A STUDY ON

CHANGES IN OXIDATIVE STRESS MARKER LEVELS IN YOUNG ADULT MALES OF DIFFERENT GENOTYPE POST FUNCTIONAL EXERCISE

BY

IGIEMEH-LUKE STEPHANIE OCHUWA
BMS2101633

DEPARTMENT OF PHYSIOLOGY,
SCHOOL OF BASIC MEDICAL SCIENCES,
COLLEGE OF MEDICAL SCIENCES,
UNIVERSITY OF BENIN.

NOVEMBER, 2025.

CHANGES IN OXIDATIVE STRESS MARKER LEVELS IN YOUNG ADULT MALES OF DIFFERENT GENOTYPE POST FUNCTIONAL EXERCISE

BY

IGIEMEH-LUKE STEPHANIE OCHUWA

BMS2101633

A PROJECT WRITTEN IN THE DEPARTMENT OF PHYSIOLOGY AND SUBMITTED TO THE SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work on was carried out by **IGIEMEH LUKE STEPHANIE OCHUWA,** with the matriculation number: **BMS2101633** in partial fulfillment for the Award of Bachelor of Science Degree (B.Sc.) in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

______________________________ ______________________

IGIEMEH-LUKE STEPHANIE OCHUWA **Date**
Student

______________________________ ______________________

DR. E. O AIHIE **Date**
Project Supervisor

______________________________ ______________________

PROFESSOR O. K. UCHE **Date**
Head of Department

______________________________ ______________________

PROFESSOR S. A. ONASANWO **Date**
External Examiner

DEDICATION

I dedicate this work to God almighty for his grace, love and mercy throughout the course of this journey. I also dedicate this work to my lovely parents, Mr. and Mrs. Luke lgiemeh, my siblings for their unwavering support, to the rest of my family who have being like my second parents and lastly to all those who supported me during the course of this work.

ACKNOWLEDGEMENT

Firstly, I want to thank God Almighty for his guidance and strength throughout this journey. I am deeply grateful to my supervisor, Dr. E.O. AIHIE for his invaluable guidance and assistance in completing this project work.

My heartfelt thanks goes to my parents Mr. and Mrs. Luke Igiemeh for their unwavering love and support during the course of this study, to my grandma, Mrs. Loretta Ukpokodu for her prayers and encouragement. My profound gratitude also goes to my lovely aunts Mrs. Joan Ikhalea and Mrs. Juliana Ukpokodu and my uncle Mr. Clement Ukpokodu for their counsel, love and financial support.

Also, to my fellow project students under my supervisor; Gold, Nosa and Ose thank you all for your support, cooperation during our short time together.

TABLE OF CONTENT

LIST OF FIGURES

ABSTRACT

The six-minute walk test (6MWT) is a widely-used submaximal field walking test used to evaluate functional exercise capacity. It assesses the distance an individual can walk on a flat, hard surface in six minutes and the final distance is recorded in meters. This study was aimed to investigate changes in oxidative stress marker levels in young adult males of different genotypes post functional exercise at the University of Benin. Forty-four (44) healthy young adult males aged 18-29 years were recruited for this study. Oxidative stress arises from an imbalance between elevated reactive oxygen species (ROS) and insufficient antioxidant defenses Oxidative stress marker levels were measured pre and post-test. The analysis was done at the chemical pathology laboratory of the University of Benin Teaching Hospital, Benin City. The statistical analysis was done using Graph Pad Prism statistical package Version 8.1. The standard error of mean (SEM) was used in tables and graphs to display the results. The dependent and independent variable means were compared using the student t-test. $P<0.05$ was accepted as significant. The results showed that there was no significant change in the oxidative stress marker levels relative to genotype.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Oxidative stress is a part of the normal metabolic process, in which the cells continuously generate free radicals and nonradical derivatives of oxygen such as hydrogen peroxide (H_2O_2), superoxide, hydroxyl free radicals, and singlet oxygen. These chemical reactive molecules that contain oxygen are also called reactive oxygen species (ROS) (Zhou *et al.,* 2022). Although ROS are by-products of the normal oxygen-related metabolism and have roles in cell signaling and homeostasis (Huang and Rusanova, 2022), they could induce tissue oxidative damage in the absence of antioxidants (Reid, 2001). For human beings, ROS accumulate with the energy metabolism related to physical activities and induce oxidative stress when their production exceeds the neutralization capacity of human tissues (Xiang *et al.,* 2022). Physical activity triggers ROS and reactive nitrogen species (RNS) production, counteracted by enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (vitamins C and E) (Meng and Su, 2024). In most studies, researchers use indicators like aldehydes, particularly malondialdehyde (MDA), catalase

(CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), and glutathione peroxidase (GPx) to measure oxidative stress (Golabi *et al.,* 2022).

The ability of walking is a well-established index of the functional capacity and the quality of life (Cazzoletti *et al.,* 2022). The six-minute walk test (6MWT) is a widely-used submaximal field walking test to evaluate functional exercise capacity (Cox, 2017).The 6-min walk distance (6MWD) test is a quick, easy and inexpensive tool for measuring functional exercise capacity and it is very useful in elderly, frail patients or those with chronic diseases who are unable to perform maximal cycle ergometer or treadmill exercise tests (Cazzoletti *et al.,* 2022). The 6MWD test has advantages over laboratory-based tests on exercise tolerance since it more closely reflects the ability to perform daily life activities and does not require sophisticated and expensive equipment (Solway *et al.,* 2001).

1.2 Justification of study

This study investigated if the 6-Minute Walk function Test (6MWT) as a functional exercise have any effect on the oxidative stress marker level and to examine if the differences in genotype can explain stress adaptation and various exercise performance in healthy adult males.

1.3 Aim of Study

This study aimed to evaluate oxidative stress marker level before and after functional exercise (6-Minute Walk test) across healthy adult males of different genotypes.

1.4 Research Questions

Several research questions guided this study. Some of which includes:

i. Does the 6-Minute Walk test enable the expression of oxidative stress markers?
ii. Are the oxidative stress markers expressed more in individuals with a specific genotype compared to others?

1.5 Specific Objectives of Study

i. To access oxidative stress marker levels before and after the 6-Minute Walk test.
ii. To identify and compare oxidative stress responses across different genotypes.

1.6 Scope Of Study

The Scope of this study on the changes in oxidative stress marker levels in young adult males of different genotype post functional exercise will involve a comprehensive review of existing literature and original research. This Study will focus on the following areas:

i. Oxidative stress marker levels (SOD, CAT, MDA)
ii. Genotype

CHAPTER TWO

LITERATURE REVIEW

2.1 Exercise and Physical Activity

Physical activity is not only limited to sports but also includes walking, running, swimming, gymnastics, dance, ball games, and martial arts, the Physical Activity Guidelines for Americans, 2nd edition, provides information and guidance on the types and amounts of physical activity that provide substantial health benefits (Piercy *et al.,* 2018). Exercise is dose dependent, meaning that people who achieve cumulative levels several times higher than the current recommended minimum level have a significant reduction in the risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events (Kyu *et al.,* 2016). Benefits of physical activity have been reported for numerous outcomes such as mortality (Abell *et al.,* 2017), cognitive and physical decline (Anderson *et al.,* 2014), glycaemic control (Chastin *et al.,* 2019), pain and disability (Alanazi *et al.,* 2018), muscle and bone strength (Adsett *et al.,* 2015), depressive symptoms (Adamson *et al.,* 2015), and functional mobility and well-being (Abdin *et al.,* 2018). Overall benefits of exercise apply to all bodily systems including immunological (Sellami *et al.,* 2018), musculoskeletal (Hagen *et al.,* 2012), respiratory (Burton *et al.,* 2004), and hormonal (Kraemer and Ratamess, 2005).

2.2 Concept of Oxidative Stress

A free radical is a type of molecule that can exist independently and contain an unpaired electron in one of its atomic orbitals. This unpaired electron is what distinguishes a free radical from other molecules. Because of this unpaired electron, free radicals are highly reactive and can easily interact with other molecules (Pooja *et al.,* 2025). This starts a chain reaction, leading to widespread damage within cells. This damage can disrupt normal cellular functions and contribute to various diseases (Phaniendra *et al.,* 2015). Two of the most common and significant types of free radicals are oxygen-centered radicals, known as reactive oxygen species (ROS), and nitrogen-centered molecules, referred to as reactive nitrogen species (RNS) (Gutteridge and Halliwell, 2018). The generation of highly reactive ROS is an important feature of the normal cellular system such as fertilization, ovulation, arachidonic acid metabolism, phagocytosis, and mitochondrial respiratory chain (Chaudhary *et al.,* 2023). The reactive nitrogen and oxygen species (RNS/ROS) play a twofold role as both toxic and beneficial compounds to the organism's system. At lower concentrations, they have beneficial effects and indulged in different physiological processes such as redox regulation, mitogenic responses, cellular signaling pathways, and an immune function while at a higher level, these reactive species generate nitrosative and oxidative stress (Phaniendra *et al.,* 2015). The ROS that are physiologically relevant include superoxide anion radicals ($O2•^{-}$), hydrogen peroxide (H2O2), hydroxyl radicals (•OH), and singlet oxygen (1O2), which are generally present in cells at low levels (Pizzino *et al.,* 2017) Oxidative stress arises from an imbalance between elevated reactive oxygen species (ROS) and insufficient antioxidant defenses (Preiser, 2012).

Antioxidants

To reduce or prevent free radical-directed oxidative damage, the human body has developed an antioxidant defence mechanism that involves free radical scavenging, and enzymatic activities to neutralize the reactive species just after they have formed. In addition, the consumption of dietary antioxidants can maintain an adequate level of antioxidants in the organism's body (Lobo *et al.,* 2010). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the primary enzymatic antioxidants present in cells that help to protect cells from ROS-induced damage (Pizzino et al., 2017). The secondary enzymatic antioxidants, such as the thioredoxin system and glutaredoxins are important in maintaining cellular redox balance and repairing oxidized products (Birben *et al.,* 2012). The nonenzymatic antioxidants would include low-molecular-weight compounds such as vitamins (vitamins A, C and E), β-carotene, uric acid (UA), α-lipoic acid, and glutathione (GSH), a tripeptide (L-g-glutamyl-L-cysteinyl-L-glycine) that comprise a thiol (sulfhydryl) group. While the primary antioxidants inhibit and scavenge oxidant formation, the other antioxidants in the body scavenge oxidants as well as repair the oxidized molecules (Vona *et al.,* 2021).

Biomarkers of Oxidative Stress

Reactive oxygen species are compounds that are difficult to measure when assessing oxidative stress, primarily due to the very short half-life, so they hardly play the role of biomarkers (Tejchman *et al.,* 2021). Biomarkers of oxidative stress can be classified as molecules that are modified by interactions with ROS in the microenvironment; and molecules of the antioxidant system that change in response to increased redox stress. DNA, lipids (including phospholipids), proteins and carbohydrates are examples of molecules that can be modified by excessive ROS *in vivo* (Ho *et al.,* 2013). Biological Of these modifications, some are known to have direct effects on function of the molecule (e.g. inhibit enzyme function), but others merely reflect the degree of oxidative stress in the local environment (Ho *et al.,* 2013). In most studies, researchers use indicators like aldehydes, particularly malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), and glutathione peroxidase (GPx) to measure oxidative stress (Golabi *et al.,* 2022).

2.3 The Six Minutes' Walk Test

The 6-Minute Walk function Test (6MWT) is a widely used, simple, and practical measure of functional exercise capacity that assesses the distance an individual can walk on a flat, hard surface in six minutes (Del Buono *et al*., 2019). It evaluates the global and integrated responses of all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism (ATS, 2002). In this low complexity, safe test, the patient is asked to walk as far as possible along a 30-m minimally trafficked corridor for a period of 6 min with the primary outcome measure being the 6-min walk distance (6MWD) measured in meters (Holland *et al.,* 2014).The test gained significant prominence and widespread acceptance following the publication of standardized guidelines by the American Thoracic Society (ATS) in 2002 (Kammin, 2022). These comprehensive guidelines provided a consistent methodology for administering the 6MWT, which was crucial for ensuring the reproducibility and comparability of results across different clinical sites and research studies (Du *et al*., 2009). The primary test outcome is the final distance walked (6MWD). Among healthy individuals, the average 6MWD is between 400 and 700 meters (Enright, 2003). This range overlaps with data reported in other populations (Britto *et al.,* 2013).Factors associated with variability in test performance among healthy subjects include weight and age (Chetta *et al.,* 2006). The contraindications to the 6MWT is having a history of an acute coronary syndrome (either unstable angina or myocardial infarction) within 30 days prior to the test as well as severe, uncontrolled hypertension and a resting heart rate greater than 120 beats per minute (American Thoracic Society, 2002).

The following table summarize key methodological guidelines, normative data, and influential factors for the 6MWT, providing a comprehensive overview for context and comparison.

Table 2.1: Key ATS Guidelines for 6MWT Administration

Aspect	Guideline/Recommendation
Test Environment	Indoors, flat, straight, hard surface, minimally trafficked corridor.
Corridor Length	Recommended 30 meters (100 feet). Shorter corridors reduce 6MWD due to more turns.
Markings	Every 3 meters, cones at turnaround points. Starting line clearly demarcated.
Equipment	Stopwatch/countdown timer, mechanical lap counter, 2 cones, movable chair, worksheets, sphygmomanometer. Optional: Pulse oximeter, Borg scale.
Patient Preparation	Comfortable clothing, appropriate shoes, use usual walking aids (document). No vigorous exercise within 2 hours prior. Light meal acceptable.
Pre-test Measurements	Resting Heart Rate (HR), Blood Pressure (Systolic and Diastolic), Oxygen Saturation (SpO_2), Perceived Exertion (Borg scale).
Instructions	"Walk as far as possible for 6 minutes." Permission to slow down/stop/rest. No running/jogging. Demonstrate turning.

Aspect	Guideline/Recommendation
Encouragement	Standardized phrases at specific time intervals (e.g., every minute).
During Test	If the patient stops during testing, the timer is not stopped, but a notation on the time of stopping and restarting should be noted. Reason for premature cessation of testing by patient documented (e.g., chest pain, intolerable dyspnea, joint, or back or leg pain). Assessor can terminate testing based on patient appearance or if oxygen saturation is <80%.
Post-test Measurements	Immediate HR, BP, SpO2, Perceived Exertion.
Safety Considerations	Assessors should be certified in basic life support and cardiopulmonary resuscitation. Reasons for immediate test termination (e.g., chest pain, intolerable dyspnea, SpO2 < 80%). Access to emergency equipment should be available. This can include a crash cart, sublingual nitroglycerin, and bronchodilators.

Table 1.0 provides a summary of the 6MWT procedural guidelines by the American Thoracic Society (ATS) (American Thoracic Society, 2002; Kammin, 2022)

CHAPTER THREE

MATERIALS AND METHODS

3.1 EQUIPMENTS AND MATERIALS

i. Countdown timer(stopwatch)
ii. Two small cones to mark turnaround point
iii. Moveable tables and chairs
iv. Sphygmomanometer
v. Latex hand gloves
vi. Methylated spirit
vii. Cotton wool
viii. Tourniquet
ix. 5ml syringe
x. Worksheets
xi. Measuring tape
xii. Plain bottle

3.2 STUDY DESIGN

This study involved forty four (44) healthy adult males between the ages of 18 years and 29 years

3.3 STUDY POPULATION AND STUDY AREA

A total number of forty-four (44) healthy males participated in this study, between the age of 18 years and 29 years from the University of Benin. This study was conducted at the physiology department in the University of Benin, Benin City.

3.4 INCLUSION CRITERIA

Criteria for inclusion included:

i. Subjects between the ages of 18 - 29years.
Normal weight subjects

3.5 EXCLUSION CRITERIA

Criteria for exclusion included:

i. Subjects who experience shortness of breath
i. Subjects who had Chronic Obstructive Pulmonary Diseases (COPD).
ii. Subjects who were underweight and overweight.

3.6 ETHICAL APPROVAL

What principles guided this research? They include:

i. Informed consent from each participants
ii. Voluntary participation of subjects
iii. Confidentiality
iv. Anonymity
v. Distribution of questionnaires to participants

3.7 ETHICAL CONSIDERATION

Ethical approval and clearance for this study was sought and obtained from the Research Ethics Committee, College of Medical Sciences, University of Benin, Benin City, Nigeria. (CMS/REC/2025/791).

3.8 METHODOLOGY

<u>Measurement of the 30m hallway</u>

i. A straight hallway was used at the department of Physiology in the University of Benin.

ii. The hallway was measured using a measuring tape to mark out 30m and calibrated at intervals of 3m.

iii. Two small cones were placed at both ends on the 30m mark to indicate the turnaround points.

Participant preparation

i. Participants were educated about the 6-minute walk test procedure.

ii. Participants were given questionnaires along with a consent form to fill.

iii. Participants were asked to wear comfortable clothing and appropriate walking shoes.

iv. Participants were asked to walk at their own pace to cover as much distance as possible in 6 minutes but not jog or run.

Six Minute Walk Test

i. The Participant's BMI was measured.

ii. The Participants were asked to rest for at least 10 minutes before their pre-test.

iii. The Participants are informed about the test procedure, including the goal of walking as far as possible within six minutes.

iv. The Participants walked back and forth the cones on the 30m hallway.

v. Standardized encouragement phrases (as per ATS guidelines) were given at set intervals to maintain consistency.

vi. The number of laps walked was counted and recorded.

vii. The total number of laps walked was multiplied by 60 meters to get the total number of meters walked.

Blood Sample Collection

After inspecting the subject's arm, and selected an appropriate venipuncture site (the antecubital fossa or forearm). The tourniquet was placed approximately 3 to 5 inches above the vein site. The subject was instructed to extend his arm and flex and relax the fist a few times to engorge the veins for easier identification. Palpate the selected view if necessary. Cleansed the area with a methylated spirit and allowed the area to dry completely. A 5ml syringe and needle was used for the blood collection, after collection, tourniquet was released and the vacutainer tube (plain bottle) was used to collect, store and transport blood samples for laboratory testing. A cotton wool moist with methylated spirit was placed over the venipuncture site firmly held in place for about one to two minutes while removing the needle until bleeding stopped. The tube were placed in the approved specimen transport bag with the requisition and transport to the laboratory as soon as possible. The analysis was done in the chemical pathology laboratory of the University of Benin Teaching Hospital, Benin City. A Superoxide Dismutase (SOD) Activity Assay Kit was used for the assessment of SOD Marker, Catalase (CAT) Activity Assay Kit was used for the assessment of CAT Marker and Malondialdehyde (MDA) Activity Assay Kit was used for the assessment of MDA Marker

SAMPLE COLLECTION AND PREPARATION

i. Collected blood samples were immediately separated from the serum and the usual precautions in the collection of venipuncture samples was observed.

ii. The blood samples were collected in a plain venipuncture tube without additives or anti-coagulants (for serum) or evacuated tubes containing EDTA or heparin.

iii. The blood was allowed to clot for serum samples and was centrifuged to separate the serum or plasma from the cells. Samples were stored by refrigeration at 2-8^{o}C for a maximum period of five (5) days.

3.9 STATISTICAL ANALYSIS

Data obtained from the 6MWT was performed with Graph Pad Prism Version 8.1. The standard error of mean (SEM) was used in the graphs to display the results. The dependent and independent variable means were compared using the student t-test. $P<0.05$ was accepted as significant.

CHAPTER FOUR

RESULTS

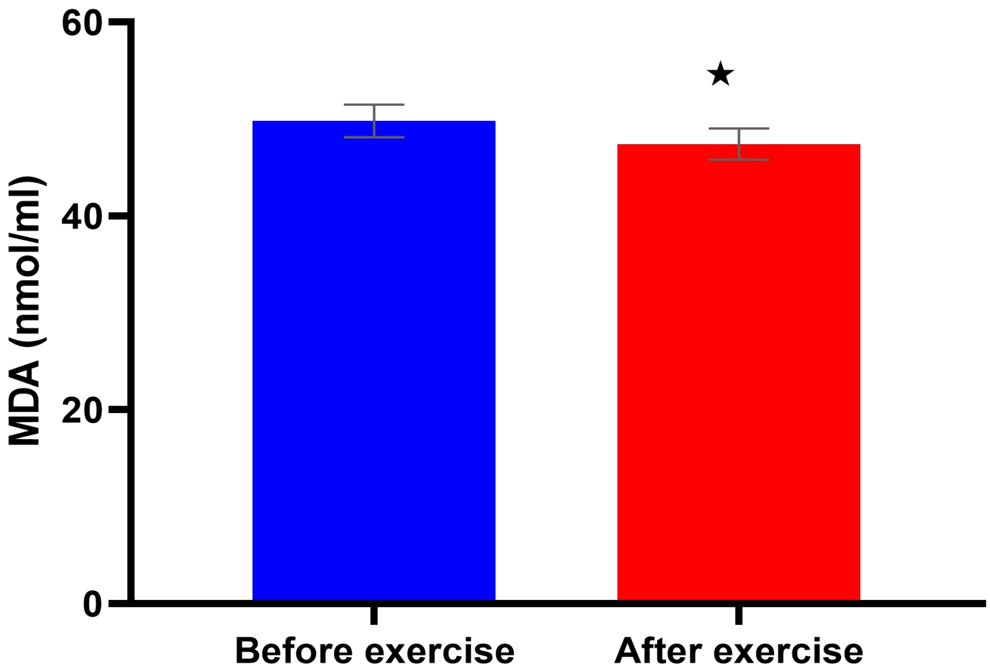

Figure 1: Showed MDA before and after six (6) minute walk exercise in young adult male

There was a significant decrease after exercise compared with before the exercise

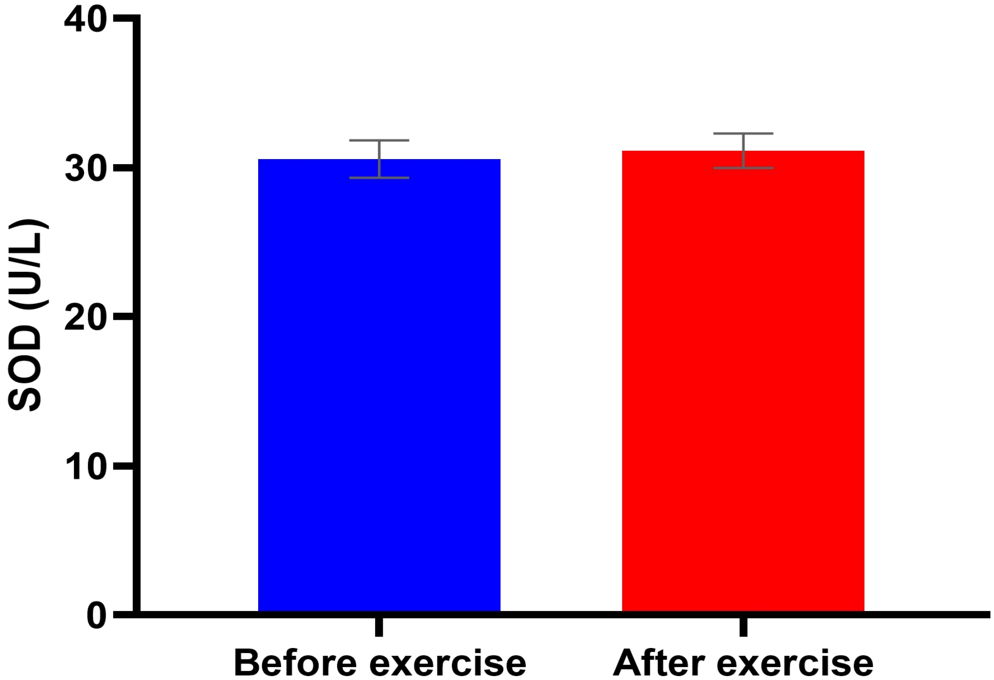

Figure 2: Showed SOD before and after six (6) minute walk exercise in young adult male

There was no significant changes after exercise compared with before the exercise

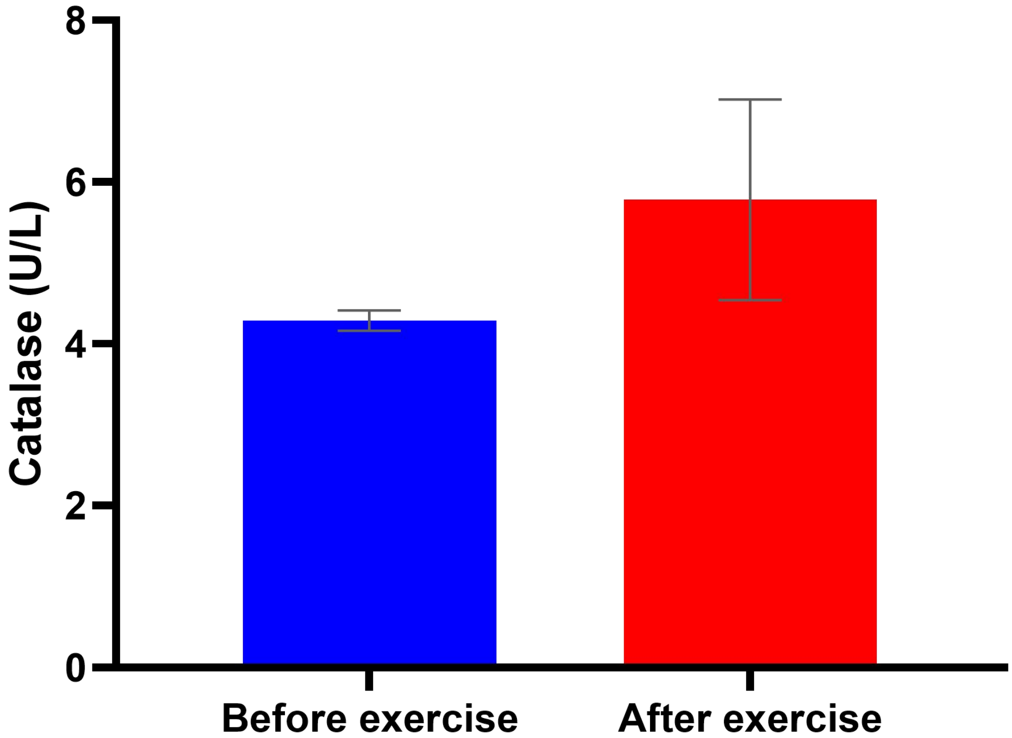

Figure 3: Showed Catalase before and after six (6) minute walk exercise in young adult male

There was no significant changes after exercise compared with before the exercise

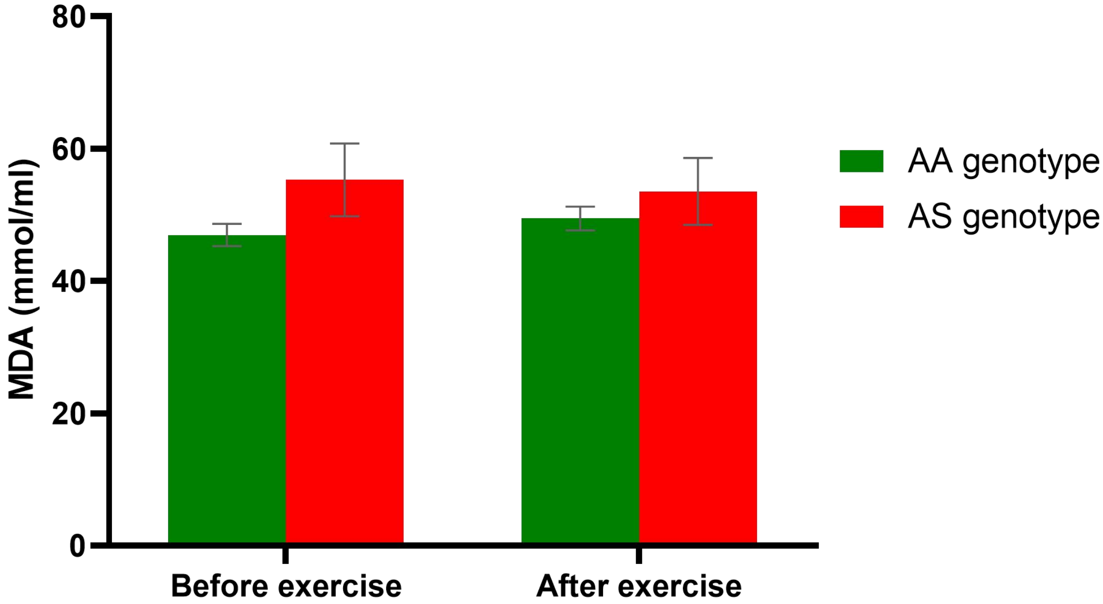

Figure 4: Comparing mean MDA level in different genotypes before and after six (6) minute walk exercise in young adult.

There were no significant different in AS genotype relative to AA genotype at pre and post exercise

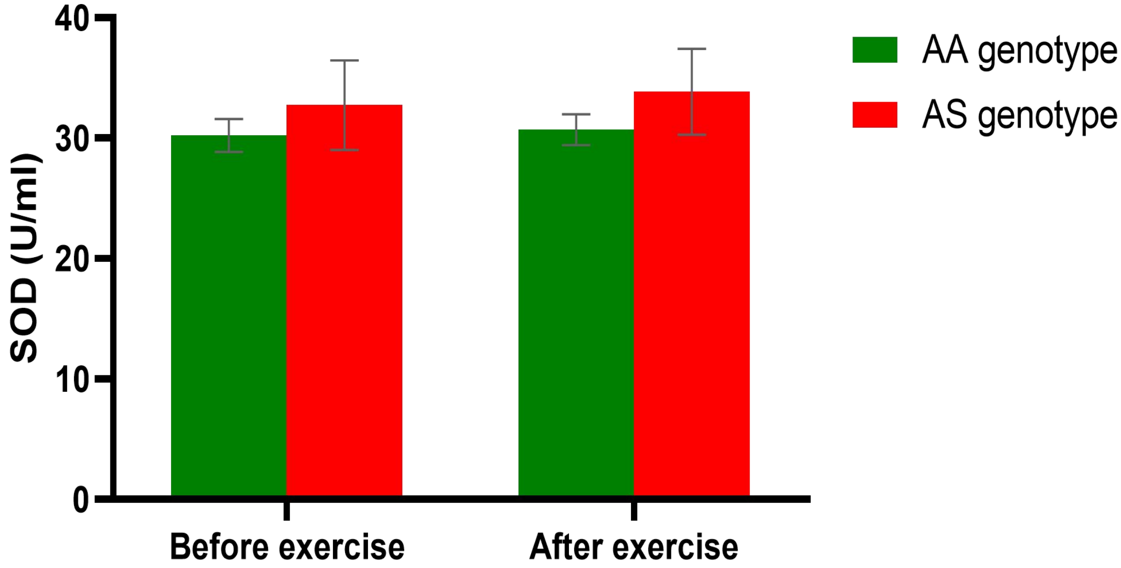

Figure 5: Comparing mean SOD level in different genotypes before and after six (6) minute walk exercise in young adult.

There were no significant different in AS genotype relative to AA genotype at pre and post exercise

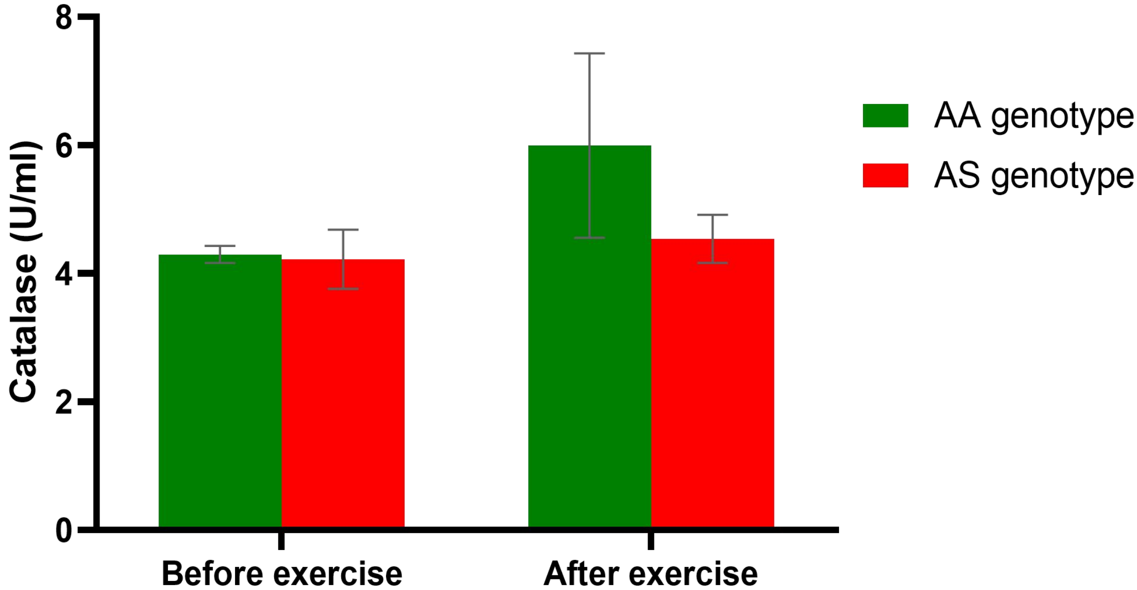

Figure 6: Comparing mean catalase level in different genotypes before and after six (6) minute walk exercise in young adult.

There were no significant different in AS genotype relative to AA genotype at pre and post exercise

CHAPTER FIVE

5.1 DISCUSSION

The six-minute walk test is easy to administer, reflects everyday activity, and is one of the predictive markers for exercise stress test performance. It is also recommended as an indicator of daily functional ability (Solway *et al.,* 2001). In figure 1 which Showed MDA before and after six (6) minute walk exercise in young adult male, there was a significant decrease after exercise compared with before the exercise. MDA is mainly formed in cells due to peroxidation of membrane lipids and then released into the extracellular space (Dalle-Donne *et al.,* 2008). According to a study carried out by Niknam *et al.,* the decrease in MDA in the exercise group may be due to the reduced formation of MDA in the cells. Because MDA is mainly formed due to mitochondrial ROS (Barrera *et al.,* 2018) improving mitochondrial function and antioxidant defense (increase GPx activity and decrease H2O2 levels) after the physical activity/ exercise may have led to a reduction in MDA formation.

While in the comparison in catalase, SOD and MDA levels in different genotypes there was no significant difference in AS genotype relative to AA genotype at pre and post exercise. HbS, which represents around 80% of total hemoglobin in SCD patients, can generate a 2-fold greater quantity of reactive oxygen species than HbA (Fearon and Faux, 2009). In sickle cell trait carriers, in whom HbS represents around 40% of total hemoglobin, It was found that oxidative stress varied little at baseline from healthy subjects and the main finding of the study done by Chirico *et al.,* emphasizes the beneficial effects of exercise training on oxidative stress in SCT carriers. In terms of antioxidant capacity, it was found that SOD and catalase was expressed similarly in both SCT carriers and control groups in responses to exercise and recovery (Monchanin *et al.,* 2008). In various situations, exercise training has been shown to decrease oxidative stress through an upregulation in the antioxidant system thereby halting the overproduction of oxidants (Pialoux *et al.,* 2009).

It is unclear whether oxidative stress and nitric oxide metabolism is impaired in SCT carriers. Although few studies have looked at changes in oxidative in this context, evidence suggests that there is little difference between SCT carriers and control subjects in response to exercise or during recovery (Monchanin *et al.,* 2008).

5.2 CONCLUSION

In conclusion, this study investigated the changes in oxidative stress marker level in young adult males of different genotype following functional exercise (6-Minute Walk test). The findings suggest that there was no significant changes in oxidative stress marker levels relative to genotype.

REFERENCE

Adamson, B. C., Ensari, I., and Motl, R. W. (2015). Effect of exercise on depressive symptoms in adults with neurologic disorders: A systematic review and meta-analysis. *Archives of Physical Medicine and Rehabilitation.* **96**(7): 1329–1338.

Abdin, S., Welch, R. K., Byron-Daniel, J., and Meyrick, J. (2018). The effectiveness of physical activity interventions in improving well-being across office-based workplace settings: A systematic review. *Public Health.* **160***:* 70–76.

Abell, B., Glasziou, P., and Hoffmann, T. (2017). The contribution of individual exercise training components to clinical outcomes in randomised controlled trials of cardiac rehabilitation: A systematic review and meta-regression. *Sports Medicine – Open.* **3**(1): 19.

Adsett, J. A., Mudge, A. M., Morris, N., Kuys, S., and Paratz, J. D. (2015). Aquatic exercise training and stable heart failure: A systematic review and meta-analysis. *International Journal of Cardiology.* **186**: 22–28.

Agarwala, P., and Salzman, S. H. (2020). Six-minute walk test: Clinical role, technique, coding, and reimbursement. *Chest*. **157**(3): 603–611.

Alanazi, M. H., Parent, E. C., and Dennett, E. (2018). Effect of stabilization exercise on back pain, disability and quality of life in adults with scoliosis: A systematic review. *European Journal of Physical and Rehabilitation Medicine.* 54(5): 647–653.

American Thoracic Society. (2002). ATS statement: Guidelines for the six-minute walk test. *American Journal of Respiratory and Critical Care Medicine.* **166**: 111–117.

Anderson, D., Seib, C., and Rasmussen, L. (2014). Can physical activity prevent physical and cognitive decline in postmenopausal women? A systematic review of the literature. *Maturitas.* **79**(1): 14–33.

Barrera, G., Pizzimenti, S., Daga, M., Dianzani, C., Arcaro, A., Cetrangolo, G. P., Giulio G., Marie Angele C., Maria G., and Gentile, F. (2018). Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. *Antioxidants***.** **7**(8):102.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., and Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal.* **5**(1): 9–19.

Britto, R. R., Probst, V. S., de Andrade, A. F., Samora, G. A., Hernandes, N. A., Marinho, P. E., Karsten, M., Pitta, F., and Parreira, V. F. **(2013).** Reference equations for the six- minute walk distance based on a Brazilian multicenter study. *Brazilian Journal of Physical Therapy.* **17**(6): 556–563.

Burton, D. A., Stokes, K., and Hall, G. M. (2004). Physiological effects of exercise. *Continuing Education in Anaesthesia, Critical Care and Pain.* **4**(6):185–188.

Cazzoletti, L., Zanolin, M. E., Dorelli, G., Ferrari, P., Dalle Carbonare, L. G., Crisafulli, E., Alemayohu, M. A., Olivieri, M., Verlato, G., and Ferrari, M. (2022). Six-minute walk distance in healthy subjects: Reference standards from a general population sample. *Respiratory Research.* **23**(1): 83.

Chastin, S. F. M., De Craemer, M., De Cocker, K., Powell, L., Van Cauwenberg, J., Dall, P., Hamer, M., and Stamatakis, E. (2019). How does light-intensity physical activity associate with adult cardiometabolic health and mortality? Systematic review with meta-analysis of experimental and observational studies. *British Journal of Sports Medicine.* **53**(6):370–376.

Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Calina, D., and Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry***.** **11**: 1158198.*

Chetta, A., Zanini, A., Pisi, G., Aiello, M., Tzani, P., Neri, M., and Olivieri, D. (2006). Reference values for the 6-min walk test in healthy subjects 20–50 years old. *Respiratory Medicine.* **100**(9):1573–1578.

Chirico, E. N., Martin, C., Faës, C., Féasson, L., Oyono-Enguéllé, S., Aufradet, E., Dubouchaud, H., Francina, A., Canet-Soulas, E., Thiriet, P., Messonnier, L., and Pialoux, V. (2012). Exercise training blunts oxidative stress in sickle cell trait carriers. *Journal of Applied Physiology.* **112**(9): 1445–1453.

Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D., and Milzani, A. (2006). Biomarkers of oxidative damage in human disease. *Clinical chemistry*. **52**(4): 601-623.

Del Buono, M. G., Arena, R., Borlaug, B. A., Carbone, S., Canada, J. M., Kirkman, D. L., Garten, R., Rodriguez-Miguelez, P., Guazzi, M., Lavie, C. J., Abbate, A. (2019). Exercise Intolerance in Patients With Heart Failure. *Journal of the American College of Cardiology*. **73**(17): 2209–2225.

Du, H., Newton, P. J., Salamonson, Y., Carrieri-Kohlman, V. L., and Davidson, P. M. (2009). A review of the six-minute walk test: Its implication as a self-administered assessment tool. *European Journal of Cardiovascular Nursing.* **8**(1): 2–8.

Enright, P. L. (2003). The six- minute walk test. *Respiratory Care.* **48**(8): 783–785.

Fearon, I. M., and Faux, S. P. (2009). Oxidative stress and cardiovascular disease: Novel tools give (free) radical insight. *Journal of Molecular and Cellular Cardiology.* **47**(3): 372–381.

Gutteridge, J. M. C., and Halliwell, B. (2018). Mini-review: Oxidative stress, redox stress, or redox success? *Biochemical and Biophysical Research Communications.* **502**(2): 183–186.

Hagen, K. B., Dagfinrud, H., Moe, R. H., Østerås, N., Kjeken, I., Grotle, M., and Smedslund, G. (2012). Exercise therapy for bone and muscle health: An overview of systematic reviews. *BMC Medicine.* **10**(1): 167.

Holland, A. E., Spruit, M. A., Troosters, T., Puhan, M. A., Pepin, V., Saey, D., McCormack, M. C., Carlin, B. W., Sciurba, F. C., Pitta, F., Wanger, J., MacIntyre, N., Kaminsky, D. A., Culver, B. H., Revill, S. M., Hernandes, N. A., Andrianopoulos, V., Camillo, C. A., Mitchell, K. E., Lee, A. L., Hill, C. J., and Singh, S. J. (2014). An official European Respiratory Society/American Thoracic Society technical standard: Field walking tests in chronic respiratory disease. *European Respiratory Journal.* **44**(6): 1428–1446.

Huang, Z., and Rusanova, O. M. (2022). Cardiorespiratory system in the context of regular exercise in kayaking. *Physical Activity and Health.* **6**(1): 124-135.

Kammin, E. J. (2022). The 6-minute walk test: Indications and guidelines for use in outpatient practices. *The Journal for Nurse Practitioners.* **18**(6): 608–610.

Kraemer, W. J., and Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine.* **35**(4): 339–361.

Kyu, H. H., Bachman, V. F., Alexander, L. T., Mumford, J. E., Afshin, A., Estep, K., Moyer, L., and Forouzanfar, M. H. (2016). Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: Systematic review and dose-response meta-analysis for the Global Burden of Disease Study 2013. *BMJ. 354*: i3857.

Monchanin, G., Serpero, L. D., Connes, P., Tripette, J., Wouassi, D., Francina, A., Massarelli, R., Gozal, D., Thiriet, P., and Martin, C. (2008). Plasma levels of adhesion molecules ICAM-1 and VCAM-1 in athletes with sickle cell trait with or without alpha-thalassemia during endurance exercise and recovery. *Clinical Hemorheology and Microcirculation.* **40**(2): 89–97.

Niknam, A., Gaeini, A. A., Hamidvand, A., Jahromi, M. K., Oviedo, G. R., Kordi, M., and Safarpour, F. (2025). High-intensity functional training modulates oxidative stress and improves physical performance in adolescent male soccer players: a randomized controlled trial. *BMC sports science, medicine and rehabilitation*. **17**(1):38.

Phaniendra, A., Jestadi, D. B., and Periyasamy, L. (2015). Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry.* **30**(1): 11–26.

Pialoux, V., Brown, A. D., Leigh, R., Friedenreich, C. M., and Poulin, M. J. (2009). Effect of cardiorespiratory fitness on vascular regulation and oxidative stress in postmenopausal women. *Hypertension***.** **54**(4): 1014–1020.

Piercy, K. L., Troiano, R. P., Ballard, R. M., Carlson, S. A., Fulton, J. E., Galuska, D. A., George, S. M., and Olson, R. D. (2018). The physical activity guidelines for Americans. *JAMA.* **320**(19): 2020–2028

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity***.** **2017**(1): 8416763.

Pooja, G., Shweta, S., and Patel, P. (2025). Oxidative stress and free radicals in disease pathogenesis: A review. *Discovery Medicine.* **2**:104.

Reid, M. B. (2001). Invited review: Redox modulation of skeletal muscle contraction: What we know and what we don't. *Journal of Applied Physiology.* **90**(2): 724–731.

Sellami, M., Gasmi, M., Denham, J., Hayes, L. D., Stratton, D., Padulo, J., and Bragazzi, N. (2018). Effects of acute and chronic exercise on immunological parameters in the elderly aged: Can physical activity counteract the effects of aging? *Frontiers in Immunology.* **9**:2187.

Tejchman, K., Kotfis, K., and Sieńko, J. (2021). Biomarkers and mechanisms of oxidative stress—Last 20 years of research with an emphasis on kidney damage and renal transplantation. *International Journal of Molecular Sciences*. **22**(15): 8010.

Vona, R., Pallotta, L., Cappelletti, M., Severi, C., and Matarrese, P. (2021). The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. *Antioxidants.* **10**(2): 201.

Xiang, L., Mei, Q., Wang, A., Shim, V., Fernandez, J., and Gu, Y. (2022). Evaluating function in the hallux valgus foot following a 12-week minimalist footwear intervention: A pilot computational analysis. *Journal of Biomechanics*. **132**: 110941.

Zhou, Z., Chen, C., Teo, E.-C., Zhang, Y., Huang, J., Xu, Y., and Gu, Y. (2022). Intracellular oxidative stress induced by physical exercise in adults: Systematic review and meta-analysis. *Antioxidants*. **11**(9): 1751.

APPENDIX

DEPARTMENT OF PHYSIOLOGY
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN
BENIN CITY
6 MINUTES WALK TEST QUESTIONNAIRE

Subject Session

1. Are you currently taking any medications? [ ] Yes [ ] No
If yes, please specify: ____

2. Have you had an ECG test in the last 5 months?[ ] Yes [ ] No
If yes, what was the result? ________________________________

3. Have you had any recent surgery? [ ] Yes [ ] No
If yes, please specify the type and date: ____

4. Have you had a heart attack in the last 4 weeks? [ ] Yes [ ] No
5. Have you experienced chest pain in the last 4 weeks? [ ] Yes [ ] No
6. Do you mind if a blood sample is taken for this test? [ ] Yes [ ] No
7. Please describe your current state of health: ____

8. Do you smoke? [ ] Yes   [ ] No
If yes, how many cigarettes per day? ____________________

9. Do you experience shortness of breath? [ ] Yes   [ ] No
If yes, please describe when it occurs and how severe it is: ____
____
____
____

10. Are you asthmatic? [ ] Yes   [ ] No

11. When was your last meal?
Please signify in hours: ____

WORK-SHEET

(ONLY Tick number 1. The rest of the data will be filled by the Project Student in charge)

1. Would you like to participate in the 6 minute walk test from the beginning to end?
Yes [ ]   No [ ]

2. Subject: _____________

3. Age: ______________

4. Sex: [ ] Male   [ ] Female

5. Date of Birth: ________________________________

6. Blood Group: [ ] A   [ ] AB   [ ] O   [ ] Other ________________

7. Height: ______________ cm, Weight: ______________ kg

8. BMI: ________________ Kg/m2, Category: ________________

9. SpO2 Pre: _______________% Post: ___________________%

10. Pulse Rate: Pre: _______________bpm, Post: __________________ bpm

11. Borg Scale: Pre: _____________, Post: _______________

12. FVC: __________ /____________L   FEV1:___________ /___________L

PEF: _________/_________L/s   FEV1%:_________/_________%

FEF25:_________/_________L/s   FEF2475:__________/__________

FEF75:__________/___________

13. Blood pressure reading (systolic/dystolic) Pre: _________________ mmHg, Post: _______________mmHg

14. Blood glucose Pre: ___________ mg/dl, Post: _____________ mg/dl

15. Number of laps (*60metres) and final partial lap: __________________________

16. Total distance Covered in 6 minutes: _____________ Meters

17. Genotype: [ ] AA [ ] AS [ ] SS [ ] Other _______________
(Procedure: Electrophoresis)

18. Cortisol: Pre: _____________ nmol/L, Post: ______________ nmol/L
(Procedure: Cortisol Competitive ELISA technique)

19. Oxidative stress Test
a. MDA: Pre: ________________, Post: _________________

b.CAT: Pre: ________________, Post: _________________

c. SOD: Pre: ________________, Post: _________________

20. Full Blood Count:

a. WBC Pre: ________________, Post: _____________________

b. LYM% Pre: ________________, Post: _____________________

c. MON% Pre: ________________, Post: _____________________

d. NEU% Pre: ________________, Post: _____________________

e. EOS% Pre: ________________, Post: _____________________

f. BAS% Pre: ________________, Post: _____________________

g. RBC Pre: ________________, Post: _____________________

h. HCB Pre: ________________, Post: _____________________

i. HCT Pre: ________________, Post: _____________________

j. MCV Pre: _________________, Post: ______________________

k. MCH Pre: _________________, Post: ______________________

l. MCHC Pre: _________________, Post: ______________________

m. RDWC Pre: _________________, Post: ______________________

n. RDWS Pre: _________________, Post: ______________________

o. PLT Pre: _________________, Post: ______________________

p. MPV Pre: _________________, Post: ______________________
q. PCT Pre: _________________, Post: ______________________
r. PDW Pre: _________________, Post: ______________________
s. PLCR Pre: _________________, Post: ______________________

21. Stopped or paused before 6 minutes (Yes / No), Reason: _____________________

22. Other symptoms at the end of the test (Angina, dizziness, hip, leg or calf pain): _____________________________________

...