albeit late (a mean of 9 h after onset), manifested no beneficial or detrimental effects. One potential reason for lack of benefit from thrombolytic therapy in these patients with non-Q-wave infarction is a too-late initiation of therapy.
Nevertheless, an early diagnosis in these patients is extremely difficult to obtain, given that the EKG is nondiagnostic and that myocardial infarction cannot be excluded by the results for total CKMB or troponin T or I until at least 8–12 h after onset of symptoms [30]. The rapid CKMB subform assay, which reliably diagnoses myocardial infarction within 3–6 h from onset of symptoms, has the potential to triage these patients [31, 32] for therapeutic modalities including early thrombolytic therapy. Extrapolating the present results to humans would indicate that patients with unstable angina and increased CKMB are indeed undergoing myocardial necrosis. The increase in total plasma CK in these dogs occurred primarily because of release of myocardial CKMM (tissue subform CKMM-3) rather than of CKMB (tissue subform CKMB-2). Nevertheless, it is reasonable to assume that CKMB release into plasma also reflects infarction, given that MM and CKMB have essentially the same function and molecular masses and are both located in the cytosol. By analogy, an increase of the tissue subform CKMB-2 in plasma as detected by the CKMB subform assay should also reflect infarction. However, the CKMB subform assay per se was not utilized in this study, and its sensitivity relative to total CK for detection of injury in the dog remains unknown. Nevertheless, these results in the conscious dog, showing that increased plasma CK does not occur with myocardial ischemia but does occur with myocardial infarction, provide strong indirect support for the clinical interpretation that increased plasma CK activities reflect myocardial necrosis.

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