



RESEARCH ARTICLE

SERUM LEVELS OF MYOGLOBIN IN OBESE SUBJECTS ATTENDING UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL

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ABSTRACT

**Background:** The study was carried out to determine the serum concentrations of Myoglobin in obese subjects attending University of Port Harcourt Teaching Hospital.

**Methods:** One hundred and Eighty Five (185) obese subjects of BMI >30 had their Myoglobin determined and compared with 160 age and sex matched controls.

**Results:** The myoglobin concentration of 14.33+15.61 in Obese subjects was higher than 13.59+3.69 obtained in the Control (P>0.05).

**Conclusion:** The result of this study suggests that serum level of Myoglobin is increased in Obese subject.

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INTRODUCTION

Obesity is a chronic disease which develops gradually and is fuelled by environmental factors such as nutrition, physical inactivity, drugs and genetic influence (Olshanky *et al.*, 2005). Obesity is a chronic life style disease which develops gradually over several years and fully established confers severe consequences on physical and psychological health (National Heart, 1998) Obesity and overweight affects approximately 1.7 billion people worldwide and over 135 million people are in Europe (Mokdad *et al.*, 2001). Globally, it is estimated that about 300 million people are obese (IASO, 2003), and in the United States of America, obesity affects over 60 million people which translate to 34% (Flegal *et al.*, 2002). It is also estimated that 325,000 people in the United States die annually from obesity related health complications (Kuczmarski *et al.*, 1994). It is for these reasons, that obesity has become a major health issue for public discourse in most developed western countries. WHO (WHO, 2010) classified obesity as Normal Weight(BMI of 18.5 – 24.9kg/m<sup>2</sup>),Over Weight(BMI of 25.0 – 29.9kg/m<sup>2</sup>),Grade 1 obesity (BMI of 30.0 – 34.9kg/m<sup>2</sup>),Grade 2 obesity(BMI of 35.0 – 39.9kg/m<sup>2</sup>) and Grade 3 (morbid obesity) BMI of 40kg/m<sup>2</sup> and above. BMI does not directly measure body fat, but correlates with the amount of body fat.

Athletes may have a BMI that identifies them as obese, though in the real sense they do not have excess body fat (National Institute of Health and Clinical Excellence, 2006). However, recent reports from various studies indicate an increasing prevalence (Copper *et al.*, 1997; Akpa *et al.*, 2006). Some recent studies are documented with alarming prevalence rates of 71.6% in females and 50.5% in males in a population of hypertensive patients, figures similar to or higher than that from developed countries (Amodu *et al.*, 2005). In sub-Sahara Africa, available data shows an increasing prevalence in obesity.

More than 25% of urban men and 50% of urban women in Cameroun were either overweight or obese (Yeong *et al.*, 2006). About 6.5% of men and 19.5% of women were obese using body mass index (12). Body mass index provided the highest prevalence of obesity in men and the waist-to-hip ratio the lowest prevalence (3.2%) (Yeong *et al.*, 2006). Among women, using waist-to-hip ratio and waist circumference yielded the highest prevalence of obesity (28%) and body mass index the lowest (19.5%) (Yeong *et al.*, 2006). Thus, obesity (a disease) previously thought to have low prevalence in Nigeria because of its association with wealth and affluence has risen over the last decade or more to levels that now constitute an epidemic threat. Obese individuals have problems with respiratory system and sometimes with the cardiovascular system.

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Cardiac markers are biomarkers used to evaluate heart function (Rao *et al.*, 1999). 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 (Rao *et al.*, 1999). The myoglobin is an oxygen binding protein of cardiac and skeletal muscle with a molecular mass of 17,800 Da (Fred *et al.*, 2006). It is a low molecular weight protein and its cytoplasmic location probably accounts for its early appearance in the circulation following muscle (heart or skeletal) injury (Fred *et al.*, 2006). There is no difference in the myoglobin protein localisation in the heart and the skeletal muscle (Fred *et al.*, 2006). Serum myoglobin methods are unable to distinguish the tissue of origin because the protein is identical (Fred *et al.*, 2006). The value of myoglobin assay in myocardial infarction is its early appearance in serum after myocardial infarction (Karras *et al.*, 2001). Since the interval between onset of symptoms and clinical presentation is variable, it has been suggested that multiple biomarkers are needed to enable detection of myocardial infarction in patients who present early or late after the onset of pain (Wendi *et al.*, 2010). Currently, myoglobin most effectively fits the role of an early marker (Karras *et al.*, 2001). A rise in myoglobin is detectable in blood as early as 1 to 2 hours after symptom onset and is highly sensitive for myocardial infarction diagnosis (Karras *et al.*, 2001). If myoglobin concentration remains within the reference range 8 hours after onset of chest pain, acute myocardial infarction can essentially be ruled out. Myoglobin is significantly more sensitive than CK and CK-MB activities during the first hours after chest pain onset. It starts to rise within 1-4 hours and detectable in essentially all acute myocardial infarction patients between 6 and 9 hours from chest pain onset, returning to baseline level between 18-24 hours of onset (Karras *et al.*, 2001). Thus serum myoglobin is not cardiac specific and patients with renal failure or injury, trauma or diseases involving the skeletal muscle can have abnormal concentration in the absence of myocardial infarction (Wendi *et al.*, 2010). There is now a consensus that myoglobin should be used as an early marker, but should be in conjunction with specific marker such as troponin I or T (Fred and Allan, 2006). The present study seeks to determine the serum concentration of Myoglobin in the obese (with BMI > or =30) aged 20-40 years

## MATERIALS AND METHODS

### 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 were used while the control will consist of age matched apparently healthy individuals who are not obese, with BMI less than 25. The inclusion Criteria for the study include (i) Aged 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 (17)

$$n = \frac{Z^2 pq}{d^2}$$

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%

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

$$= 185 \text{ participants}$$

### 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 Myoglobin. 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

Myoglobin estimation was done by enzyme linked immunosorbent assay using a myoglobin ELISA assay reagent produced by Diagnostic Automation Inc, USA.

**Principle:** This is based on the principle of a solid phase enzyme linked immunosorbent assay which utilizes a unique monoclonal antibodies directed against distinct antigenic determinant on the myoglobin molecules. Mouse monoclonal antimyoglobin antibody is used for solid phase immobilisation (on the microtitre wells).A goat antimyoglobin antibody is in the antibody enzyme(Horseradish peroxidase) conjugate solution. The sample reacts simultaneously with the two antibodies resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme linked antibodies.

**Procedure:** A 1 in 10 dilution of sample and controls were done prior to start of the analysis. Twenty microlitre (20ul) of appropriate calibrators, controls and samples were pipette into assigned well. Two hundred microlitre (200ul) of enzyme conjugate reagents was added to each well and swirled for 30 seconds to mix. It was covered with a plastic wrap and incubated for 45minutes at 25°C.The content of the microplate was decanted and blotted dry with absorbent paper. The content was washed five times using distilled water while One hundred microlitre (100ul) of tetramethyl benzidine (TMB) was added to the wells, mixed and incubated for 20minutes at 25°C. One hundred microlitre (100ul) of stop solution was added to each well, mixed gently for 30 seconds and read using a micro plate reader at 450nm.

**Table 1. Myoglobin in obese subjects**

Parameter	Control	Obese	t	P Value
Myoglobin	13.59±3.69	14.33±15.61	-0.400	0.666

**Table 2. Myoglobin in different gender of obese**

Gender	Control	Obese	t	P
Male	17.11±13.35	16.19±20.50	0.295	0.709
Female	12.03±3.59	13.46±3.13	2.290	0.026

**Table 3. Myoglobin in different classes of obese at different age groups**

Age Group	Control	Class 1 Obese	P Value	Control	Class 2 Obese	P Value	Control	Class 3 Obese	P Value
20-25	12.38 ± 4.04	11.13 ± 3.99	0.405	0.00 ± 0.00	0.00 ± 0.00	1.000	0.00 ± 0.00	0.00 ± 0.00	1.000
26-30	24.68 ± 41.38	10.65 ± 1.91	0.255	24.68 ± 41.38	10.96 ± 0.79	0.275	0.00 ± 0.00	0.00 ± 0.00	1.000
31-35	12.92 ± 3.32	12.92 ± 3.70	1.000	12.92 ± 3.32	25.63 ± 23.83	0.104	12.93 ± 3.32	40.55 ± 56.86	0.137
36-40	24.39 ± 25.58	12.45 ± 4.55	1.000	24.39 ± 25.58	10.73 ± 1.56	0.115	24.39 ± 25.58	11.42 ± 1.15	0.146

The concentrations of the unknown was extrapolated from the standard curve using the concentrations of calibrator

### 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.

### RESULTS

The myoglobin concentration of 14.33±15.61 in obese subjects was not significantly different from 13.59±3.69 in the control as shown in Table 1.

The male control has Myoglobin concentration of 17.11±13.35 while the obese male had Myoglobin concentration of 16.19±20.50. Female obese had Myoglobin concentration of 13.46±3.13 while the control concentration was 12.03±3.59 as shown in Table 2.

The myoglobin concentration of controls for Obese class 1 in age group 20-25years was 12.38 ± 4.04 while it was 24.68 ± 41.38, 12.92 ± 3.32 and 24.39 ± 25.58 in age groups 26-30years, 31-35years and 36-40years while it was 11.13 ± 3.99, 10.65 ± 1.91, 12.92 ± 3.70 and 12.45 ± 4.55 respectively for obese class 1 in age groups 26-30years, 31-35years and 36-40years.

The myoglobin concentration of controls for Obese class 2 in age group 20-25years was 0.00 ± 0.00 while it was 24.68 ± 41.38, 12.92 ± 3.32 and 24.39 ± 25.58 in age groups 26-30years, 31-35years and 36-40years while it was 0.00 ± 0.00, 10.96 ± 0.79, 25.63 ± 23.83 and 10.73 ± 1.56 respectively for obese class 2 in age groups 26-30years, 31-35years and 36-40years.

The myoglobin concentration of controls for Obese class 3 in age group 20-25years was 0.00 ± 0.00 while it was 0.00±0.00, 12.93 ± 3.32 and 24.39 ± 25.58 in age groups 26-30years, 31-35years and 36-40years while it was 0.00 ± 0.00, 0.00±0.00, 40.55±56.86 and 11.42±1.15 respectively for obese class 3 in age groups 26-30years, 31-35years and 36-40years as shown in Table 3.

### DISCUSSION

The result of this study showed no significant different in serum concentration of Myoglobin in obese and controls. Myoglobin though have good sensitivity (90-100%) (Karras and Kane, 2001) if myocardial infarction occurs within 4-12 hours has poor specificity because of large the large quantity of the protein in skeletal muscle. Specific shortfalls with the use of Myoglobin apart from its reduced heart tissue specificity is that its diagnostic window ends after 24 hours thus late presentation can lead to a misdiagnosis. This is the reason myoglobin is actually measured as a negative predictor of acute myocardial infarction (Bhatti *et al.*, 2001). There was significant difference in Myoglobin concentrations of Female Obese and their control. This may be as a result of more fat than muscle in females. Myoglobin has the most effective role as an early marker as a rise in myoglobin concentration in the blood is usually detected as early as 1 to 2 hours after the onset of symptoms and is highly sensitive for diagnosis of myocardial infarction. The result of this study showed no significant different in serum concentration of Myoglobin in all the age groups in obese and controls. Some studies (De Lemos *et al.*, 2010; Sanders *et al.*, 2011) has shown that cardiac markers are elevated in obese subjects suggesting that an Obese patient with coronary syndrome will require a different reference range for the diagnosis of acute coronary syndrome.

### Conclusion

The result of this study showed no significant different in serum concentration of Myoglobin in obese and controls.

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