Serum levels of creatine kinase-mb in obese subjects attending university of Port Harcourt teaching hospital

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Introduction

Obesity has become an important health issue globally with its negative impact on health and especially in its contribution to cardiovascular disease morbidity and mortality.1 Cardiovascular diseases are the second most common cause of death in adults in sub-Saharan Africa second to infectious diseases.2 Obesity is a major risk factor for several of these Cardiovascular diseases.3 Obesity increases adverse cardiac events in many ways.3,4 Many adipokines and other chemical mediators like tumour necrotic factor – alpha, interleukin 6, plasminogen activator inhibitor, oestrogens, leptin, angiotensinogen, retinal binding protein and insulin like growth factor -1 are present in increased concentrations in obese patients. These have various adverse effects on the cardiovascular system by creating a pro-inflammatory and prothrombotic state as well as causing endothelial damage and vascular hypertrophy.3,4

Cardiac markers are biomarkers used to evaluate heart function.5 They are often discussed in the context of myocardial infarction. Most of the early markers identified were enzymes, as a result, the term cardiac enzymes is sometimes used.6 However, not all of the cardiac markers currently used are enzymes, e.g. troponin is not an enzyme but a cardiac marker, therefore a useful clinical laboratory test for detecting and predicting acute myocardial event or minor myocardial injury.6

Creatine kinase-MB (CK-MB) has the most specificity for cardiac muscle even though it accounts for only 3-20% of the total Creatine Kinase activity in the heart.7 CK-MB is a valuable tool for the diagnosis of acute myocardial infarction because of its relatively high specificity for cardiac injury.9 Extensive experience with CK-MB has established it as a benchmark and gold standard for other cardiac markers. However it takes at least 4-6 hours from onset of chest pain before CK-MB activities increase to significant levels in the blood. Peak levels occur at 12-24 hours, and serum activities usually return to baseline levels with 2-3 days.9

During recent years, CK-MB activity assays have been increasingly replaced by CK-MB mass assays that measure the protein concentration of CK-MB rather than its catalytic activity. To increase specificity of CK-MB for cardiac tissue, it has been proposed that a ratio (relative index) of CK-MB mass/CK activity can be calculated. If this ratio exceeds 3, it is indicative of acute myocardial infarction rather than skeletal muscle damage.9

The present study seeks to determine the serum concentration of CK-MB in the obese (with BMI > or =30) aged 20-40 years

Methods and Material

Subjects

One hundred and eighty five (185) subjects made up of staff of the University of Port Harcourt Teaching Hospital, (UPTH) as well as patients attending the General Out Patients Clinics and Metabolic Clinics of UPTH (with average age 34.74±4.33 and BMI of 32.04±1.58) were used while the control will consist of age (Average age 34.14±3.45 and BMI of 22.46±1.65) matched apparently healthy individuals who are not obese, with BMI less than 25.

The inclusion Criteria for the study include (i) Age between 20 and 40 and (ii) Subjects who have given their consent in writing while Exclusion Criteria include (i)Subjects less than 20 years(to exclude adolescent fat)(ii)Subjects more than 40 years(to exclude middle age fat spread(iii)Subjects with diagnosed cardiac, liver or renal disease( excluded by history, as indicated in the questionnaire)(iv)Diabetics (excluded by fasting plasma glucose) and (v) Hypertensives.

Sample size determination

Sample size is determined using the formula

\[ n = \frac{Z^2 pq}{d^2} \]

Where \( n = \) sample size minimum
\( Z = 95\%\) confidence interval = 1.96
\( p = \) proportion of the target population 0.14
\( q = 1.0 - p \)
\( d = \) with, degree of accuracy (95% interval) = 0.05%

Therefore \( n = \frac{(1.96)^2 \times 0.14 \times 0.86}{(0.05)^2} \)

\( = 185 \) participants

Sample Collection and Procedures

A 5 ml fasting sample was collected by venepuncture from each subject with 1ml decanted into labeled fluoride oxalate bottles for glucose analysis and the remaining put into a plain
bottle for analysis of the Creatine kinase-MB. Serum urea and creatinine was also done to exclude renal pathology since renal failure leads to fluid retention which can give an altered BMI. The Serum was separated from cell by centrifugation at 2,500g for 15 minutes and samples for assay analyzed as soon as possible.

### Biochemical analysis

CK–MB estimation was done by enzyme linked immunosorbsent assay using a CK-MB ELISA assay reagent produced by Diagnostic Automation Inc, USA.

**Principle:** The immobilization takes place at the surface of a microplate well through the interaction of stepvadin coated on the wall and exogenously added biotinylated monoclonal anti CK-MB antibody. upon mixing biotin labeled antibody, the enzyme labeled antibody and a serum containing the native antigen.Reaction results between the native antigen and antibodies without completion or stearic hindrance to form a soluble sandwich complex. Simultaneously the complex is deposited to the well through the high affinity reaction of stepvadin and biotinylated antibody. After equilibrium is attained the antibody fraction is separated from unbound antigen by decantation. The enzyme activity in the antibody bound fraction is directly proportional to the native antigen concentration.

**Procedure:** Twenty five microlitre (25ul) of appropriate calibrators, controls and samples were pipette into assigned well. One hundred microlitre (100ul) of CK-MB enzyme reagents was added to each well and swirled for 30 seconds to mix. It was covered with a plastic wrap and incubated for 15minutes at 25°C. The content of the microplate was decanted and blotted dry with absorbent paper. The content was washed thrice using three hundred microlitre (300ul) of wash buffer while One hundred microlitre (100ul) of substrate was added to the wells and incubated for 15 minutes at 25°C. Fifty microlitre(50ul) of stop solution was added to each well, mixed gently for 20 seconds and reading using a micro plate reader at 450nm. The concentrations of the unknown was extrapolated from the standard curve using the concentrations of calibrators.

### Statistical Analysis

The data generated from the study will be analyzed using the statistical package for social sciences (SPSS) version 17.0. Values will be expressed as mean ± standard deviation. The student t-test will be used to compare mean differences between obese and control subjects.

**Result**

The CK Mb of 2.91±0.29 in control was significantly different from 2.76±0.30 in obese subjects as shown in Table 1 below.

### Table 1: CK Mb in obese subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Obese</th>
<th>T</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK Mb</td>
<td>2.91±0.29</td>
<td>2.76±0.30</td>
<td>3.695</td>
<td>0.000</td>
</tr>
</tbody>
</table>

The male control has CK Mb concentration of 2.88±0.31 while the obese male had CK Mb concentration of 2.78±0.29. Female obese had CK Mb concentration of 2.86±0.24 while the control concentration was 2.73±0.29 as shown in table 2 below.

### Table 2: CK Mb in different gender of obese

<table>
<thead>
<tr>
<th>Gender</th>
<th>Control</th>
<th>Obese</th>
<th>T</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2.88±0.31</td>
<td>2.78±0.29</td>
<td>1.795</td>
<td>0.070</td>
</tr>
<tr>
<td>Female</td>
<td>2.73±0.29</td>
<td>2.86±0.24</td>
<td>2.649</td>
<td>0.011</td>
</tr>
</tbody>
</table>

The CK Mb concentration of controls for Obese class 1 in age group 20-25years was 2.88±0.30 while it was 2.80±0.42, 2.85±0.32 and 2.91±0.22 in age groups 26-30years, 31-35years and 36-40years while it was 2.76±0.24, 2.70±0.14, 2.74±0.22 and 2.78±0.38 respectively for obese class 1 in age groups 26-30years, 31-35years and 36-40years. The Ck Mb concentration of controls for Obese class 2 in age group 20-25years was 0.00±0.00 while it was 2.79±0.42, 2.85±0.32 and 2.91±0.22 in age groups 26-30years, 31-35years and 36-40years while it was 0.00±0.00, 2.92±0.19, 2.85±0.24 and 2.82±0.35 respectively for obese class 2 in age groups 26-30years, 31-35years and 36-40years. The Ck Mb concentration of controls for Obese class 3 in age group 20-25years was 0.00±0.00while it was 0.00±0.00, 2.86±0.32 and 2.91±0.23 in age groups 26-30years, 31-35years and 36-40years while it was 0.00±0.00, 0.00±0.00, 2.56±0.19 and 2.48±0.30 respectively for obese class 3 in age groups 26-30years, 31-35years and 36-40years as shown in table 3 below.

### Table 3: CK-MB in different classes of obese at different age groups

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Control</th>
<th>Class 1 Obese</th>
<th>P Value</th>
<th>Control</th>
<th>Class 2 Obese</th>
<th>P Value</th>
<th>Control</th>
<th>Class 3 Obese</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25</td>
<td>2.88±0.24</td>
<td>2.76±0.24</td>
<td>0.221</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>26-30</td>
<td>2.80±0.22</td>
<td>2.70±0.14</td>
<td>0.442</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>31-35</td>
<td>2.85±0.32</td>
<td>2.74±0.22</td>
<td>0.186</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>36-40</td>
<td>2.91±0.22</td>
<td>2.78±0.38</td>
<td>0.122</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Discussion**

Cardiac markers help Physicians to assess acute coronary syndrome and to identify and manage high risk patients. The national academy of Clinical Biochemistry (NACB) recommends that 2 biochemical markers be used for routine diagnosis of acute myocardial infarction. One should be a marker that is elevated in the blood after a few hours and the other a marker that remain elevated in blood for several days. Myoglobin and CK-MB appear early in the blood but also disappear in blood after two days. Troponin is present 8hours to 10 days after acute myocardial infarction when the actual time of myocardial episode cannot be ascertained.

The result of this study showed that CKMB concentration was found to be lower in the Obese subjects. The result of this study further showed that CKMB concentration in Obese male and Obese females were not different from their respective controls. The CKMB is found mainly in the heart muscle unlike CKMM which is found more in large quantities in the skeletal muscle.In a related study during an exercise induced study, the total CK was found to be significantly higher in Obese subjects compared with controls, while the CKMB was not significantly different between the two groups. It was agreed that the increase in the total CK of the Obese was due to the stress of the total body musculature.

**Conclusion**

The study has shown that CKMB concentration was lower in Obese. Therefore obese subjects should have their CKMB concentration determined to ascertain their cardiac nature.

**References**