HAEMATOLOGICAL CHANGES IN HEALTHY YOUNG ADULT DURING FUNCTIONAL EXERCISE

(6 MINUTES WALK TEST).

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SCHOOL OF BASIC MEDICAL SCIENCE

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BENIN CITY

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CERTIFICATION

This is to certify that this project titled "**HAEMATOLOGICAL CHANGES IN HEALTHY YOUNG ADULT DURING FUNCTIONAL EXERCISE (6 MINUTES WALK TEST)**" has been carried out by **ASAZOBOR MIRACLE O**., with matriculation number **BMS1909998** in partial fulfillment of the requirements for the award of Bachelor of Science Degree (B.Sc) in department of Physiology, at the School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Edo state, Nigeria.

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I also extend sincere appreciation to all those who, in one way or another, contributed

DEDICATION

I dedicate this work to God Almighty, my Creator, my constant source of strength, and the wellspring of my inspiration, wisdom, and understanding. It is by His grace alone that I have been able to embark on and complete this journey. Through His guidance, I have found direction; on His wings, I have soared.

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ABSTRACT

Functional exercise, typified by the Six-Minute Walk Test (6MWT), provokes a significant physiological stress response to meet the demands of submaximal exertion. The haematological system is highly sensitive to such stress, withchanges in erythrocyte, leukocyte, and platelet parameters serving as key indicators of fluid shift, immune mobilization, and hemostatic activation. The aim of this study was to evaluate the acute effects of functional exercise (Six-Minute Walk Test) on key haematological indices in healthy young adult males. A pre-post intervention study was conducted involving healthy young adult male volunteers. Baseline venous blood samples were collected into EDTA tubes for a full blood count analysis. Participants subsequently performed the 6MWT as per the American Thoracic Society guidelines. A second venous blood sample was collected immediately post-test for repeat haematological analysis. Statistical significance was determined using a paired T-test. The study revealed a specific pattern of haematological response. No significant changes were observed in total white blood cell count, lymphocyte count, granulocyte count, red blood cell count, mean corpuscular haemoglobin concentration, or haematocrit value post-exercise. However, a significant reduction was recorded in key erythrocyte indices, including haemoglobin concentration, mean corpuscular haemoglobin (MCH), and mean cell volume (MCV) immediately following the 6MWT. This study demonstrates that functional exercise induces a defined hematological profile in healthy young males, characterized by significant reductions in hemoglobin, MCH, and MCV without changes in total cell counts. These findings provide a crucial normative baseline for clinical interpretation of exercise tests and highlight the sensitivity of erythrocyte indices to submaximal exertion.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The ability to perform sustained physical activity is a fundamental component of human health and a prerequisite for engaging in the myriad tasks of daily living. This ability is scientifically conceptualized as functional capacity, which represents an individual's integrated capacity to perform aerobic work (Arena *et al*., 2007). It is not the measure of a single organ system but rather reflects the coordinated and efficient interplay of the pulmonary, cardiovascular, and musculoskeletal systems to deliver and utilize oxygen for sustained metabolic activity (Baillot *et al*., 2015). A high functional capacity is associated with overall well-being, a reduced risk of chronic disease, and the ability to maintain an independent and active lifestyle.

The gold-standard physiological metric for quantifying functional capacity is the maximal oxygen uptake, or $\dot{V}O_{2max}$. This parameter defines the maximum rate at which an individual can take up, transport, and utilize oxygen during exhaustive, large-muscle-mass exercise.

Because maximal exercise typically involves only about half of the body's total musculature, $\dot{V}O_{2max}$ is generally understood to be limited by central factors, specifically, the heart's maximal capacity to pump oxygenated blood, rather than by the peripheral muscles' ability to extract oxygen. For comparative purposes across individuals of different body sizes, $\dot{V}O_{2max}$ is commonly expressed relative to body weight, in units of millilitres of oxygen per kilogram of body weight per minute (m*L* . kg^{-1}. min^{-1}). In clinical and applied settings, functional capacity is also frequently expressed in Metabolic Equivalents (METs), where one MET represents the energy expenditure of the body at rest, approximately equal to 3.5 /m*L* .kg^{-1}. min^{-1} of oxygen consumption (Arena *et al*., 2007).

Functional capacity is not a static attribute; it is modulated by a host of intrinsic and extrinsic factors. In healthy, non-athletic individuals, aerobic capacity exhibits a predictable decline with age, averaging about 10% per decade after young adulthood (Arena et al., 2007). This decline accelerates significantly in the elderly, potentially exceeding 20% per decade, and is mediated by age-associated reductions in maximal heart rate, stroke volume, and blood flow to skeletal muscle. Sex also plays a significant role; at any given age, men typically demonstrate a $\dot{V}O_{2max}$ that is 10-20% greater than that of women. This difference is largely attributable to physiological distinctions, including a higher average hemoglobin concentration, a greater proportion of lean muscle mass, and a larger stroke volume in men (Waltz *et al*., 2013).

Three key changes characterize the immediate hematological response to exercise. First is the phenomenon of hemoconcentration, a direct consequence of a shift in plasma fluid from the intravascular to the interstitial space. This decrease in plasma volume, which can range from 5% to 20%, leads to a relative increase in the concentration of all elements that cannot easily pass through the capillary walls, including red blood cells, leukocytes, platelets, and large plasma proteins (Waltz *et al*., 2013). Second is a pronounced leukocytosis, or an increase in the total number of circulating white blood cells. This response is immediate and involves the rapid mobilization of various leukocyte subsets into the bloodstream, a key component of the body's integrated stress response (Meyer-Lindemann *et al*., 2023). Third is a state of thrombocytosis, characterized by an increase in the number of circulating platelets. The magnitude and specific characteristics of these hematological shifts are exquisitely sensitive to the intensity and duration of the exercise stimulus, making it imperative to characterize the response to specific, standardized exercise protocols (Neves *et al*., 2015).

1.2 Statement of the Problem

While the 6MWT is a globally standardized tool for assessing functional capacity and the general hematological responses to exercise are broadly understood, a critical intersection of these two areas remains unexplored. The existing body of literature reveals several significant gaps that preclude a complete understanding of the physiological effects of this common test.

First, there is a recognized deficiency in population-specific normative data for the 6MWT, particularly for young adults. Multiple studies have demonstrated that reference equations for 6MWD, which are largely derived from and are highly dependent on age in middle-aged and older cohorts, perform poorly when applied to younger populations (Halliday *et al*., 2020). In healthy adults aged 18-50, age has a minimal effect on 6MWT performance, rendering existing predictive models inaccurate. This highlights a clear need for the collection of fundamental physiological data, including hematological responses, within this specific demographic to establish a valid frame of reference.

Second, the vast majority of research in exercise hematology has utilized protocols involving maximal or near-maximal exertion, such as graded exercise tests to exhaustion, or high-intensity interval training (Neves *et al*., 2015). The precise, time-course hematological changes induced by a self-paced, continuous, and submaximal walking test like the 6MWT have not been systematically characterized.

Therefore, the central problem is a lack of fundamental, normative data describing the precise acute hematological changes, encompassing erythrocyte, leukocyte, and platelet parameters, in direct response to a standardized functional exercise test (the 6MWT) within a healthy young adult population. This baseline data is essential for the accurate interpretation of 6MWT results in clinical populations, where hematological abnormalities are common, and for a more nuanced understanding of the physiological impact of moderate, sustained physical activity that is representative of daily life.

1.3 Aim of the Study

The aim of this study was to evaluate the acute effects of functional exercise (Six-Minute Walk Test) on key haematological indices in healthy young adult males.

1.4 Objectives of the Study

1. To determine the baseline haematological parameters (Full Blood Count) in healthy young adults.
2. To assess the acute changes in erythrocyte indices (Haemoglobin, Haematocrit, RBC count) immediately following the 6MWT.

3. To evaluate the effect of the 6MWT on leukocyte count and differentials (Neutrophils, Lymphocytes, Monocytes).

1.5 Research Question

What are the baseline haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, and platelet count in healthy young adults?

What is the effect of the 6MWT on Hb, PCV, RBC count, WBC count, differentials, and platelet indices immediately post-exercise?

1.6 Justification of the Study

The Six-Minute Walk Test (6MWT) is a globally recognized, standardized tool for assessing functional exercise capacity. While its general physiological demands are understood, a critical and detailed investigation into its acute effects on the haematological system in healthy young adults is warranted for several compelling reasons:

Existing literature on exercise hematology is predominantly based on studies involving **maximal or high-intensity exercise** (e.g., graded exercise tests to exhaustion, high-intensity interval training). The haematological response to a self-paced, submaximal, and functional exercise like the 6MWT has not been systematically characterized. Furthermore, reference equations and normative data for the 6MWT itself are often derived from older or clinically impaired populations, making them inaccurate for healthy young adults. This study is justified by the critical need to establish a **foundational, population-specific baseline** for haematological changes in this demographic.

1.7 Significance of the Study

By establishing a clear set of normative hematological responses to the 6MWT in healthy young adults, this study will create a crucial reference standard. This baseline will be invaluable for future research and clinical practice involving populations where both functional capacity and hematological status are compromised, such as in individuals with sickle cell disease, chronic fatigue syndrome, heart failure, or chronic obstructive pulmonary disease. It will allow clinicians and researchers to more accurately distinguish between a normal, adaptive physiological response to the test and a potentially pathophysiological one, leading to more precise interpretations and better-informed clinical decisions.

From a physiological perspective, the findings will enhance our fundamental understanding of how the hematological system responds to moderate, sustained exercise that closely mimics the demands of daily living. This contrasts with the large body of existing literature focused on maximal exertion, providing a more nuanced view of the body's response to functional activity. Methodologically, this research will contribute to the deeper physiological characterization of the 6MWT, reinforcing its validity and expanding its utility as a robust tool for both clinical assessment and scientific inquiry.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HEMATOLOGICAL ADAPTATIONS TO ACUTE SUBMAXIMAL EXERCISE

The onset of physical exercise triggers an immediate and significant redistribution of body fluids. The most prominent of these shifts is a movement of plasma fluid out of the intravascular compartment (the blood vessels) and into the interstitial space, particularly within and around the contracting muscles (Idowu *et al*., 2024). This process, which results in a state of transient hemoconcentration, is governed by fundamental principles of fluid dynamics, primarily the Starling forces across the capillary membrane.

During exercise, several factors combine to drive this fluid shift. First, the increase in heart rate and blood pressure raises the hydrostatic pressure within the capillaries, effectively pushing fluid out into the surrounding tissues (Neves *et al*., 2015). Second, the metabolic activity of the muscles produces a large number of osmotically active particles (metabolites such as lactate, phosphate, and potassium ions), which accumulate in the interstitial fluid. This increases the osmotic pressure of the interstitial space, pulling water out of the capillaries to balance the concentration gradient.

The result is a rapid decrease in the circulating plasma volume, which can range from 5% to as much as 20%, with the magnitude of the decrease being dependent on the intensity and duration of the exercise (Idowu *et al*., 2024). Because large molecules such as plasma proteins (e.g., albumin, fibrinogen) and all blood cells (erythrocytes, leukocytes, and platelets) are too large to easily pass through the capillary endothelium, they are left behind in a smaller volume of plasma. This leads to a relative increase in their concentration in the blood (Ivanov, 2022; Waltz *et al*., 2013). This exercise-induced hemoconcentration is a key mechanism underlying many of the acute hematological changes observed during physical activity. The process begins almost instantaneously with the start of exercise and, following cessation of activity, the plasma volume typically returns to its baseline level within 30 to 60 minutes as fluid shifts back into the vasculature (Ivanov, 2022).

2.1.1 CHANGES IN RED BLOOD CELL COUNT, HEMOGLOBIN, AND HEMATOCRIT

Red blood cells (erythrocytes) are the most numerous cells in the blood, and their primary function is the transport of oxygen from the lungs to the tissues, a role mediated by the iron-containing protein hemoglobin (Hb). An individual's total red cell mass and, by extension, their total hemoglobin mass, is a primary determinant of the blood's oxygen-carrying capacity and is therefore a critical factor for aerobic exercise performance (Mairbäurl, 2013).

In response to a single, acute bout of exercise, the key erythrocyte parameters, red blood cell (RBC) count, hemoglobin concentration, and hematocrit (Hct; the percentage of blood volume occupied by red cells), all show a significant and immediate increase (Idowu *et al*., 2024). This increase, which can be in the range of 10-30% following short, intense exercise, is not the result of the production of new red cells (erythropoiesis), which is a process that takes days to weeks (Neves *et al*., 2015). Instead, this acute rise is almost entirely a passive consequence of the exercise-induced hemoconcentration described previously. As plasma volume decreases, the existing red cells become more concentrated, leading to higher measured values for RBC count, Hb, and Hct (Okon *et al*., 2025).

It is crucial to differentiate this acute, transient response from the chronic adaptations seen with long-term endurance training. Highly trained athletes often exhibit resting hemoglobin and hematocrit values that are at the lower end of the normal range or even slightly below it (Ihegihu *et al*., 2022). This condition, often termed "sports anemia" or "pseudoanemia," is not a pathological state but rather a beneficial physiological adaptation. Endurance training stimulates a chronic expansion of the plasma volume that is proportionately greater than the training-induced increase in total red cell mass (Mairbäurl, 2013). This hemodilution reduces blood viscosity, which in turn decreases the workload on the heart and may enhance blood flow and thermoregulation during exercise (Waltz *et al*., 2013).

The baseline hemoglobin level, even within the normal physiological range, has a direct and significant impact on functional exercise capacity. Studies have demonstrated a strong, independent, and linear relationship between hemoglobin concentration and 6MWD (Ivanov, 2022; Waltz *et al*., 2013). A lower hemoglobin level means a lower oxygen-carrying capacity, which can lead to reduced oxygen delivery to the muscles, earlier onset of fatigue, and consequently, a shorter distance walked. The effect is clinically meaningful; it has been shown that a decrease of approximately two grams per deciliter in hemoglobin concentration is associated with a reduction in 6MWD of about 50 meters, a change that is considered significant for patients.[48] This underscores the importance of the hematological system in determining functional performance.

2.1.2 EXERCISE-INDUCED LEUKOCYTOSIS

The response of the immune system, specifically the white blood cells (leukocytes), to acute exercise is one of the most dramatic and complex of all physiological systems. A single bout of exercise induces a rapid and substantial increase in the number of circulating leukocytes, a phenomenon known as exercise-induced leukocytosis (Neves *et al*., 2015; Sand, 2013). Different leukocyte subsets are mobilized with distinct magnitudes and time courses, driven by a complex interplay of neuroendocrine signals and hemodynamic forces. The following table provides a summary of the expected acute hematological changes:

Table 2. 1: Summary of Acute Hematological Changes Following Submaximal Exercise

Parameter	**Immediate Post-Exercise Change**	**Primary Mechanism**	**Typical Recovery Time**
Plasma Volume	↓ (5-20%)	Fluid shift to interstitium	30-60 minutes
Hematocrit (Hct)	↑ (10-30%)	Hemoconcentration	30-60 minutes

Parameter	**Immediate Post-Exercise Change**	**Primary Mechanism**	**Typical Recovery Time**
Hemoglobin (Hb)	↑ (10-30%)	Hemoconcentration	30-60 minutes
RBC Count	↑ (10-30%)	Hemoconcentration	30-60 minutes
Total WBC Count	↑↑ (50-100%)	Demargination, Mobilization	Biphasic (see below)
Lymphocytes	↑↑ (Immediate)	Catecholamine-driven demargination	Biphasic: Lymphopenia 1-3h post
Neutrophils	↑ (Slight, immediate) then ↑↑ (Delayed)	Demargination, then Cortisol-driven release	> 24 hours
Monocytes	↑ (Immediate)	Catecholamine-driven demargination	~ 1 hour
Platelet Count	↑	Demargination (splenic	~ 24 hours

Parameter	Immediate Post-Exercise Change	Primary Mechanism	Typical Recovery Time
		contraction)	

The response of the lymphocyte population to acute exercise is characteristically biphasic. The initial phase is an **instantaneous and pronounced lymphocytosis**, where the number of circulating lymphocytes can increase by 50-100% or more. This surge begins immediately with the onset of exercise and typically peaks at or shortly after its cessation, lasting for approximately 45 to 60 minutes (Meyer-Lindemann *et al*., 2023). This mobilization is not equal across all lymphocyte subsets. The most dramatic increases are seen in Natural Killer (NK) cells and cytotoxic (CD8+) T-lymphocytes, which are highly sensitive to the effects of stress hormones (Sand, 2013).

This initial lymphocytosis is followed by a second phase: a **transient lymphopenia**. Within one to three hours after the completion of exercise, the number of circulating lymphocytes, particularly NK and CD8+ T-cells, drops significantly, often to levels below the pre-exercise baseline (Meyer-Lindemann *et al*., 2023). For many years, this post-exercise lymphopenia was interpreted as a period of immune suppression, leading to the "open window" theory, which posited an increased susceptibility to infection in the hours following strenuous exercise. This lymphopenia is now understood not as a suppression of the immune system, but as a strategic **redeployment** of effector lymphocytes out of the circulation and into peripheral tissues (such as the lungs, gut, and skin) (Arena *et al*., 2007; Sand, 2013). This process is thought to enhance immune surveillance, allowing these potent immune cells to patrol for potential pathogens or tissue damage in the periphery.

The responses of the myeloid lineage cells, monocytes and neutrophils, follow different pathways. Monocytes exhibit a response pattern similar to that of lymphocytes: a rapid increase in their circulating numbers immediately following exercise, which typically resolves and returns to baseline within about an hour (Meyer-Lindemann *et al*., 2023b).

The neutrophil response is characterized by its delayed nature. While there may be a small, immediate increase in neutrophil count due to demargination and hemoconcentration, the most significant change is a **delayed neutrophilia**. The number of circulating neutrophils begins to rise in the hour following exercise, peaks several hours later, and can remain elevated for 24 hours or more before returning to baseline. This second wave of leukocytosis is not due to the mobilization of cells already in the vasculature, but rather to the release of mature neutrophils from the large reserve pool stored in the bone marrow (Risøy *et al*., 2003).

2.1.3 CATECHOLAMINES, CORTISOL, AND HEMODYNAMIC FACTORS

The distinct kinetics of the different leukocyte subsets are driven by different underlying mechanisms, primarily neuroendocrine and hemodynamic in nature. The immediate, rapid mobilization of lymphocytes (especially NK and CD8+ cells) and monocytes is largely orchestrated by the surge in **catecholamines**, adrenaline (epinephrine) and noradrenaline (norepinephrine), that accompanies the onset of exercise (Meyer-Lindemann *et al*., 2023). These hormones bind to β2-adrenergic receptors (β2-AR) expressed on the surface of these leukocytes, which weakens their adhesion to the endothelial cells lining the blood vessel walls (Sand, 2013).

This effect, combined with the **hemodynamic shear forces** generated by the exercise-induced increase in cardiac output and blood flow, effectively "washes" these marginated cells off the vessel walls and into the active circulation, causing the rapid leukocytosis (Meyer-Lindemann *et al.*, 2023).

The delayed neutrophilia, on the other hand, is primarily mediated by a different stress hormone: **cortisol** (Sand, 2013). Cortisol levels rise more slowly during exercise and peak in the recovery period. Cortisol acts to mobilize neutrophils from the bone marrow storage pool and also appears to inhibit their apoptosis and migration out of the bloodstream, leading to their accumulation in the circulation for many hours post-exercise *(*Risøy *et al.*, 2003). Other factors, such as the inflammatory cytokine Interleukin-6 (IL-6) released from contracting muscles, also contribute to stimulating this neutrophil release (Neves *et al.*, 2015). The magnitude of this entire neuroendocrine and subsequent leukocyte response is known to be highly dependent on the intensity and duration of the exercise stimulus (Neves *et al.*, 2015).

2.1.4 EXERCISE-INDUCED THROMBOCYTOSIS AND ACTIVATION STATE

Platelets (thrombocytes) are small, anucleated cell fragments that play a central role in hemostasis and thrombosis. Similar to other formed elements of the blood, their circulating numbers are acutely affected by exercise. A single bout of physical activity consistently and immediately causes an increase in the peripheral platelet count, a state known as **thrombocytosis** (Belviranli *et al.*, 2017; Neves *et al.*, 2015).

The mechanisms underlying this rapid increase are twofold. First, the general hemoconcentration that occurs with exercise leads to a passive, relative increase in platelet concentration (Niazi *et* *al*., 2025). Second, and more significantly, is the active mobilization of platelets from storage pools. The spleen serves as a major reservoir for platelets, sequestering up to one-third of the total platelet mass. The sympathetic nervous system activation and catecholamine release that occur during exercise can cause splenic contraction, which expels these stored platelets into the general circulation, contributing to the observed thrombocytosis (Neves *et al*., 2015).

2.2 DEFINITION OF 6-MINUTE WALK TEST (6MWT)

The 6-minute walk test (6MWT) is defined as a practical test designed to evaluate functional exercise capacity by measuring the distance an individual can walk on a flat, hard surface in a period of 6 minutes (Kammin, 2022). This standardized assessment tool has gained well-known acceptance in both clinical and research settings due to its simplicity, cost-effectiveness, and ability to provide meaningful information about an individual's functional status and exercise tolerance (Du *et al*., 2009). The test is classified as a submaximal exercise test because it does not require participants to exercise to exhaustion, making it suitable for a wide range of populations, including healthy young adults such as university students (Du *et al.*, 2009). The six-minute walk test (6MWT), originally introduced by Butland et al. in 1982 as a modified alternative to the 12-minute walk test developed by McGavin et al., has gained widespread recognition for its utility in evaluating functional capacity in adults with limited physical endurance (Abdulkadir *et al*., 2019). The fundamental principle underlying the 6MWT is that it reflects the functional exercise level required for most daily activities, as these activities are typically performed at submaximal levels rather than at maximum exertion (American Thoracic Society, 2002). This characteristic makes the test particularly relevant for assessing functional capacity in real-world contexts, where individuals must sustain moderate levels of activity over extended periods. The test's focus on endurance rather than peak performance provides valuable insights into an individual's ability to maintain physical activity levels that are required for normal daily functioning, academic activities, and recreational pursuits (Du *et al*., 2009). The 6MWT is distinguished from other exercise tests by its self-paced nature, allowing participants to control their walking speed and rest as needed during the test period. This self-regulation aspect is particularly important when assessing healthy populations, as it enables individuals to optimize their performance based on their perceived capabilities and comfort levels (Puente-Maestu, 2020). The test outcome is primarily measured as the total distance walked in 6 minutes, though additional parameters such as heart rate response, oxygen saturation, perceived exertion, and symptoms can also be monitored to provide a more comprehensive assessment of functional capacity (Du *et al*., 2009).

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). Prior to this standardization, variations in test protocols often led to inconsistent findings, making it challenging to synthesize data and establish reliable reference values. The ATS guidelines thus transformed the 6MWT into a robust and dependable tool for both clinical assessment and scientific inquiry. These standardized protocols ensure consistency in test administration across different settings and populations, enabling meaningful comparison of results and facilitating the development of normative values (Kammin, 2022). The ATS guidelines specify precise requirements for the testing environment, equipment, participant preparation, test instructions, and encouragement procedures, all of which are essential for obtaining reliable and valid results (American Thoracic Society, 2002).

The 6-Minute Walk Test (6MWT) is a simple, safe, and inexpensive submaximal exercise test used to assess functional capacity and cardiorespiratory fitness (Du *et al*., 2009). It evaluates the distance a person can walk on a flat surface in six minutes, providing valuable information on exercise tolerance and oxygen saturation (Akinpelu *et al*., 2023). The 6MWT has shown a strong positive correlation with VO_2max, indicating its effectiveness in measuring aerobic fitness (Garcia *et al*., 2015). It can be used to monitor progress in functional capacity and endurance for various populations, including young asymptomatic adults and hypokinetic workers (Pollentier *et al*., 2010). The test is well-tolerated by patients and has proven to be reproducible (Zotter-Tufaro *et al*., 2015). Adapting the 6MWT as a self-administered assessment tool could potentially enhance clinicians' ability to monitor functional status and promote self-management in chronic heart disease patients (Du *et al*., 2009).

The fundamental strength of the 6MWT lies in its ability to assess functional capacity at a submaximal level, which is a key differentiator from maximal exercise tests such as cardiopulmonary exercise testing (CPET) (Shabbir et al., 2021). While maximal tests provide information on peak exercise capacity, the 6MWT more closely reflects the physical demands of daily living activities. This characteristic makes the 6MWT exceptionally well-tolerated by a broad spectrum of individuals, including those who may be unable to perform more strenuous, high-intensity tests due to various health conditions or low fitness levels. Its ease of administration and patient comfort contribute significantly to its practicality in diverse clinical and research environments (Du *et al*., 2009). Furthermore, the 6MWT provides a holistic assessment of functional capacity by integrating the responses of multiple physiological systems during a submaximal, real-world activity. This is a profound advantage (Shabbir *et al*., 2021). The test evaluates the coordinated function of the cardiac system (heart's pumping efficiency), the pulmonary system (lung capacity and gas exchange), peripheral and systemic circulation (blood flow and oxygen delivery to tissues), neuromuscular units (nerve-muscle coordination), the 6MWT does not merely measure an isolated physiological parameter, but rather assesses how effectively all these vital systems work in concert to support a common, everyday activity like walking (Akinpelu *et al.*, 2023).

2.2.1 PRINCIPLE OF THE 6-MINUTE WALK TEST

The 6-Minute Walk Test (6MWT) serves as an invaluable tool for evaluating the integrated responses of several key physiological systems during sustained physical activity. The 6-Minute Walk Test (6MWT) provides a comprehensive snapshot of an individual's exercise tolerance and functional reserve (Kammin, 2022). Firstly, the 6MWT assesses the efficiency and reserve of the cardiopulmonary system (Shabbir *et al*., 2021). During the six minutes of walking, the heart's ability to pump oxygenated blood effectively to the working muscles is challenged, and the lungs' capacity for efficient gas exchange (taking in oxygen and expelling carbon dioxide) is evaluated. The distance covered reflects the combined capacity of these vital organs to support aerobic activity (Pollentier *et al*., 2010). Secondly, the test reflects the integrity of **peripheral and systemic circulation**. Adequate blood flow throughout the body, particularly to the extremities, is essential for delivering oxygen and nutrients to the active muscles and removing metabolic waste products. The 6MWT provides an indirect measure of how well this circulatory network supports sustained muscular effort (Shabbir *et al*., 2021).

Thirdly, insights are gained into the function of neuromuscular units and muscle metabolism (Sperandio *et al*., 2015). The test challenges the muscles' ability to generate and sustain force, as well as their metabolic pathways for energy production (aerobic and anaerobic) (Motl *et al.*, 2012). It indicates how efficiently muscles can perform repetitive work without succumbing to excessive fatigue, reflecting their endurance and coordination. The distance walked (6MWD) is thus a direct outcome of the integrated and global responses of all these physiological systems working in concert, making it a robust and ecologically valid measure of overall exercise tolerance and functional status (Shabbir *et al*., 2021). The test reveals age-related differences, with sexagenarians achieving greater walking distances and oxygen uptake than septuagenarians, while maintaining similar walking efficiency. Metabolically, the 6MWT comprises two distinct phases: the first 3 minutes involve mixed aerobic and anaerobic work, while the last 3 minutes represent steady-state aerobic metabolism (Motl *et al*., 2012). In children with juvenile idiopathic arthritis, the 6MWT elicits responses at 80-85% of peak heart rate and oxygen uptake, indicating intense submaximal exercise (Paap *et al*., 2005).

2.2.2 STANDARDIZATION BY THE AMERICAN THORACIC SOCIETY (ATS) AND ITS SIGNIFICANCE

In 2002, the American Thoracic Society (ATS) published official guidelines for the administration of the six-minute walk test (6MWT), which were later updated in a 2014 joint technical statement and systematic review by the ATS and the European Respiratory Society (ERS). Standardization of test protocols is critical to ensure the reliability and reproducibility of results (Kammin, 2022). The 6MWT should ideally be conducted along a straight, flat corridor with minimal foot traffic and a length of at least 30 meters, aligning with the reference standards established in normative studies (Agarwal *et al*., 2023). While validated reference equations exist for shorter track lengths, such as 20 meters and 10 meters, these adjustments are generally reserved for clinical settings with spatial limitations. The configuration and length of the walking track significantly influence test outcomes. For instance, in patients with chronic obstructive pulmonary disease (COPD), walk distances were notably longer on 30-meter tracks compared to 10-meter tracks. Furthermore, continuous track designs such as oval, square, or circular layouts yield longer distances due to reduced time spent turning (Beekman *et al*., 2013). Although treadmills can be used to accommodate space constraints and facilitate continuous monitoring, they may underestimate walking capacity due to patient unfamiliarity (Kammin, 2022).

The meticulous standardization of the 6MWT by the American Thoracic Society (ATS) is paramount for ensuring the reliability, reproducibility, and comparability of results across different clinical settings, research studies, and patient populations. Despite its apparent simplicity, the test demands strict adherence to a detailed protocol, as even minor methodological deviations can significantly influence the 6MWD (Agarwala and Salzman, 2020). This emphasizes a crucial understanding: while the 6MWT is lauded for its simplicity and practicality, its accurate and reproducible administration is far from trivial. This implies that proper training and strict adherence to protocol are paramount, especially in research settings, to avoid confounding variables and ensure data integrity (Kammin, 2022). The "simplicity" often attributed to the 6MWT refers primarily to its accessibility and tolerability for patients, not to the ease of its administration for researchers or clinicians. For these professionals, the detailed ATS guidelines are not merely suggestions but fundamental requirements for obtaining scientifically valid and generalizable findings. Any deviation, even seemingly minor, can introduce significant bias and invalidate comparisons. Comprehensive training for administrators and strict adherence to protocol are essential to harness the true value of the 6MWT as a scientific instrument (American Thoracic Society, 2002).

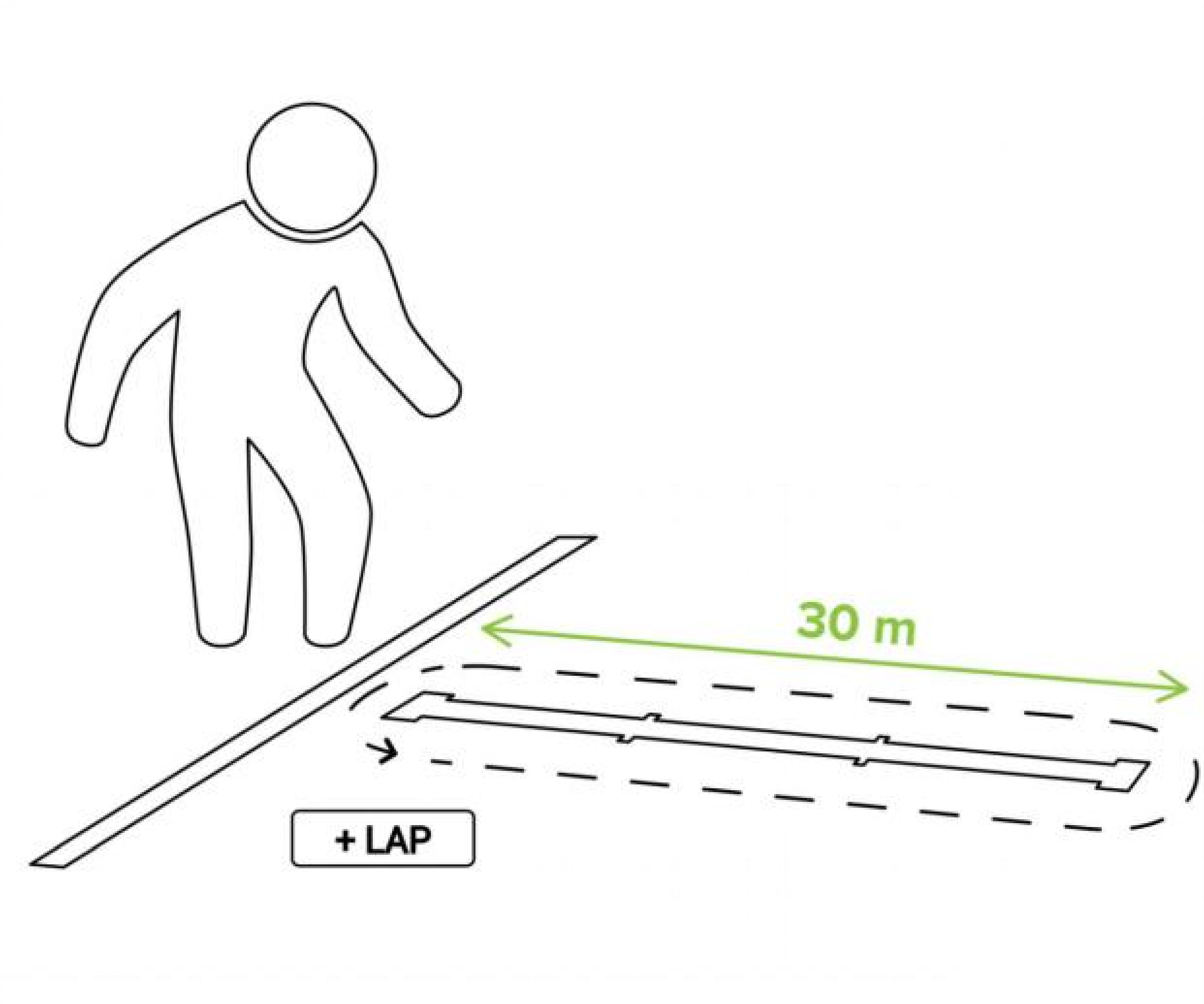

Figure 2. 1: The 6-Minute Walk Test (6MWT) walking course Source: (**https://support.mesimedical.com/app/uploads/2023/09/6MWT-01-01-1536x1283.jpg**) Accessed: 18/07/2025

Key aspects of this standardization include:

Testing Environment: The ATS guidelines strongly recommend performing the 6MWT indoors, along a flat, straight, hard-surfaced, and minimally trafficked corridor. The ideal corridor length is 30 meters (approximately 100 feet). Clear markings should be placed every 3 meters along the corridor, and turnaround points at each end must be distinctly indicated, typically with bright orange traffic cones (Kammin, 2022). The rationale for a 30-meter corridor is critical: shorter corridors necessitate more frequent turns, which can reduce the total 6MWD due to the time and effort expended in changing direction (AgarwalaandSalzman, 2020). Studies have shown that continuous (oval) tracks can result in longer walk distances compared to straight tracks, further emphasizing the impact of track layout (Kammin, 2022). The use of a treadmill is explicitly not recommended, as it prevents patients from self-pacing, leading to shorter and non-interchangeable distances compared to corridor tests (American Thoracic Society, 2002).

Equipment: Essential equipment for a standardized 6MWT includes a stopwatch or countdown timer, a mechanical lap counter to accurately track completed laps, two small cones for turnaround points, a movable chair (for resting if needed), and worksheets on a clipboard for recording data (AgarwalaandSalzman, 2020). Additionally, a sphygmomanometer for blood pressure measurement, a source of oxygen for emergency use, and a telephone for rapid emergency response are crucial safety provisions. A pulse oximeter for measuring heart rate (HR) and oxygen saturation (SpO2) is highly recommended, as is the Borg Breathlessness Scale for assessing perceived exertion (RPE) (American Thoracic Society, 2002).

Patient Preparation: Prior to the test, participants should be instructed to wear comfortable clothing and appropriate walking shoes. They should continue their usual medical regimen, and any regular walking aids (e.g., cane, walker) must be used during the test and documented (American Thoracic Society, 2002). To ensure consistent effort and avoid acute fatigue, vigorous exercise should be avoided within two hours preceding the test. A light meal is generally acceptable before morning or early afternoon tests. Resting vital signs, including heart rate, blood pressure (systolic and diastolic), oxygen saturation, and perceived exertion (using the Borg scale), should be accurately recorded before the test commences (AgarwalaandSalzman, 2020).

Instructions and Encouragement: Standardized instructions are paramount to ensure that all participants understand the test's objective and perform with consistent effort. The patient is clearly told, "The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway." They are also informed that they may get out of breath or tired and are permitted to slow down, stop, or rest as necessary, but should resume walking as soon as they are able (American Thoracic Society, 2002). Running or jogging is explicitly prohibited. Crucially, standardized verbal encouragement must be provided at specific intervals (e.g., every minute) to optimize effort and minimize variability in performance due to motivational factors. This consistent encouragement helps to standardize the intensity of effort across different individuals and studies, which has been identified as a significant factor influencing 6MWD (AgarwalaandSalzman, 2020; American Thoracic Society, 2002; Kammin, 2022).

Endpoints and Safety Considerations: The test concludes precisely at the six-minute mark, at which point the total distance walked is measured to the nearest meter. Immediately following the test, post-test physiological parameters (heart rate, blood pressure, oxygen saturation, and perceived exertion) are recorded again. If a patient stops during the test, the timer is not paused; the time of stopping and restarting, along with the reason for cessation, must be documented. Reasons for immediate test termination include intolerable dyspnea, chest pain, leg cramps, staggering, diaphoresis, or a pale/ashen appearance. A persistent drop in SpO2 below 80% is also a critical stopping criterion. Test administrators must be certified in basic life support and cardiopulmonary resuscitation to manage potential complications, although the 6MWT is generally considered a safe test with rare complications (American Thoracic Society, 2002; Kammin, 2022; Shabbir *et al*., 2021).

The ATS guidelines also address factors affecting reproducibility and reliability (American Thoracic Society, 2002). A "learning effect" is commonly observed, where individuals tend to walk slightly farther in a second test compared to the first, often showing a 2-8% improvement (AgarwalaandSalzman, 2020). To account for this, it is recommended to perform at least two tests, with the best distance recorded, and to allow adequate rest (a minimum of 30 minutes) between tests if conducted on the same day. Inconsistent verbal encouragement or variations in track layout and length can also significantly influence results, further underscoring the need for strict adherence to the standardized protocol (American Thoracic Society, 2002; Pepera *et al*., 2023).

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

Table 2. 2: 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.
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 2.2 provides a summary of the 6MWT procedural guidelines by the American Thoracic Society (ATS) (American Thoracic Society, 2002; Kammin, 2022).

Table 2. 3: Normative 6MWD Values by Age and Sex (General Healthy Adult Populations)

Age Group (Years)	Population/Source	Mean 6MWD (meters) - Male	Mean 6MWD (meters) - Female	Key Findings/Notes
18-30	Healthy Individuals (General)	554.32 ± 28.69	491.31 ± 50.39	Males significantly higher 6MWD than females.
18-35	Apparently Healthy Nigerian Young Adults (Ile-Ife)	608.2 ± 207.79	565.9 ± 69.49	Overall mean 619.41 ± 125.76m. Males higher.
18-40	North-African Subjects	680 ± 70 (overall SD)	N/A	BMI, sex, age influenced distance. Norms from other populations not reliable.
18-50	Healthy Young Adults (Two-center study)	Median 637m (IQR 584-686m) overall	N/A	Age had minimal impact in this group. Weak correlation with height, weight, BMI.
20-59	Mixed Populations	736 (88.3)	674 (76.8)	Significant sex differences observed.
40-49	Healthy Adults (7 countries)	611 ± 85	N/A	Part of a larger cohort study.

45-70	Apparently Healthy Nigerian Adults	405.67 ± 26.76	353.80 ± 39.30	Overall mean 378m. Males significantly higher. Age and height significant predictors.
60-69	Community-dwelling Elderly	572	538	Decline with age evident.
60+	Mixed Populations	599 (125.4)	550 (115.0)	Significant sex differences observed.

Table 2.3 provides a summary of normative data, and influential factors for the 6MWT with comprehensive overview for context and comparison (Akinpelu *et al.*, 2023; Bourahli et al., 2016; Garcia *et al*., 2015; Ihegihu *et al.*, 2022; Paap *et al*., 2005; Sperandio *et al*., 2015)

2.3 APPLICATIONS OF THE 6-MINUTE WALK TEST (6MWT) IN YOUNG ADULTS

The 6-Minute Walk Test (6MWT) serves as a highly valuable and practical tool for establishing baseline functional capacity in healthy young adults. This baseline measurement provides a quantitative starting point against which future changes in fitness can be objectively assessed. For instance, it is widely employed to monitor the effectiveness of physical training programs, lifestyle interventions, or rehabilitation protocols over time (Shabbir *et al*., 2021). An increase in the 6MWD following an intervention is a clear indicator of improved aerobic capacity and endurance (*6 Minute Walk Test*, 2021). Clinically, a meaningful change in 6MWD, signifying a real improvement in a patient's condition, is often considered to be around 30 meters, though some studies suggest values up to 45 meters in specific populations. This responsiveness to change makes the 6MWT an effective outcome measure for evaluating the efficacy of various health and fitness initiatives (Casanova *et al*., 2011).

The 6MWT was initially developed for patients with cardiopulmonary diseases such as Chronic Obstructive Pulmonary Disease (COPD), heart failure, cystic fibrosis, and pulmonary hypertension (AgarwalaandSalzman, 2020), its application has significantly expanded to include healthy adult populations. This broader use is driven by the need to establish robust reference values for different demographics. For healthy adults aged 18-30 years, reported 6MWD values typically range from 400 to 700 meters (Kammin, 2022). More specific studies provide normative data; for example, apparently healthy Nigerian young adults (18-35 years) in Ile-Ife have shown a mean 6MWD of 619.41 ± 125.76 meters (Ihegihu *et al*., 2022), while a two-center study of young adults (18-50 years) reported a median 6MWD of 637 meters (Casanova *et al*., 2011). Beyond general fitness assessment, the 6MWT is also pertinent for young adults in specific clinical contexts. It is utilized in the assessment and monitoring of various neuromuscular conditions, such as Charcot-Marie-Tooth disease and myasthenia gravis, providing valuable information on functional changes over time (Kammin, 2022). Furthermore, in contemporary healthcare, it has found new applications in monitoring recovery, particularly in patients recovering from conditions like post-COVID-19 pneumonia, where cardiopulmonary endurance may be compromised (Agarwal *et al*., 2023). The 6MWT also demonstrates a good correlation with peak oxygen uptake (VO2 max), which is considered the gold standard for aerobic capacity measurement. This correlation positions the 6MWT as a reliable proxy for aerobic fitness, making it a valuable tool even when more complex and expensive CPET is not feasible (Kammin, 2022).

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 Materials and Instruments

The following materials and instruments were used for data collection and analysis:

1. **Haematological Analysis:** Automated Haematology Analyzer (for Full Blood Count and indices including Hb, HCT, RBC, MCV, MCH, WBC, and differentials).
2. **Blood Collection:** Sterile venepuncture sets (needles, syringes), tourniquet, antiseptic swabs, and **K3EDTA tubes**.
3. **6MWT Equipment:** A calibrated 30-meter walk track, marking cones, a stopwatch, and data collection forms.
4. **Safety Equipment:** A movable chair for resting, sphygmomanometer, and a first-aid kit.

3.2 Study Area

This study was conducted at the University of Benin, Benin City, Edo State, Nigeria. The 6MWT was administered in a suitable, flat, and unobstructed 30-meter corridor within the university premises, adhering to the American Thoracic Society (ATS) guidelines.

3.3 Study Design

This study employed a **pre-post intervention design** (also known as a within-subjects or repeated-measures design). This design was selected because it allows for the comparison of haematological parameters in the same individuals under two conditions: at rest (pre-test) and immediately after a standardized exercise intervention (post-test). This approach effectively controls for inter-individual variability and enhances the statistical power to detect changes resulting from the intervention.

3.4 Study Population

3.4.1 Participants

The study participants comprised forty-four (44) healthy adult male students of the University of Benin. Participants were recruited through voluntary response and word-of-mouth.

3.4.2 Inclusion Criteria

a) Male students of the University of Benin.

b) Aged between 18 and 29 years.

c) Apparently healthy with no known chronic illnesses.

3.4.3 Exclusion Criteria

Individuals were excluded from the study if they met any of the following criteria:

a) History of any acute illness within the four (4) weeks preceding the study.
b) Known haematological, cardiovascular, pulmonary, or metabolic disorders.
c) Regular smokers.

d) On any medication that could influence haematological parameters.
e) Inability to understand or perform the 6MWT as per instructions.

3.5 Ethical Considerations

Ethical approval for this study was obtained from **the** Research Ethics Committee, College of Medical Sciences, University of Benin, Benin City**.** Before the commencement of the study, the purpose, procedures, potential risks, and benefits were thoroughly explained to all prospective participants. Written informed consent was obtained from each participant, affirming their voluntary agreement to partake in the study. Confidentiality of all participant data was maintained throughout the research process.

3.6 Study Procedure

The procedure for each participant was completed in a single session and followed these sequential steps:

1. Pre-Test Assessment:

1) Participants were instructed to avoid vigorous exercise for at least two hours prior to testing.
2) Upon arrival, they were allowed to sit and rest for **10 minutes** to establish a physiological baseline.
3) Following rest, a **pre-test venous blood sample (3-5 mL)** was drawn from the antecubital vein using standard venepuncture technique under aseptic conditions.
4) The blood was collected into a **tri-potassium Ethylenediaminetetraacetic acid (K3EDTA) tube** to prevent coagulation.

5) This baseline sample was labeled and stored appropriately for subsequent Full Blood Count (FBC) analysis.

2. The Six-Minute Walk Test (6MWT):

1) Immediately after blood collection, participants performed the 6MWT as per the standardized **American Thoracic Society (ATS) 2002 guidelines**.
2) The test was conducted on a **flat, straight, 30-meter long corridor**, with the course length clearly marked every 3 meters and turnaround points indicated with cones.
3) Standardized instructions were given: *"The object of this test is to walk as far as possible for 6 minutes. You may slow down, stop, and rest if necessary, but please resume walking as soon as you are able."*
4) Standardized phrases of encouragement (e.g., "You are doing well") were provided at regular intervals.
5) The total distance walked in meters **(6MWD)** was recorded at the end of the test.

3. Post-Test Assessment:

1) Immediately upon completion of the 6-minute walk period, **a** second venous blood **sample (3-5 mL)** was collected from the participant using the same procedure as the pre-test sample into a K3EDTA tube.
2) This post-test sample was similarly labeled for immediate FBC analysis.

3.7 Data Analysis

The data generated from the haematological analysis were collated and statistically analyzed.

Data analysis was performed using GraphPad Prism version 8.1 statistical software. Continuous data were summarized and presented as Mean ± Standard Error of the Mean (SEM)**.** A paired sample T-test was used to compare the pre-test and post-test values of all haematological parameters within the same group of participants. **A** p-value of less than 0.05 ($p < 0.05$) was considered statistically significant for all analyses.

CHAPTER FOUR

4.0 RESULTS

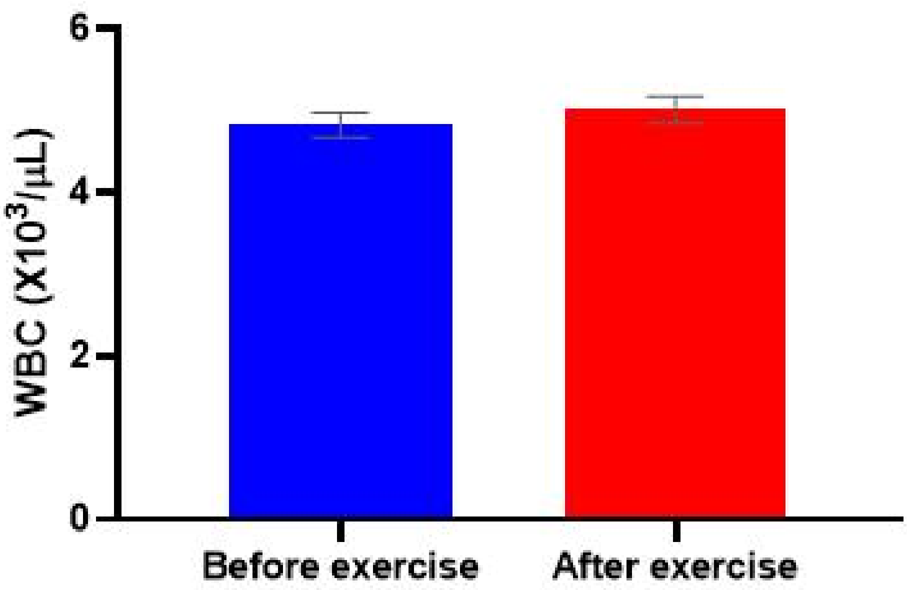

Figure 4. 1: Showed white blood cells count before and after six (6) minute walk exercise in young adult male.

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

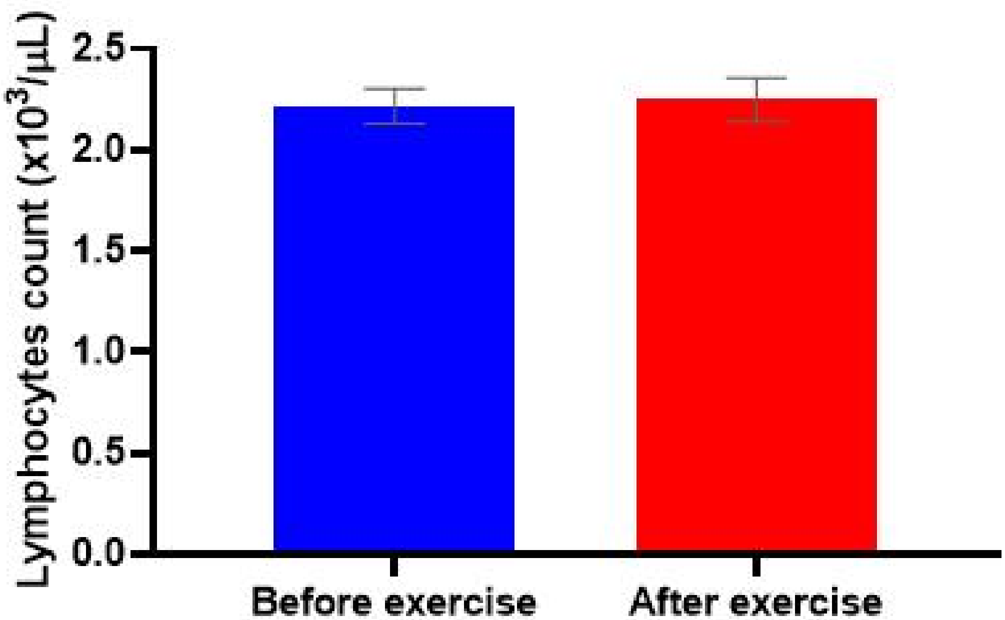

Figure 4. 2: Showed lymphocytes count before and after six (6) minute walk exercise in young adult male.

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

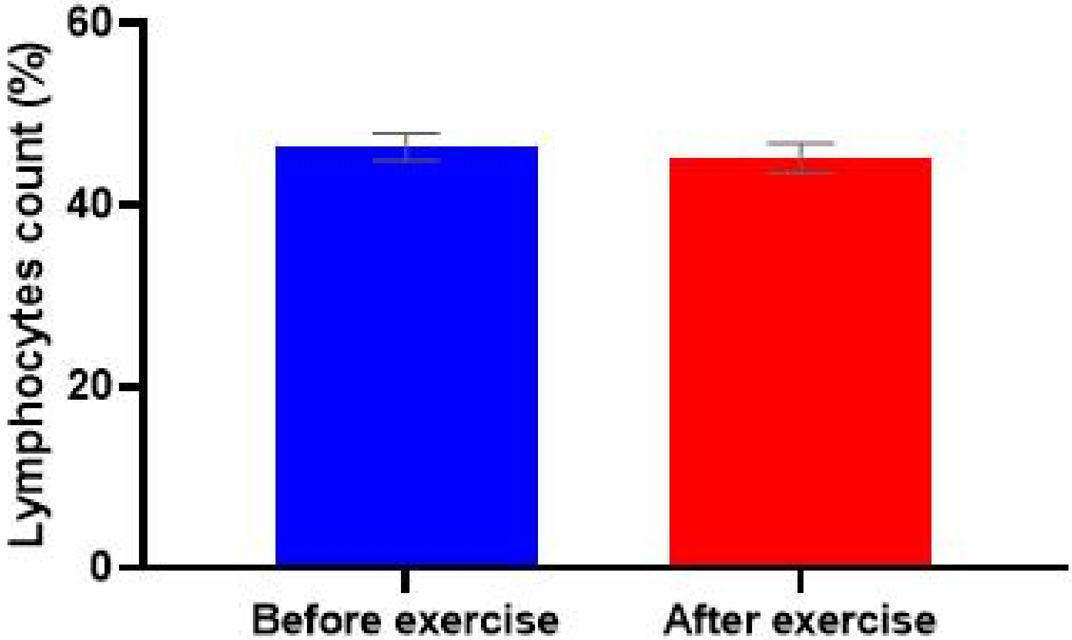

Figure 4. 3: Showed percentage lymphocytes count before and after six (6) minute walk exercise in young adult male.

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

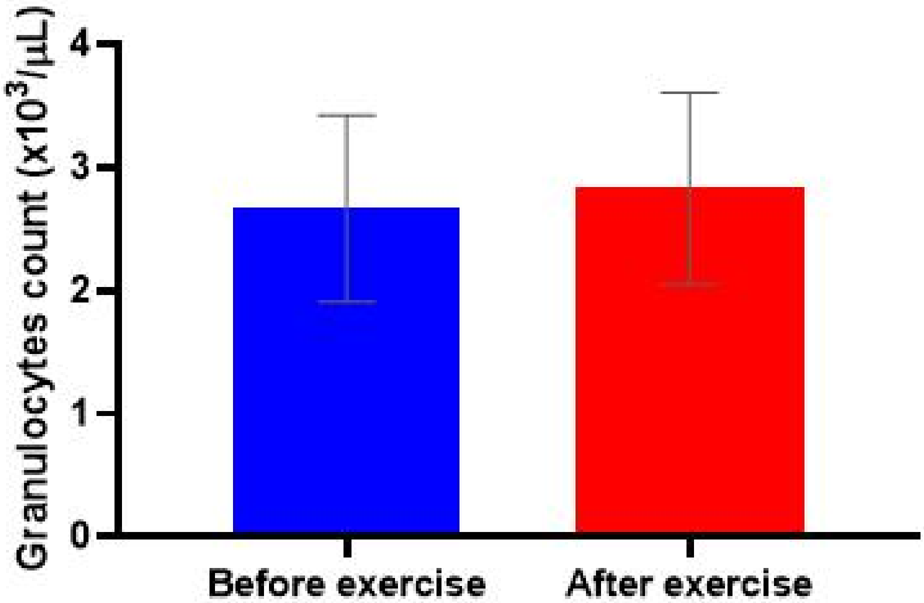

Figure 4. 4: Showed granulocytes count before and after six (6) minute walk exercise in young adult male.

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

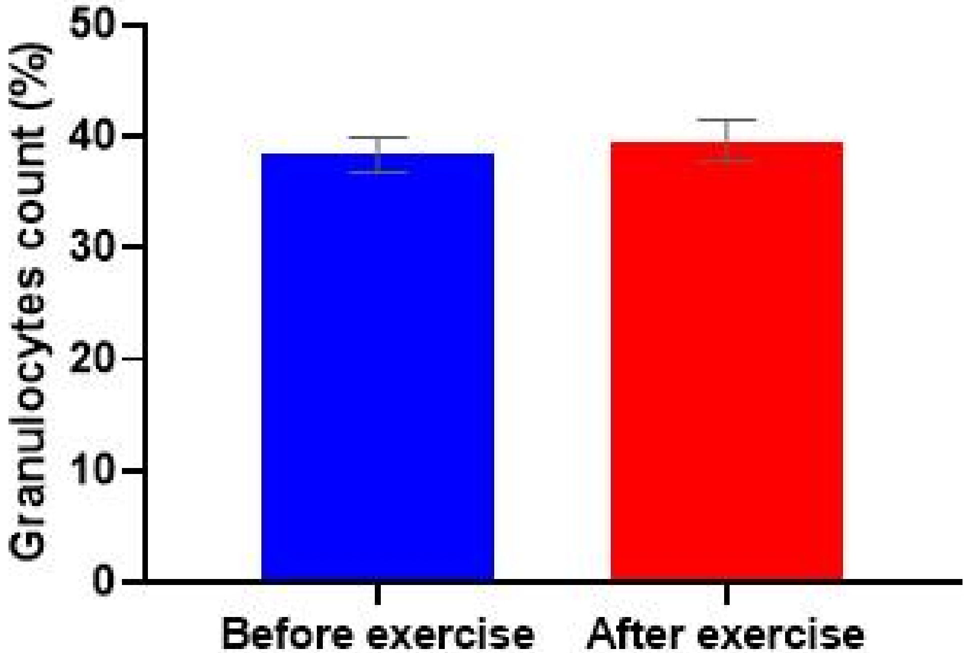

Figure 4. 5: Showed granulocytes count before and after six (6) minute walk exercise in young adult male.

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

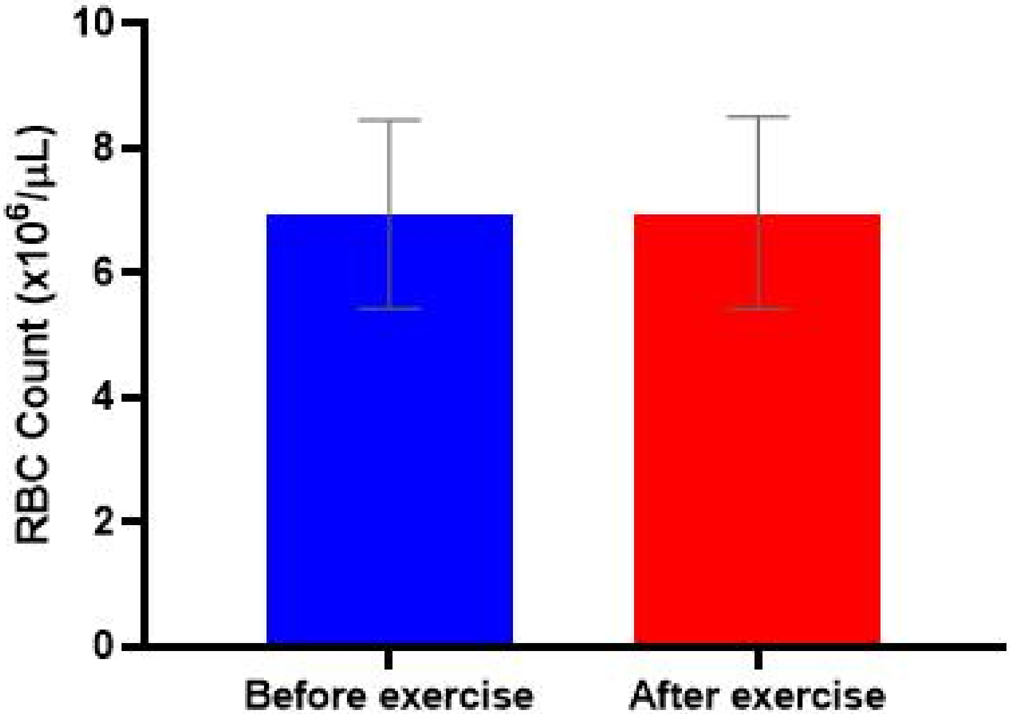

Figure 4. 6: Showed RBC count before and after six (6) minute walk exercise in young adult male.

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

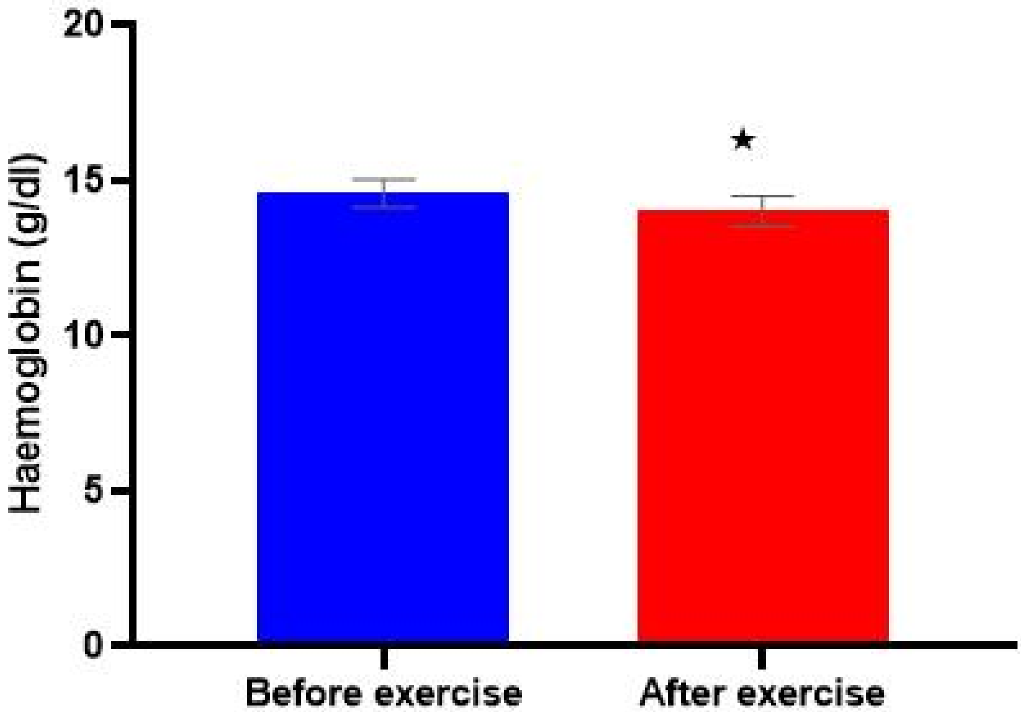

Figure 4. 7: Showed haemoglobin concentration before and after six (6) minute walk exercise in young adult male.

There was a significant reduction after exercise relative to before the exercise.

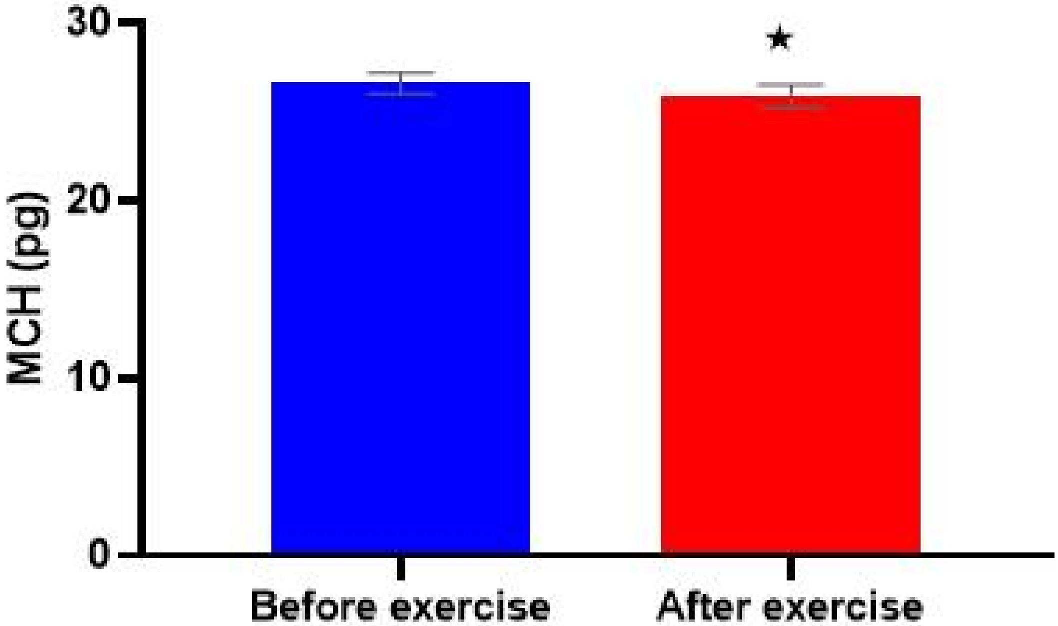

Figure 4. 8: Showed MCH before and after six (6) minute walk exercise in young adult male.

There was a significant reduction after exercise relative to before the exercise.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study aimed to evaluate the acute effects of the Six-Minute Walk Test (6MWT) on key haematological indices in healthy young adult males. The findings reveal a significant reduction in key erythrocyte indices, hemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH), and Mean Cell Volume (MCV), immediately following the exercise, with no significant changes observed in total White Blood Cell (WBC) count, lymphocyte count, granulocyte count, or Red Blood Cell (RBC) count.

5.1.1 Erythrocyte Response: Hemodilution and Hemoconcentration

The most prominent finding of this study is the significant post-exercise decrease in hemoglobin concentration. This finding directly contrasts with the classical understanding of acute exercise hematology, where an increase in hemoglobin and hematocrit is expected due to **hemoconcentration** (a reduction in plasma volume from fluid shifting into the interstitial spaces) (Mairbäurl, 2013). This phenomenon is well-documented in high-intensity or maximal exercise (Wardyn *et al*., 2008).

The observed reduction in Hb, coupled with unchanged RBC count, suggests a different mechanism may be at play during submaximal functional exercise like the 6MWT. One plausible explanation is a rapid, exercise-induced **hemodilution**, where plasma volume expands faster than the concentration of red blood cells can adjust, leading to a dilutional effect on hemoglobin concentration (Okon *et al*., 2025). Furthermore, the significant decreases in MCH and MCV suggest that the exercise may have induced subtle changes in the red blood cell population, possibly through the influx of younger, smaller red cells. Intense exercise is known to cause mechanical hemolysis of older, more fragile RBCs, and while the 6MWT is submaximal, it may still trigger a mild effect, leading to a proportional increase in younger RBCs which are characteristically smaller (Mairbäurl, 2013). This shift would be seen as a reduction in the average MCV and MCH.

5.1.2 Leukocyte Response: The Absence of Leukocytosis

The results showed no significant change in total white blood cell count, lymphocyte count, or granulocyte count following the 6MWT. This finding aligns with some studies that utilize submaximal protocols but contrasts sharply with research involving maximal exertion. Exercise-induced leukocytosis is a well-known response primarily driven by the surge in stress hormones like catecholamines and cortisol, which mobilize white blood cells from marginal pools and bone marrow (Wardyn *et al*., 2008).

The absence of leukocytosis in this study is a critical indicator that the physiological stress imposed by the self-paced, submaximal 6MWT was insufficient to trigger a significant neuroendocrine response or a systemic inflammatory state. This distinguishes the 6MWT from high-intensity exercises and underscores its nature as a mild, functional stressor. It suggests that in healthy young adults, the immune system is not markedly perturbed by this level of activity.

5.2 Conclusion

In conclusion, this study demonstrates that functional exercise in the form of the 6MWT induces a defined hematological profile in healthy young males, characterized by significant reductions in hemoglobin, MCH, and MCV without concurrent changes in total white or red blood cell counts**.** This pattern challenges the conventional expectations derived from maximal exercise studies and underscores the unique nature of the submaximal exercise response.

The results suggest that the 6MWT's primary hematological impact may be related to fluid shifts and negligible RBC population dynamics rather than the pronounced hemoconcentration and immune cell mobilization seen in exhaustive exercise.

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