Serum β-enolase in acute myocardial infarction

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SUMMARY The enzyme β-enolase (αβ and ββ forms) is present in skeletal and heart muscle and
catalyses the glycolysis of 2-phosphoglycerate to phosphoenolpyruvate. The enzyme was mea-
sured in serum samples from patients with acute myocardial infarction, angina pectoris, con-
gestive heart failure, and idiopathic cardiomyopathy. Serum concentrations of β-enolase were
significantly increased in acute myocardial infarction but not in the other cardiovascular diseases.
Activity peaked approximately 12 to 14 hours after an acute attack of chest pain, and then gradu-
ally decreased as the patient recovered. The rise and fall in β-enolase concentration were faster
and steeper than those of creatine kinase activity, particularly in patients in whom activities of
both these enzymes were less high. The assay of β-enolase, which is highly specific and sensitive,
has considerable advantages for the early diagnosis of myocardial infarction and the diagnosis of
a second episode of myocardial infarction because β-enolase concentration increases very early
and rapidly and clears quickly.

These data imply that serum β-enolase may be a more effective marker for early myocardial
infarction, particularly in milder cases, than measurement of creatine kinase activity.

Mammalian enolase (2-phospho-D-glycerate hydro-
lase, EC 4.2.1.11) occurs as dimers composed of
three immunologically distinct subunits—α, β, and
γ.1,2 γ-enolase (αγ and γγ forms) is found in neuronal
tissues and neuroendocrine cells and β-enolase (αβ
and ββ forms) is found mainly in heart and skeletal
muscle.3,4 The characteristic distribution of these
enolases suggests that they may be useful as disease
markers.5

Using a sensitive enzyme immunoassay method
Kato et al have recently confirmed that β-enolase in
serum is a useful marker for human muscle dis-
eses.5 We have measured immunoreactive
β-enolase in serum samples from patients with vari-
ous cardiovascular diseases to determine whether
this enzyme could be used as a marker for acute
myocardial infarction.

Patients and methods

We studied 26 consecutive patients (aged 38–84, 21
men and 5 women) with a first acute attack of myo-
cardial infarction and without neuromuscular dis-
eases. They were admitted to the coronary care unit
of our hospital. We also studied 10 patients (all men,
aged 36–61) with angina pectoris, eight with con-
gestive heart failure not caused by acute myocardial
infarction, and three patients with idiopathic cardio-
myopathy. Cardiac disease was diagnosed by the
attending cardiologist according to standard criteria,
which included clinical history, electrocardio-
graphic and echocardiographic changes, and bio-
chemical assessment. We excluded patients with
cardiogenic shock and those on cardiotonic medica-
tion, except for dopamine, which did not affect this
enzyme. We also studied 20 normal subjects (15 men
and 5 women, aged 20–70) and 10 patients with renal
failure or hepatic disease. Blood samples were col-
lected every two hours for 34 hours after admission
from the cubital vein via an indwelling tube contain-
ing anticoagulant. Within 30 minutes of collection serum was separated from the blood samples by centrifugation at 3000 rpm for 10 minutes. Plasma was obtained from citrated blood by centrifugation.

We used the $\beta$-enolase immunoassay described by Kato et al.\textsuperscript{4} The assay system uses polystyrene spheres coated with immobilised purified antibodies to $\beta$ subunit and $\beta$ subunit antibodies labelled with $\beta$ D-galactosidase from Escherichia coli. The $\beta$-enolase assay is specific and there is no cross-reaction with other forms of enolase.\textsuperscript{5} Intramuscular injections and cardioversion did not markedly alter concentrations of serum $\beta$-enolase.

Serum creatine kinase (EC 2.7.3.2) activity was assayed spectrophotometrically with Testomar-C CK mono from Calbiochem-Behring, or a Centrifichem CK reagent set from Baker Instruments Co, by coupling the reaction with hexokinase and glucose-6-phosphate dehydrogenase.\textsuperscript{6} The normal value of total creatine kinase activity is $<35$ mU/ml.

Technetium-99m pyrophosphate scintigraphy of the myocardium was carried out according to the method of Bonte et al.\textsuperscript{7,8} in order to assess the size of the infarct. There was a good correlation between infarct size and creatine kinase activity; $y = 12.2x - 12.8$, $r = 0.81$, $p < 0.001$.

Patients with acute myocardial infarction were divided into two groups. Those in whom the concentration of $\beta$-enolase was normal on admission and peak creatine kinase activity ($\leq 300$ mU/ml) and peak $\beta$-enolase concentration ($\leq 110$ ng/ml) tended to be low were identified as mild cases. Those with a higher than normal concentration of $\beta$-enolase on admission, higher peak of creatine kinase activity ($>300$ mU/ml), and peak $\beta$-enolase ($>110$ ng/ml) concentration were defined as severe cases. There were 13 patients in each group.

**Results**

The mean (SD) serum concentration of $\beta$-enolase in the 20 normal subjects was $5.6 (3.1)$ ng/ml (range 1.6–16.7 ng/ml). Concentrations of $\beta$-enolase were normal in haemolysed serum samples. In the 26 patients with acute myocardial infarction the serum concentration of $\beta$-enolase correlated well with serum creatine kinase activity ($y = 0.41x + 34.67$, $r = 0.813$).

The peak concentration of $\beta$-enolase in serial serum samples from patients with acute myocardial infarction was significantly raised (4–22 times higher than the normal value) (figs 1 and 2). $\beta$-enolase concentration peaked 12–14 hours after an acute attack of chest pain lasting more than an hour and then gradually fell during recovery. Changes in serum $\beta$-enolase concentration resembled changes in serum creatine kinase activity in acute myocardial infarction (figs 1 and 2). In 13 mild cases the increase in $\beta$-enolase concentration occurred before the increase in creatine kinase activity and the rise and fall in $\beta$-enolase concentration were steeper than those of creatine kinase activity (fig 1a and b). Comparison of the $t$ values of the gamma variate function for the changes in $\beta$-enolase concentration and creatine kinase activity confirmed these data. In 13 severe cases the difference in the increase in $\beta$-enolase concentration and creatine kinase activity showed a
β-enolase and myocardial infarction

![Graph](image)

**Figure 2** Mean (SE) serum β-enolase concentrations and creatine kinase activity in 13 patients with severe acute myocardial infarction. Increases in creatine kinase activity and β-enolase concentrations were significantly higher than normal from 4 to 34 hours and from 2 to 34 hours respectively after the onset of acute myocardial infarction. Normal values are shown by the shaded area.

Although the mean value in patients with acute hepatitis was twice that in normal subjects, most values came within the limits of normal variation (mean ± 2SD). We found a significant (p < 0.05) cor-

**Table** Mean (SE) β-enolase concentration in normal subjects before and after treadmill exercise and in patients with cardiovascular, hepatic, or renal disease

<table>
<thead>
<tr>
<th></th>
<th>β-enolase concentration (ng*</th>
<th>ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects (20)</td>
<td>5.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Treadmill exercise test (7):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>4.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Angina pectoris (10)</td>
<td>5.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure (8)</td>
<td>4.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Idiopathic cardiomyopathy (3)</td>
<td>2.1 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Acute hepatitis and liver cirrhosis (10)</td>
<td>10.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Renal failure (10)</td>
<td>3.3 ± 0.5</td>
<td></td>
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</tbody>
</table>

*β/β enolase equivalent.*
relation between the peak concentration of $\beta$-enolase and ejection fraction (fig 4) and infarct size measured by scintigraphy both in mild and severe cases.

**Discussion**

Many investigators have reported an increase in the activity of several enzymes in the serum of patients with acute myocardial infarction and have confirmed a relation between peak activity of the serum enzyme (particularly creatine kinase) and the size of the myocardial infarct. The activity of glycolytic enzymes, such as pyruvate kinase and enolase was also reported to be increased in acute myocardial infarction. But serum $\beta$-enolase had not been evaluated as a marker in acute myocardial infarction. We used a highly sensitive assay for $\beta$-enolase. We demonstrated that the concentration of serum $\beta$-enolase rose in an early phase of acute myocardial infarction. The increase correlated well with changes in creatine kinase activity and the increase in $\beta$-enolase was specific for acute myocardial infarction; it did not occur in patients with other kinds of heart disease who did not have concurrent neuromuscular disorders in which serum concentrations of $\beta$-enolase are raised.

Serum concentrations of $\beta$-enolase were normal in patients with acute hepatitis and renal failure, which suggests that the increase that we found in patients with myocardial infarction was not caused by a decrease in the degradation rate of this enzyme. We believe that release from necrotic heart muscle rather than from blood cells accounts for the increase in $\beta$-enolase because $\beta$-enolase is not found in blood cells.

The peak concentration of $\beta$-enolase paralleled that of creatine kinase MB and myoglobin. The $\beta$-enolase assay system may be more simple and sensitive than assays for creatine kinase and myoglobin. A very early and rapid increase in concentration after the onset of infarction and a quick clearance may make $\beta$-enolase a better marker than creatine kinase for the early diagnosis of infarction and the diagnosis of a second episode of necrosis occurring more than a day after the first episode. In cases of acute myocardial infarction in which creatine kinase activity is not very high it may be difficult to detect myocardial infarction, and in such patients the assessment of serial changes in serum $\beta$-enolase concentration may be more useful for the early detection of acute myocardial infarction.

The time required to assay $\beta$-enolase concentrations can be reduced to less than an hour if the reaction temperature is raised and the size of the polystyrene balls is increased. This enzyme immunoassay is cheaper than a radioimmunoassay and does not require radioactive reagents.

Serum $\beta$-enolase concentration has been successfully used to estimate the size of myocardial infarcts produced in dogs by experimentally induced myocardial infarction (unpublished observations).

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**References**

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**Fig 4** Correlation between peak serum concentrations of $\beta$-enolase and ejection fraction in patients with acute myocardial infarction (mild and severe cases).
\[ \beta \text{-enolase and myocardial infarction} \]


Serum beta-enolase in acute myocardial infarction.

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