EFFECTS OF FUNCTIONAL EXERCISE ON CORTISOL LEVELS IN HEALTHY ADULT MALES

BY

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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.

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CERTIFICATION

This is to certify that this project work on **"EFFECTS OF FUNCTIONAL EXERCISE ON CORTISOL LEVELS IN HEALTHY ADULT MALES"** was carried out by **EDENYA CHUKWUNARU ELKANAH,** with matriculation number: **BMS2005092**; in partial fulfillment for the Award of Bachelor of Science (B.Sc.) Degree in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City.

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DEDICATION

This project work is dedicated to God Almighty who made everything possible for me, for His unfailing love and endless protection throughout the course of my programme. I wholeheartedly dedicate this project work to my beloved parents. Their unwavering support, endless sacrifices, and constant encouragement have been the foundation of this journey.

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ABSTRACT

One well established measures of functional ability and quality of life is the ability to walk. The six-minute walk test (6MWT) is a simple, low-tech, safe and well established, self-paced assessment tool to quantify functional exercise capacity in adults. The test is self-paced, with standardized instructions and encouragement being given as patients walk as far as possible for about six minutes through a flat corridor. The final distance is recorded in meters. This study was aimed to investigate the effect of functional exercise on cortisol levels in healthy adult males at the University of Benin. Forty-five (45) healthy adult males aged 18-29 years were recruited for this study. A straight hallway was used at the University of Benin, Physiology Department. The hallway was measured using a measuring tape to mark out 30m and calibrated at intervals of 3m. Two small cones were placed at both ends on the 30m mark to indicate the turnaround points.The subjects' Body Mass Index (BMI) was calculated before the test. The pre and post-test vitals were measured and recorded. The Subjects walked back and forth the cones on the 30m hallway and the number of laps walked was counted, recorded and then multiplied by 60 meters to get the total number of meters walked. A 5ml syringe was used for blood collection from the subjects, after collection, the vacutainer tube (plain bottle) was used to collect, store and transport blood samples for laboratory testing. Cortisol 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.A CORTISOL ELISA TEST KIT was used for the assessment of the cortisol level test. The statistical analysis was done using Graph Pad Prism statistical package Version 8.1. The standard error of mean (SEM) was used in 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 cortisol levels before and after the six minute walk test and there was a weak negative correlation between distance covered and blood cortisol level. Furthermore, individual stress response profiles, genetic and hormonal fluctuations can all affect how much cortisol an individual produces in response to various stressors. The cortisol response to exercises intensities are influenced by various factors such as age, gender, time of day and level of physical activity.

CHAPTER ONE

INTRODUCTION

1.0 Background Of The Study

One well established measures of functional ability and quality of life is the ability to walk (Cazzoletti *et al.*, 2022). The six-minute walk test (6MWT), is a simple, low-cost, submaximal exercise that is used to assess patients' functional capacity in the event of moderate or severe cardiopulmonary disease (Wang *et al.,* 2022). The ability of individuals with chronic respiratory diseases to exercise is assessed using a variety of field walking tests. Among these, the 6-minute walk test (6MWT) is a crucial study that offers prognostic, therapeutic response, and functional data that are helpful in the treatment of patients with heart and respiratory conditions. It requires no special training on the part of the staff performing it, items and equipment available at any clinician's office or hospital may be used, and is safe and well-tolerated by most patients at any stage of disease, with the test being highly reflective of usual daily activity and exercise performance (Holland *et al.,* 2024).It is particularly useful in assessing and monitoring chronic obstructive pulmonary disease (COPD) and has a role in managing patients with other conditions, including those with diffuse parenchymal lung diseases and pulmonary arterial hypertension. Overall, it is an inexpensive test that can provide a wealth of data with potential impact on the treatment of several conditions. The test is self-paced, and patients walk as far as they can through a level hallway for roughly six minutes while receiving standardized instructions and encouragement. According to the American Thoracic Society (2002), the final distance is measured in meters.

The 6MWT may be used to distinguish between healthy and ill people based on a variety of demographic and physiological factors (Dourado, 2011). However, age, height, weight, sex and race appear to be the major independent variables that can alter the 6MWT in healthy individuals, and these traits have been suggested for therapeutic application (Vaish *et al.,* 2013). In addition to be used to assess patients' functional state on a single occasion, the six-minute walk test (6MWT) has also been used to predict morbidity and death (American thoracic society, 2002).

1.2 Cortisol

Even though cortisol is commonly recognized as the stress hormone of the body, it affects many bodily systems. It is the primary glucocorticoid that is expelled from the adrenal cortex's zona fasciculata layer. The hypothalamus-pituitary-adrenal axis controls cortisol secretion as well as production. Cortical insufficiency, like Addison's disease, or excess cortisol diseases, like Cushing syndrome, can result from a loss of control. Cholesterol is converted into the steroid hormone cortisol. It is produced in the adrenal cortex's zona fasciculata layer. The anterior pituitary releases adrenocorticotropic hormone (ACTH), which increases LDL receptors and the activity of cholesterol desmolase, the rate-limiting step in the synthesis of cortisol that transforms cholesterol to pregnenolone. Most glucocorticoids are inactive and attached to either albumin or corticosteroid-binding globulin (CBG) (Ramamoorthy and Cidlowski,2016). The inactive form is converted to its active form by 11-beta-hydroxysteroid dehydrogenase 1 (11-beta-HSD1) in most tissues, while 11-beta-HSD2 inactivates cortisol back to cortisone in the kidney and pancreas. (Ramamoorthy and Cidlowski,2016).

1.2 Justification Of Study

This study investigates if six-minute walk test as a functional exercise have any effect on cortisol levels in healthy adults male. It is unclear whether natural or stress-induced fluctuations in cortisol levels significantly influence 6MWT. By measuring variables such as distance covered, BMI, we can ascertain an healthy adult individual fitness and identify any limitations or improvements that may not be detected during routine physical activities.

1.3 Aim Of Study

The study is aimed to evaluate the effect of functional exercise on cortisol levels in healthy young male adult subjects.

1.4 Research Questions

1. Does the six minute walk test have an effect on cortisol level in healthy young male adult subjects?
2. Is there a significant correlation between distance covered and cortisol levels?

1.5 Specific Objectives

1. To evaluate the effects of functional exercise capacity on cortisol levels of healthy young male adult subjects.
2. To ascertain if there is a significant correlation between distance covered and cortisol levels?

CHAPTER TWO

LITERATURE REVIEW

Balke developed a simple walk test with a time limit in the 1960s (Balke, 1963; Guyatt *et al.,* 1985), which Cooper later modified to a twelve-minute walk test. Because the twelve-minute walk (12MWT) was too taxing for patients with heart and respiratory issues (Butland *et al.,* 1982), a six-minute walk test (6MWT) was created. This test is easier to administer, better tolerated, and more representative of everyday activities of living (Solway *et al.,* 2001). In 1985, the six-minute walk test was developed for the purpose of evaluating exercise before prescribing it.

Assessing a participant's walking distance on a level, firm surface in six minutes at their own pace is the aim of the 6MWT. The final objective is to walk as far as you can (Fell *et al*., 2022). According to American Thoracic Society (ATS) recommendations from 2002 state that participants are free to go at their own pace over a 30-meter pathway, pausing or resting as necessary. In order to keep participants engaged, the guidelines also recommend adopting standard expressions of encouragement (Crapo *et al.,* 2002). The 6MWT is well-liked due to its simplicity, absence of specialized skills or equipment, and resemblance to daily tasks (Belle *et al.,* 2012; Chetta *et al.,*2006). In many therapeutic intervention studies, the 6MWT was also discovered to be a useful tool for evaluating the development of functional exercise capacity.

When prescribing exercise for both initial evaluation and recording functional results after program completion, the six-minute walk test, as initially designed, can be used in a cardiac rehabilitation setting (Wright *et al.,* 2021). The distance walked may be used as an outcome measure and a sign of sickness, severity, and prognosis in clinical trial testing for medical and surgical procedural therapy (Venkatesh *et al.,* 2011).

2.1 History Of The Six Minute Walk Test (6MWT)

The six minute walk test (6MWT) was first developed in 1976 to assess the exercise capacity of COPD patients. It has been shown to be dependable, affordable, secure and user-friendly assessment tool (Casanova *et al.,*2007). This low-complexity, safe test involves giving the patient instructions to walk as far as they can for six minutes down a 30-meter, lightly-trafficked corridor; the major outcome measure is the six-minute walk test, which is measured in meters (Agarwala and Salzman, 2020).

2.2 The Fundamentals Of Six Minute Walk Test As A Functional Exercise

The six-minute walk test (6MWT), a submaximal exercise test, is an efficient way to evaluate a patient's functional ability and ability to perform daily living activities that are significantly impacting their quality of life. (Guyatt *et al.,* 1991; Solway *et al.,*2001).

The 6MWT has been conducted under various conditions, such as in straight or circular tracks of varying lengths, indoors or outdoors (Brooks *et al.,*2003; Pepera *et al.,* 2013). Unlike other walk tests, the patients while conducting the 6MWT can pace themselves under their own tolerance of the intensity, but the pause times should be documented and taken into consideration after the test (Enright, 2003). The verbal encouragements are another crucial component. To encourage the patients to perform well on the test, the doctors will employ a series of conventional phrases of encouragement at predetermined points.

2.3 Physiology Of The Six Minute Walk Test (6MWT)

Although it does not allow for the identification of the mechanisms underlying exercise limitation, Baddini-Martinez (2018) describes the six-minute walk test as a general indicator of system integration encompassing the neurological system as well as the respiratory, cardiovascular, and locomotor systems.

The exchanges between the heart and lungs during exercise are the main physiological processes that are significant to the test (Casano and Anjum,2023).

According to Jean-Pierre and Alian (2011), pulmonary circulation is the process by which blood is transferred from the heart to the lungs and back again. It involves 100% of the cardiac output. Gaseous exchanges between the pulmonary alveoli and capillaries allow carbon dioxide to be taken out of the circulation and added to alveolar gas. The blood is then returned to the left side of the heart via the four pulmonary veins and from the heart to the systemic circulation.

The process of matching minute ventilation to cardiac output in response to varying exercise intensity levels involves the neurological and musculoskeletal system as well. This is accomplished through reflex responses that modify cardiac and pulmonary activity to the levels of oxygen consumption required by the degree of exertion (Casano and Anjum,2023).

2.3.1 Purpose Of Six Minute Walk Test

Reduced functional capacity in heart failure patients results in a worse prognosis and a decreased quality of life. The six-minute walking test is a widely used and well-tolerated method for identifying patients with heart failure. Without the need for specialized equipment or extra training for medical professionals, the six-minute walk test assesses a person's submaximal level of functional ability while they walk for six minutes on a level, hard surface. It evaluates each system's reaction to exercise (Giannitis *et al.,* 2019).

The 6-minute walk test has been studied in terms of morbidity and mortality in patients with pulmonary arterial hypertension and heart failure populations in particular. Patients with advanced diseases and multiple comorbidities, such as those with heart failure, chronic obstructive pulmonary disease, or cystic fibrosis, who are unable to complete more demanding exercise tests, may benefit from using the test to evaluate their functional status (Giannitis *et al.,* 2019). A reduction in estimated glomerular filtration rate may be utilized as an early marker to identify diminishing functional capacity in patients with heart failure (Vuckovic and Puzantian, 2017). Renal insufficiency has been indicated as a limiting factor to exercise capacity in patients with heart failure (McCollough *et al.,*2010).

2.4 Factors That Affect Performance In The Six-Minute Walk Test

1. **Health factors**: health factors like Heart Failure: Patients suffering from heart failure typically experience a decrease in their ability to engage in physical activity, with exercise intolerance and generalized fatigue being two prominent indicators of this condition. Consequently, these individuals often find it challenging to partake in physical exercise, as they fear that doing so may worsen their symptoms (Pollentier *et al*., 2010).

2. **Age**: People frequently discover that they are less able to engage in physical activities as they age (Marcell, 2003). Their bodies undergo changes over time, which is why this occurs. They might not have as much energy or strength, and their ability to move around might not be as good as it once was. These changes can make it tougher for individuals to conduct ordinary chores like walking, climbing stairs, or even getting dressed. Older persons may therefore require more assistance with these tasks, which may affect their level of independence (Guralnik *et al.,* 1995).

3. **Anxiety**: Anxiety can trigger a physiological response known as hyperpnea, which is characterized by rapid and shallow breathing. This exaggerated breathing pattern can lead to exertional dyspnea, or shortness of breath during physical activity. Experiencing dyspnea during exercise can deter individuals from engaging in the test or limit the intensity and duration of the 6MWT (Cooper, 2006).

4. Muscle Mass: Muscles undergo a number of changes as humans age, such as a loss of bulk, strength, endurance, flexibility, and coordination. Their capacity to walk more effectively over longer distances is directly impacted by these changes. While decreasing endurance causes exhaustion during activities like walking more quickly, reduced muscle mass results in decreased strength and power (Maden-Wilkinson *et al.,* 2015).

2.5 Body Mass Index (BMI)

Measurements of height and weight are used in mathematical computations to calculate body mass index (BMI), which is used to evaluate a person's health (Oniszczenko and Stanisławiak, 2019). It provides a simple and quick way to screen individuals for their weight category, which broadly categorizes them into underweight, normal weight, overweight, or obese (Oniszczenko and Stanisławiak, 2019). It is used to assess the risk of developing chronic conditions such as diabetes, hypertension, depression, and cancer because excess body weight, especially in the form of fat, is associated with an increased risk of these health problems (Coletta *et al.,* 2016). Adipose tissue (fat) and lean mass (muscle, bone, organs, etc.) are two distinct components that have opposing effects on health and are included in the Body Mass Index (BMI), which is calculated based on total body weight. Body fat, or adipose tissue, has been connected to detrimental health outcomes like an elevated risk of long-term conditions like diabetes, heart disease, and high blood pressure. Conversely, better health outcomes are linked to retained lean mass, which comprises muscles and other non-fat components. Lean mass preservation is advantageous for a number of reasons. First of all, because muscles are essential for strength, movement, and general bodily function, it is associated with better levels of physical fitness. Lean mass can also improve metabolic health and weight management by increasing caloric expenditure, which means the body burns more calories even when at rest. Additionally, those with greater lean mass frequently have greater exercise capacity, which makes it easier and allows them to stay active for longer. Longevity and good health depend on adipose tissue and lean mass remaining balanced. While excess body fat can be detrimental to health, preserving lean mass through exercise and proper nutrition can have numerous benefits, including improved physical fitness, higher caloric expenditure, and better overall survival. (Adams *et al.,* 2006).

Classification	BMI (kg/m2)
Underweight	<18.5
Normal weight	18.5–24.9
Overweight	25.0–29.9
Obese	≥30
Obese I	30.0–34.9
Obese II	35.0–39.9
Obese III	≥40

Table I: BMI classification of adult weights based on WHO

2.6 INDICATIONS

The 6MWT is mainly recommended for evaluating the response to treatment in moderate-to-severe cardiac or pulmonary illness, according to the American Thoracic Society (2002). It can also be used for risk classification prior to surgery. Patients who walked less than 70% of their estimated distance during the preoperative evaluation for pulmonary surgery, for instance, were more likely to experience lung problems after the procedure (Santos *et al.,* 2016). The 6MWT has additional purposes based on guidelines, such as assessing a patient's prognosis based on the underlying condition and their exercise tolerance (Thoracic Society of America, 2002). The 6MWT can be used to evaluate conditions that specifically affect any of the several organ systems that are engaged in physical activity. The 6MWT has been utilized extensively in individuals with COPD among lung disorders (Celli *et al.,* 2016: Aalstad *et al*., 2018). Other conditions in which it can be helpful include idiopathic pulmonary fibrosis, cystic fibrosis, and sarcoidosis (Martin *et al.,* 2013: Baughman and Lower, 2007; Du Bois *et al.,* 2011). Heart failure and peripheral artery disease are

cardiovascular conditions with a significant burden of morbidity and mortality, where the 6MWT can be a useful adjunct in their assessment and management. (Shah *et al.,* 2001: Mc

Dermott *et al.,* 2014). It is often used in the context of cardiac rehabilitation, being reflective of clinical change (Bellet *et al.,* 2012).

Functional impairment resulting from conditions affecting the neurological and muscular systems, such as fibromyalgia, Parkinson disease, multiple sclerosis, and spinal muscular atrophy, can also be reliably evaluated with the 6MWT (Pankoff *et al.,*2000: Falvo and Earhart,2009: Goldman *et al.,*2008: Montes *et al.,* 2010). Furthermore, it has been found to be highly predictive of ambulatory capacity after total knee arthroplasty (Ko *et al.,* 2013).

2.7 Contraindications

Although the American Thoracic Society (2002) states that the only known absolute contraindication to the 6MWT is a history of acute coronary syndrome (either unstable angina or myocardial infarction) within 30 days before the test, other absolute contraindications that apply to field walking tests generally, such as syncope, acute respiratory failure, or the presence of a noncardiopulmonary condition that may limit or worsen with exercise, should also be taken into consideration (Holland *et al.,* 2014).

Relative contraindications include severe, uncontrolled hypertension and a resting heart rate greater than 120 beats per minute (ATS Committee on proficiency Standards for Clinical Pulmonary Function Laboratories, 2002). Like any other test, the 6MWT is self-paced, which would lessen the possibility of adverse outcomes brought on by otherwise excessive exercise levels. However, clinician judgment must still be utilized when determining whether to proceed with testing.

2.8 Clinical Significance

The final distance walked (6MWT) is the main test result (Holland *et al.,* 2014). The average 6MWT in healthy individuals is 400-700 metres (Enright,2003). This range overlaps with data reported in other populations (Britto *et al.,*2013: Chetta *et al.,*2006). Factors associated with variability in test performance among healthy subjects include weight and age (Chatta *et al.,* 2006: Enright,2003: Sherrill, 1998).

However, most of the variability remains unexplained (ATS, 2002). The longest distance walked by the patient should be taken as the actual result since there is a learning effect associated with improved walking distance when at least two tests are conducted

(Eright,2003). Other factors that may affect the 6MWD in healthy adults include the methodology for 6MWT and the percentage of peak heart rate achieved (Holland *et al.,*2014).

It is important to take into account the limitations of the 6MWT as an exercise capacity assessment. Because of its design, the study is unable to pinpoint the mechanisms behind a patient's exercise intolerance or the cause of dyspnea or hypoxemia. Furthermore, although it correlates with this cardiopulmonary exercise test value in certain patients, it is unable to predict peak oxygen uptake (ATS, 2002).

In the absence of specific clinical clues or an established diagnosis, a low 6MWD is a non-specific finding. In such situations, the cause of the abnormal finding should be investigated, with attention to the cardiac, pulmonary, or musculoskeletal systems, as guided by the patient's medical history (ATS,2002).

The 6MWD has been established as a significant marker of disease severity for COPD patients since it directly correlates with lower quality of life indices, respiratory and functional impairment, and survival. It also reflects the clinical response to treatments such as lung volume reduction surgery. However, it does not have the same role for pharmacological interventions (Celli *et al.,*2016). For chronic lung diseases in general, the average 6MWD ranges between 300 and 450 meters (Celli *et al.,*2016). In COPD specifically, a 6MWD of 350 meters or less is inversely correlated with the risk of exacerbation, hospitalization, and mortality (Celli *et al.,*2016). In one study, the minimal difference in walking distance associated with a change in perceived clinical decline or improvement was a mean of 54 meters (Redelmiere *et al*., 1997).

2.8.1 Cortisol

Glucocorticoid receptors are present in almost all tissues in the body. Therefore, cortisol is able to affect nearly every organ system;

1. Nervous
2. Immune
3. Cardiovascular
4. Respiratory
5. Reproductive
6. Musculoskeletal
7. Integumentary (Kadmiel and Cidlowski, 2013).

Cortisol has many functions in the human body, such as mediating the stress response, regulating metabolism, the inflammatory response, and immune function (Oakley and Cidlowski, 2013).

2.9 Immune and Stress Responses

Glucocorticoids have a number of actions in the immune system. For example, they induce apoptosis of proinflammatory T cells, suppress B cell antibodies production, and reduce neutrophil migration during inflammation (Kadmiel, 2013). The human body is continually responding to internal and external stressors. The body processes the stressful information and elicits a response depending on the degree of threat. The body's autonomic nervous system is broken down into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). In times of stress, the SNS gets activated. The SNS is responsible for the fight or flight response, which causes a cascade of hormonal and physiological responses.

The amygdala is responsible for processing fear, arousal, and emotional stimuli to determine the appropriate response. If necessary, the amygdala sends a stress signal to the hypothalamus (Hakamata *et al.,* 2017). A spike in catecholamines, including epinephrine, is then released by the adrenal glands once the brain triggers the SNS. Effects like elevated respiratory and heart rates follow from this. The hypothalamus triggers the HPA axis as long as the body believes the stimuli pose a threat. The adrenal cortex releases cortisol, which enables the body to maintain its high level of alertness. The body gets its energy acutely from the catabolic processes of cortisol (Lee *et al.,* 2015).

2.10 Glucose and Protein Homeostasis

Important intracellular and systemic pathways are regulated by blood glucose levels. Blood glucose is more readily available to the brain when glucocorticoids, such cortisol, are present. The pancreas, fat tissue, muscle, and liver are all impacted by cortisol. Elevated cortisol levels in the liver reduce glycogen production and promote gluconeogenesis (Kuo *et al.,* 2015). Gluconeogenesis is a metabolic pathway that results in the production of glucose from glucogenic amino acids, lactate, or glycerol 3- phosphate found in triglycerides. Gluconeogenesis reverses glycolysis, a cytoplasmic pathway used to convert glucose into pyruvate molecules. This pathway is used to release energy through substrate-level phosphorylation and oxidation reactions. Unlike glycolysis, gluconeogenesis becomes active when the body needs energy. Muscles have their own internal glycogen supply that allows them to respond to changes in ATP requirements rapidly. In the presence of cortisol, muscle cells decrease glucose uptake and consumption and increase protein degradation; this supplies gluconeogenesis with glucogenic amino acids In adipose tissues, cortisol increases lipolysis.

Lastly, cortisol acts on the pancreas to decrease insulin and increase glucagon, a peptide hormone secreted by the pancreatic alpha cells to increase liver glycogenolysis, liver gluconeogenesis, liver, ketogenesis, lipolysis, and decrease lipogenesis; it also increases the activity of glucagon, epinephrine, and other catecholamines. Lipolysis is a catabolic process that releases glycerol and free fatty acids, which can be utilized in B oxidation and as an energy source for other cells as they continue to produce glucose (Exton, 1979).

2.11 Mechanism of Action of Cortisol

The release of cortisol is under control of the hypothalamus-pituitary-adrenal (HPA) axis. Corticotropin-releasing hormone (CRH) is released by the paraventricular nucleus (PVN) of the hypothalamus (Ramamoorthy, Cidlowski, 2016). It then acts on the anterior pituitary to release adrenocorticotropic hormone (ACTH), which subsequently acts on the adrenal cortex. In a negative feedback loop, sufficient cortisol inhibits the release of both ACTH and CRH. The HPA axis follows a circadian rhythm. Thus, cortisol levels will be high in the morning and low at night (Ramamoorthy , Cidlowski, 2016). Steroid hormones, such as cortisol, are primary messengers. They can cross the cytoplasmic membrane because of their fat-soluble properties. Cell membranes are composed of phospholipid bilayers; these prevent fat-insoluble molecules from passing through. Once cortisol passes through the cell membrane and enters into the cell, it binds to specific receptors in the cytoplasm. In the absence of cortisol, the glucocorticoid receptor binds to an Hsp90 chaperone protein in the cytosol. The binding of cortisol to the glucocorticoid receptor dissociates the Hsp90. The cortisol-receptor complex then enters the nucleus of the cell and affects gene transcription (Ramamoorthy , Cidlowski, 2016).

2.12 .1 Clinical Significance

Cortisol levels are continuously monitored in the body to maintain homeostasis. Unregulated levels can be detrimental.

2.12.2 Hypercortisolism

Cushing syndrome occurs when the human body is exposed to high cortisol levels for an extended period of time. The different etiologies for Cushing syndrome can be categorized as ACTH-dependent or ACTH-independent. In the ACTH-dependent subtypes, there is an excess of ACTH due to either a pituitary tumor or an ectopic source, such as a neuroendocrine tumor (Raff and Carroll, (2015). In both cases, the overproduction of ACTH stimulates the adrenal gland to produce excess cortisol. In the ACTH-independent subtypes, there is an endogenous etiology and an exogenous etiology. The endogenous cause is usually due to a tumor on the adrenal gland, which leads to excess cortisol production. The exogenous cause is due to excessive oral or injectable corticosteroid use (Raff and Carroll, (2015). Oral corticosteroids, such as prednisone, increase the amount of cortisol in the body. They are prescribed to help alleviate symptoms associated with chronic inflammatory diseases, such as systemic erythematous lupus (SLE) and rheumatoid arthritis. The symptoms of Cushing syndrome are dependent on how elevated the cortisol levels are. Common signs and symptoms of excess cortisol include weight gain (especially in the face and abdomen), fatty deposits between the shoulder blades, diabetes, hypertension, hirsutism in women, proximal muscle weakness, and osteoporosis (Lila *et al*., 2015) The treatment for Cushing syndrome is dependent on the cause. The most common treatment is through surgical intervention. However, glucocorticoid-receptor antagonists are also an option when there are contraindications to surgery.

2.12.3 Hypocortisolism

Primary adrenal insufficiency, also known as Addison disease, is most commonly caused by autoimmune adrenalitis. (Michels and Michels 2014) Other causes include malignancy, infection, or adrenal hemorrhage. Autoimmune adrenalitis results from the body attacking its adrenal cortex (Neary and Nieman 2010). Secondary adrenal insufficiency is due to insufficient production of ACTH from the anterior pituitary gland. This can be caused by pituitary disease, but the most common cause is due to suppression of the HPA axis from chronic exogenous glucocorticoid use (Wojcik *et al*., 2019) Tertiary adrenal insufficiency is due to a lack of CRH release from the hypothalamus. Symptoms of adrenal insufficiency include fatigue, weight loss, hypotension, and hyperpigmentation of the skin (Alexandraki *et al.,* 2022). Since aldosterone will also be deficient, laboratory results will show hyperkalemia. Glucocorticoid replacement therapy, such as hydrocortisone, is required to treat the symptoms of hypocortisolism. It is important to remember to increase the dosage for acute stressors, such as illness and surgery, to avoid adrenal crisis (Alexandraki *et al.,* 2022).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study Area

The study was conducted in the College of Basic Medical Sciences, University of Benin, Benin City.

3.2 Exclusive Criteria

1. Subjects who were asthmatic.
2. Subjects who had Chronic Obstructive Pulmonary Diseases (COPD).
3. Subjects who were underweight and overweight.

3.3 Inclusion Creteria

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

3.4 Materials

Materials used in carrying out this exercise include:

1. Stadiometer
2. Moveable Chair and Table
3. Stopwatch
4. Worksheets
5. Two cones
6. Measuring tape

3.5 Material Used in Blood Collection

1. Plain bottle
2. Latex hand gloves

3. Methylated spirit
4. Cotton wool
5. Tourniquet
6. 5ml syringe

3.6 Subject Population

A total number of forty-five (45) healthy male undergraduate subjects participated in this study, between the age of 18 years and 29 years of the University of Benin students participated in this study.

3.7 Ethical Consideration

Informed consent form was obtained from the subject who participated in the study while confidentiality and anonymity were observed.

3.8 Measurement of 30m Hallway

1. A less frequented straight hallway was used at the Department of Physiology in the University of Benin.

2. The hallway was measured using a measuring tape to mark out 30m -and cali-brated at intervals of 3m.

3. Two cones were placed at both ends on the 30m mark to indicate the turna-round points.

3.9 Methodology

The 6-minute walk test was carried out according to the American Thoracic Standard-standardized protocol (Ajiboye *et al*., 2014)

3.10 Participants preparation

1. Comfortable clothing should be worn.
2. Appropriate shoes for walking should be worn.
3. Patients should use their usual walking aids during the test (cane, walker, etc.).
4. The patient's usual medical regimen should be continued.
5. A light meal is acceptable before early morning or early afternoon tests.
6. Patients should not have exercised vigorously within 2 hours of beginning the test.

Subjects were asked to walk at their pace for six minutes along a measured hallmark (30m) walking back and forth. During test exercise, subjects were permitted to slow down, to stop and rest if necessary. They may lean against the wall while resting but resume walking as soon as they were able to. Subjects were permitted to quit whenever they wanted.

3.11 Six Minutes Work Test

1. The Subject's BMI was measured.
2. The Subjects were asked to rest for at least 10 minutes before the test.
3. The Subjects are informed about the test procedure, including the goal of walking as far as possible within six minutes.
4. The Subjects walked back and forth the cones on the 30m hallway.
5. Standardized encouragement phrases (as per ATS guidelines) are given at set intervals to maintain consistency.
6. The number of laps walked was counted and recorded.

7. The total number of laps walked was multiplied by 60 meters to get the total number of meters walked.
8. The Subjects were congratulated on their good effort and offered a drink of water and soda with snacks.

3.12 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 CORTISOL ELISA TEST KIT was used for the assessment of a cortisol level test.

3.13 Cortisol Elisa Test

Principle of the test

Competitive Enzyme Immunoassay

A solid phase competitive ELISA is used in the cortisol test kit from Monobind Inc. The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

3.14 Specimen Collection and Preparation

Collected blood specimens was immediately separated from the serum and the usual precautions in the collection of venipuncture samples was observed. The blood was collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tubes containing EDTA or heparin. The blood was allowed to clot for serum samples and was centrifuged to separate the serum or plasma from the cells. Samples was stored by refrigeration at 2-8^{o}C for a maximum period of five (5) days. The use of contaminated devices was and also repetitive freezing and thawing was avoided.

3.14 .1 Test Procedure

Prior to assay, all reagents, serum reference calibrators and controls were at room temperature (20-27^{o}C).

1. Formatted the microplates' wells for each serum reference, control and subject's samples to be assayed in duplicate.

2. Pipetted 0.025ml (25µL) of the appropriate serum reference, control or samples into the assigned well.
3. Added 0.050ml (50µL) of the ready to use cortisol Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Added 0.050ml (50 µL) of Cortisol Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Covered and incubated for 60minutes at room temperature.
8. Discarded the content of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Added 0.350ml (350µL) of wash buffer, decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.
10. Added 0.100ml (100 µL) of working substrate solution to all wells.
11. Incubated at room temperature for fifteen (15) minutes.
12. Added 0.050ml (50 µL) of stop solution to each well and gently mix for 15-20seconds.
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader.

3.15 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 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.

CHAPTER FOUR

RESULTS

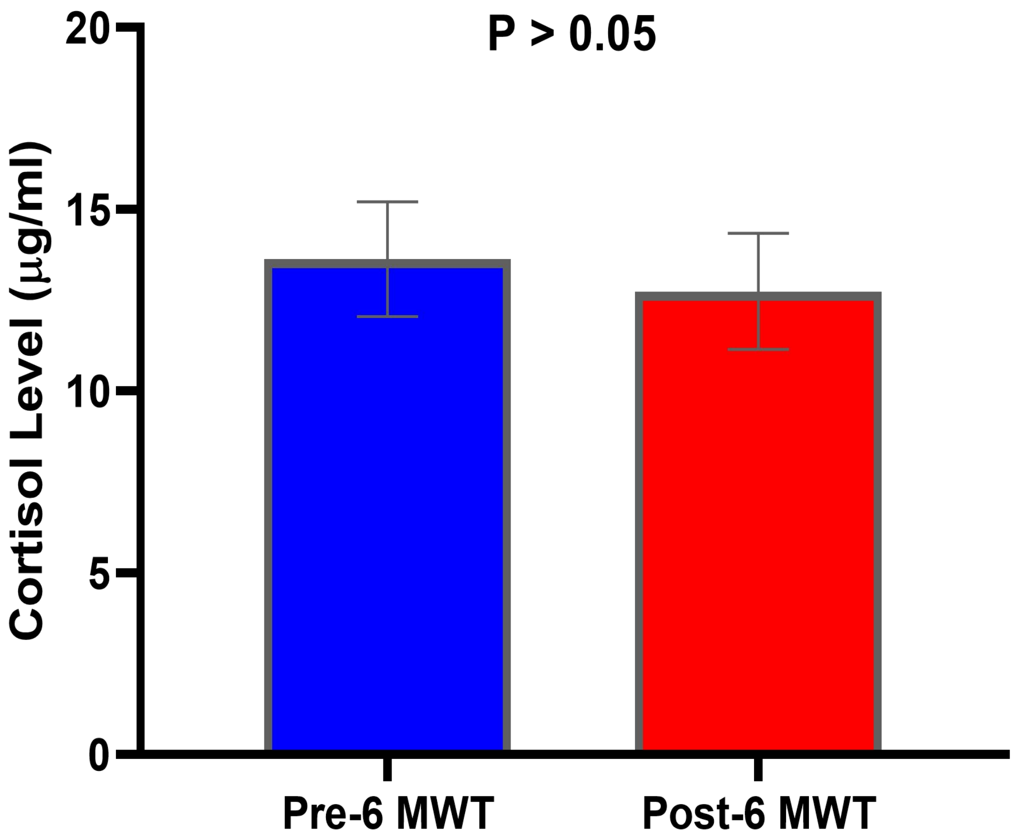

Fig 1: The plasma cortisol level in adult male individuals following six minute walk test.

There was no significant changes in post-6MWT compared with the pre-6MWT

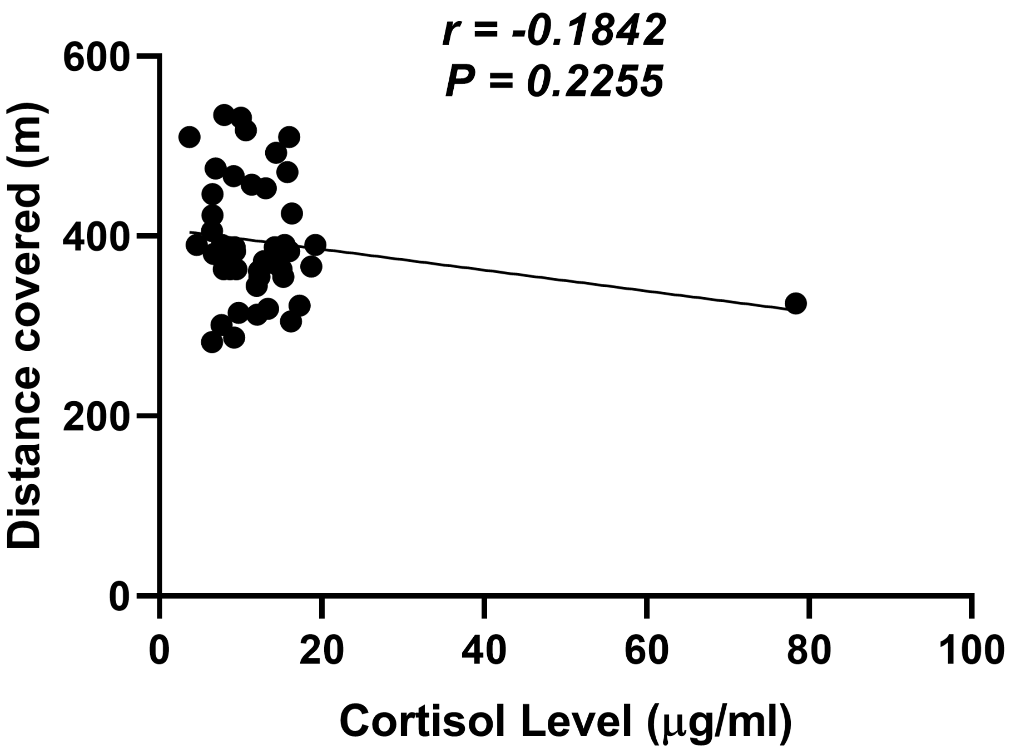

Fig 2: The relationship graph of blood cortisol level and distance covered following a six minutes test on healthy adult male individuals.

There was a weak negative correlation between distance covered and blood cortisol level

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). Cortisol levels in young adults typically follow a daily pattern, peaking in the morning and falling at night. The six minute work test (6MWT) is an assessment tool used to determine a person's exercise tolerance. It is a low-risk test that measures how far a person can walk in six minute and also useful for measuring the functional ability and fitness of people with certain health conditions.

Research in adults revealed that if a certain physical effort is exceeded, the cortisol level rises as a function of both intensity and duration (Kirschbaum and Hell hammer, 1994; Brownlee *et al.,*2005; Hill *et al.,* 2008; Gatti and De Palo, 2011). Adults who exercise at a level more than 60% of their maximal oxygen uptake (VO_2max) have been shown to release more cortisol during exercise than they do during rest. Male and female cortisol levels differ, and these differences may be variations in hormonal regulation, body composition and exercise intensity. For example, men typically have higher testosterone levels, which can raise the amount of cortisol produced during exercise. In addition, men tend to have larger muscles than women, which could lead to higher cortisol levels during exercise because of the higher metabolic demands. The results showed that there

was no significant change in cortisol levels before and after the six minute walk test and there was a weak negative correlation between distance covered and blood cortisol level.

Furthermore, the amount of cortisol that a person releases in reaction to different stressors can be influenced by their unique stress response profiles, genetics, and hormonal variations. Age, gender, time of day, and degree of physical activity are some of the variables that affect the cortisol response to exercise intensities. Cortisol levels before and after the test did not significantly change, according to this study, indicating that there is no discernible stress reaction from this brief physical exercise. Additionally, there was a weak negative correlation found between cortisol levels and the distance covered during the walk, indicating that as cortisol levels increases, the distance covered tends to decrease in the individual and it could be due to random variations rather than a true physiological effect.

5.2 CONCLUSION

In conclusion, this study investigated the effects of a six-minute walk test on healthy adult males, with findings indicating several noteworthy insights. The study showed that there was no significant change in cortisol levels prior to and after the six minute walk test and there was a weak negative correlation between distance covered and blood cortisol level.

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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):
____