COMPARATIVE EFFECT OF SIX MINUTES WALK TEST ON OXIDATIVE STRESS MARKER AND OXYGEN SATURATION IN MALE AND FEMALE YOUNG ADULTS.

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ABSTRACT

The six minute walk test is a submaximal cardiopulmonary functional exercise which can be used to establish the level of functional impairment of an individual. This study was aimed at determining the effect of six minute walk test on oxidative stress marker (catalase) and oxygen saturation in males and females. One hundred and four (104) subjects were recruited for this study, in which they were made up of 52 males and 52 females. The subjects were made to sit and rest for 10 minutes located near the starting point and their pre-walk oxygen saturation was measured using the pulse oximeter. They were then instructed to walk back and forth as fast as possible for six minute on a 30 meter walkway. After the six minute walk, the distance was measured and post walk oxygen saturation was measured. Then blood samples were taken after the walk from the subjects using 5ml syringe and transferred into a plain sample bottle. Catalase activity was determined by spectrophotometric method. Statistical analysis was done using graph pad prism version 8.1. Data were presented as mean $\pm$ SEM (standard error of mean). Unpaired student t- test was used to compare the mean of test and control values. $p<0.05$ was considered as statistically significant. The results showed no significant statistical difference between the pre- and post-walk oxygen saturation and catalase levels. It was also observed that there was no significant difference in oxygen saturation and catalase levels when the male was compared with the female subjects. Conclusively, six-minute walk test does not induce any significant reduction in oxygen saturation nor in oxidative stress in the male compared with the female subjects.

Keywords: Six – minute walk test, Oxidative stress marker, Oxygen saturation, Male, Female, Young adults.

Introduction

In the 1960s, Balke created a straightforward walk test with a set amount of time (Balk, 1963; Guyatt *et al*., 1985), which Cooper subsequently changed to a twelve minute walk test. A six minute walk test (6MWT), which is simple to administer, better tolerated and more representative of daily activities of living (Solway *et al*., 2001), was developed as a result of the twelve minute walk test (12MWT) being too exhausting when performed in patients with respiratory and cardiac problems (Butland *et al*., 1982).

The six-minute walk test is an important research study that offers functional, therapeutic response and prognostic data that is useful in the treatment of patients with respiratory and cardiac illnesses (Holland *et al*., 2014). It is extensively used because it provides an integrated picture of the cardiovascular and musculoskeletal response to exercise and is straightforward and reproducible as it

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is tolerable and safe (Holland *et al*., 2014). In addition to helping patients with diffuse parenchyma lung disorders and pulmonary arterial hypertension, it is particularly helpful in diagnosing and monitoring chronic obstructive pulmonary disease (COPD) (ATS committee, 2002). The six-minute walk test's strongest application is for monitoring how patients with moderate to severe heart or lung disease respond to therapeutic therapies. In addition to being used to assess patients' functional state on a single occasion, the six-minute walk test has also been used to predict morbidity and death (ATS committee, 2002).

The primary physiological phenomena pertinent to the test are the interactions between the heart and lungs during exercise. The neurological and musculoskeletal systems are also involved in this process, especially when it comes to the process of matching minute ventilation to cardiac output in response to changing levels of exercise intensity. This occurs through reflex responses that modify the cardiac and pulmonary activity to the levels of oxygen consumption required by the degree of exertion. Exercise tolerance will be reduced by impairment of any of these systems: the cardiovascular, pulmonary, neurological, or musculoskeletal systems (Santos *et al*., 2016).
It is generally established that oxidative stress has a role in the etiology of illnesses associated with a sedentary lifestyle, such as atherosclerosis, hypertension, diabetes mellitus, ischemic disorders, and cancer. Because oxygen free radicals destroy biological components including lipids, proteins, and DNA. Oxidative stress has been deemed hazardous (Yoshikawa & Naito, 2002).
It has been reported that gender is associated with differences in oxidative stress. Under physiological conditions, females appear to be less susceptible to oxidative stress. This may be due to the antioxidant properties of estrogen, gender differences in NADPH-oxidase activity or other mechanism(s) yet to be defined (Kander *et al*., 2017). Association between gender and oxidative stress is important because oxidative stress is implicated in many diseases that present differently in males and females.

It was reported that oxidative stress was higher in male rats than female rats (Barp *et al*., 2002). Another study showed that *in vivo* biomarkers of oxidative stress were higher in young men than in women of the same age (Ide *et al*., 2002). Similarly, it was observed that reactive oxygen species (ROS) production was higher in the vascular cells from males than in the cells from females (Matarresse *et al*., 2011). In addition, clinical and experimental data suggested a greater antioxidant potential in females over males (Bhatia *et al.,* 2012). Catalase activity levels were found to be the same between genders in the brain, heart, lung and heart, but higher in the female kidney (Chen *et al*., 2011). However, some studies showed no difference in catalase activity levels between males and females (Barp *et al*., 2002; Ide *et al*., 2002; Gomez-Perez *et al*., 2011).
Oxygen saturation measures how much hemoglobin is bound to oxygen (Hafen & Sharma, 2022). 1.36 mL of oxygen may be carried by each gram of haemoglobin (Corsini *et al*., 2022).

The sex difference in maximal oxygen consumption or VO_2 max, a crucial factor in determining aerobic capacity, has been predominantly blamed for the male advantage in endurance-exercise performance. When expressed as mL/kg/min, even the most elite women have VO_2 max values that are almost 10% lower than those observed in males with comparable elite rank (Bassett, 2002). Because exercising muscles require more oxygen, whole-body oxygen consumption (VO_2) rises as exercise intensity increases (Coyle, 1999). Women often have smaller hearts, lungs, and lower hemoglobin mass than males, restricting

their capability to supply oxygen to the working muscles, which limits their ability to meet the high oxygen demand of their male counterparts (Coyle, 1999).

Due to these varied views, this study is therefore aimed at comparing the effect of six – minute walk test on oxidative stress marker and oxygen saturation in males and females

Experimental

Materials used in the study

Stopwatch, two small Cones to mark the turn-around point, Stadiometer, Pulse Oximeter, Measuring tape for distance, Plain sample bottle, Syringe (5ml), Cotton wool, Methylated spirit, Gloves and Tourniquet were among the materials used in this study.

Study population

A total of 104 subjects were recruited for this study. They were within the ages of 17- 27 years.

Study area

This research was conducted in the University of Benin Campus.

Inclusion criteria

Following ethical approval and protocols in research, apparently healthy (52) males and (52) females students were recruited for this study. Prior to the onset of the study; all participants completed a biostatistics questionnaire to ensure suitability and provided informed consent after full study details were disclosed.

Exclusion criteria

Subjects with medical conditions such as unstable angina, myocardial infarction, Diabetes mellitus, known respiratory diseases, drug allergy, and recent thoracoabdominal surgery were excluded from this study. Also excluded were chronic alcoholics and smokers.

Six minute walk test procedure

The six minute walk test was performed indoor, along a long, flat, straight enclosed corridor with a hard surface that is seldom travelled with a walking course of 30 meter in length. The subjects were made to sit to rest for 10minutes, located near the starting point. The subject height and weight was measured using the stadiometer. Initial oxygen saturation was measured using pulse oximeter, with the subject sitting in a relaxed and upright position after prior explanation of the procedure has been given to the subjects' satisfaction. The stopwatch was set to 6 minute and the subject was position at the starting line. The subject was then instructed to walk as fast as possible at their own pace back and forth at a distance of 30 meters long for 6 minute. Once 6 minute elapses the distance walked by the subject was measured using the tape and the subjects post walk oxygen saturation was measured using the pulse oximeter, with the subjects seated in a relaxed and upright position.

Measurement of oxygen saturation

Oxygen saturation was measured using the pulse oximeter. The subjects were made to sit quietly for 10 minutes. Then the subjects were made to sit in a relaxed and upright position after prior explanation of the procedure has been given to the subjects' satisfaction. The pulse oximeter was then clipped to the end of the index finger that doesn't have nail polish or an artificial nail on, with their nail facing up. The subjects were then instructed to hold their finger still and below the level of their heart. After a few seconds a steady number for the oxygen level was observed and recorded appropriately.

Sample collection (serum assay)
5ml of blood sample was collected and transferred into a 5ml plain sample bottle. The blood sample was analyzed in the chemical pathology laboratory of the University of Benin Teaching Hospital, Benin City, Edo State.

Method of analysis
Following the collection of blood into the plain sample bottle, it was stand at 25°c for 30 minute to clot blood. Then the blood tube was centrifuged at 2000g for 15minute at 4°C. The serum was extracted preserved on ice for detection. Antioxidant enzyme assay test was done by conventional method. Antioxidant enzyme called Catalase was used to detect the enzyme Catalase in bacteria by differentiating staphylococci (Catalase - positive) from streptococci (Catalase - negative).

Estimation of catalase activity
Catalase was determined by spectrophotometric assay at OD 405nm (Cohen *et al.,* 1970).

Statistical analysis
All data were analyzed statistically using Graph pad prism version 8.1. Results were presented as mean ± SEM (standard error of mean). Unpaired student t- test was used to compare the mean of test and control values. $p < 0.05$ was considered statistically significant.

Results

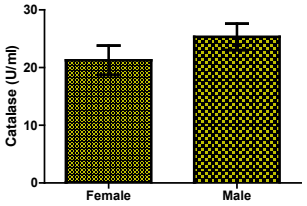


Fig.1: Effect of functional exercise on catalase in male and female young adult individuals
There was statistical increase though not significant in catalase level of male gender compared with female gender (p >0.05).

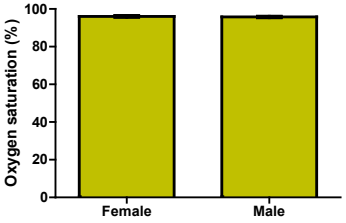


Fig.2: Effect of functional exercise on oxygen saturation in male and female adult young individuals. *There was no significant change in male gender oxygen saturation compared with female gender (p >0.05).*

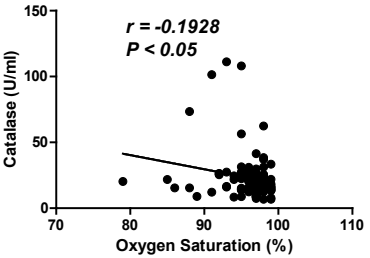


Fig.3: Relationship between catalase and the oxygen saturation by healthy adult individuals
There was significant negative correlation between catalase and oxygen saturation in healthy adult individuals.

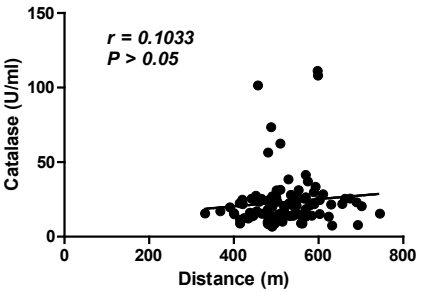


Fig.4: Relationship between catalase and the distance covered by adult individuals.
There was no significant correlation between catalase and distance covered (p >0.05).

Discussion

Figure 1 shows no statistical significance ($p>0.05$) in the levels of oxygen saturation and catalase in both the male and female subjects when compared. This agrees with the work done by Wang *et al.* (2023), who documented that physical exercise has no statistically significant effect on the level of catalase due to adaptive response of antioxidant enzyme expression to the exercise which was regulated by the dose of the physical exercise. This is contrary to the view of Lu *et al.* (2021) who observed a statistically significant increase in catalase level, but it was related to the duration of the exercise being more than 30 minutes and VO_2 max is up to 70% or more and also indicated that young age (16 – 65 years) had a statistically significant increase in the level of catalase. They documented that the intensity and duration of exercise can influence the body's response to oxidative stress (Lu *et al.,* 2021). This discrepancy may be due to individual's training status and the specific type and intensity of exercise done (Awang Daud *et al.,* 2022, Lu *et al.,* 2021).

Exponential rise of adrenaline and noradrenaline hormones with intensity is seen to be much faster than increment in exercise duration (Jacob *et al.,* 2004). Both adrenaline and noradrenaline hormones stimulate β-adrenergic receptors in the pancreas and increase glucagon secretion. Resultant high glucagon in plasma increases the blood glucose level through the mobilization of free fatty acid (FFA) from adipose tissue, mobilization of glucose from the liver, and an increase of gluconeogenesis (Awang Daud *et al.,* 2022).

Fig. 2 showed that functional exercise has no significant effect on oxygen saturation in males and females with a p-value of 0.6224. This is in agreement with the study done by Goodrich *et al.* (2018) that found no statistical significant difference in oxygen saturation between males and females attributing it to the possibility that individuals with high total hemoglobin mass (tHb) may experience more severe desaturation during exercise due to the presence of high blood volume. However, Reybrouck & Fagard (1999) observed that females have significantly higher value for mixed venous oxygen saturation which contributes to the lower arteriovenous oxygen difference to that of males; that could be as a result of their smaller muscle mass, lower capillary density and lower oxidative potential. The relationship between catalase and oxygen saturation in subjects used in this study was found to be negatively correlated which was significant (Fig. 3). This was in agreement with Radak *et al.* (2013) stating that this could be as a result of an increase in antioxidant response in an inverse relationship as catalase level increases, oxygen saturation decreases (Kinnula & Crapo, 2003). Fig. 4 showed the relationship between catalase and distance covered by subjects having a p-value of 0.1033 ($p >0.05$), which was not statistically significant. This observation agrees with the study done by Delrieu *et al.* (2021) that found no statistically significant changes in the level of catalase in relation to distance covered during exercise and this could be as a result of low intensity of exercise. It also implies that catalase level and activity is not dependent on distance covered and a determinant of submaximal exercise performance (ATS Committee, 2002).

Conclusion

The six minute walk test though a cardiopulmonary functional exercise had no significant impact on the level of catalase and oxygen saturation in males compared to females.

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