ANDROGEN LEVEL, ATHEROGENIC LIPID INDEX AND PROFILE AMONG AGING MEN IN BENIN CITY

BY

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A THESIS WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCESUNIVERSITY OF BENIN, BENIN CITY.

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DECLARATION

I, **IRUOBE LAURETTA,** hereby declare that this is the original work carried out by me in the Department of Medical Laboratory Science and it has not been submitted to any University for award of any degree.

Name of Candidate: **IRUOBE, LAURETTA**

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Signature of Candidate: ----------

CERTIFICATION

We the undersigned hereby certify that this Thesis "Androgen Level, Atherogenic Lipid Index and Profile among Aging Men in Benin City" presented by Iruobe Lauretta (PG/BMS1512097) has been duly examined and found to fulfill the requirements for the award of the degree of Masters in Clinical Chemistry of the Department of Medical Laboratory Science of the University of Benin, Benin City, Nigeria.

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DEDICATION

This project is dedicated to GOD Almighty for HE alone is worthy of praise for HIS mercies and sustaining Grace.

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LIST OF ABBREVIATIONS

ADT	Androgen Deprivation Therapy
AIP	Atherogenic Index of plasma
ANOVA	Analysis of variance
BMI	Body Mass Index
BP	Blood Pressure
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DHT	Dihydrotestosterone
DHEA	Dehydroepiandrosterone (DHEA)
ED	Endothelial Dysfunction
FBG	Fasting Blood Glucose
ELISA	Enzyme Linked Immunosorbent Assay
FESTAC	Festival of Art and Culture
GnRH	Gonadotropin-releasing hormone
GRP	Group
HDL	High Density Lipoprotein
HDLC	High Density Lipoprotein Cholesterol
HG	Hypogonadism

HPG `	Hypothalamic Pituitary Gland
IHD	Ischemic Heart Disease
IR	Insulin Resistance
LDL	Low Density Lipoprotein
LDLC	Low Density Lipoprotein Cholesterol
MetS	Metabolic Syndrome
PARA	Parameter
SBP	Systolic Blood Pressure
SD	Standard Deviation
SHBG	Sex Hormone- Binding Globulin
SIG	Significant
T2DM	Type II Diabetes Mellitus
TC	Total Cholesterol
TG	Triglyceride
TNF	Tumor Necrosis Factor
TRT	Testosterone Replacement Therapy
TT	Total Testosterone
VAL	Value
VLDLC	Very Low Density Lipoprotein Cholesterol

ABSTRACT

Aging men with low plasma Testosterone concentration could be at risk of cardiovascular disease (CVD): a leading cause of one third of deaths worldwide. Dislipidaemia, high blood pressure, smoking, diabetes, obesity, and physical inactivity are the major risk factors that cause CVD. Of these risk factors, dyslipidaemia which is described as elevated plasma concentration of lipids is the major risk factor and predictor of CVD. The major plasma lipids are Cholesterol (TC), Triglycerides (TG), high density lipoprotein cholesterol (HDL- C) and Low density lipoprotein cholesterol (LDL-C) and they have all been incriminated as aetiological factors in cardiovascular diseases. This study was conducted to determine the relationship between plasma total testosterone and atherogenic lipid profile in predicting cardiovascular diseases of men in our study group. A total of 188 apparently healthy male subjects resident in Benin City, Nigeria, aged between 18 and 75 years, were selected for this study. The subjects were divided into three (3) groups - Group A (control); male participants aged 18 -39 years (n = 94), Group B (test); male participants aged 40 - 59 years (n = 47) and Group C (test); male participants aged 60 - 75 years (n = 47). Blood samples were collected after an overnight fast; TT was assayed using the Enzyme Linked Immunosorbent Assay (ELISA) technique; fasting blood glucose (FBG) and lipids (TG, TC, and HDL-C) were assayed using enzyme – based colorimetric methods. LDL-C, Body mass index (BMI), Atherogenic index of plasma (AIP) were calculated using appropriate formulae, systolic and diastolic blood pressures were measured using a sphygmomanometer. Data were analyzed using SPSS version 21 software. TT levels were observed to be lower with increasing age and this was statistically significant, ($P < 0.001$). The concentrations of Fasting Blood Glucose, lipids (TC, TG, and LDL-C), AIP and BMI were observed to be significantly higher with increasing age, respectively, ($P < 0.001$). The values of SBP and DBP were also observed to be higher with age and these were significant statistically, ($P < 0.001$). TT correlated negatively and significantly ($P < 0.05$) with Age ($r = - 0.626$, $P = 0.000$), TC ($r = - 0.250$, $P = 0.015$), LDLC ($r = - 0.247$, $P = 0.017$), but it was observed to correlate positively and significantly with DBP, ($r = 0.205$, $P = 0.047$). AIP correlated positively and significantly with Age ($r = 0.0261$, $p = 0.011$), TC ($r = 0.404$, $p = 0.000$), TG ($r = 0.816$, $p = 0.000$), LDLC ($r = 0.473$, $p = 0.000$) but negatively and significantly with HDLC ($r = - 0.492$, $p = 0.000$).This study showed that TT is associated with atherogenic lipid; it may therefore be considered a risk factor and a predictive marker for men who are at risk for cardiovascular disease.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background study

The leading cause of one third of deaths worldwide is cardiovascular disease and aging men with low plasma Testosterone concentration could be at risk of developing cardiovascular disease (Deaton *et al.,* 2005). The development of coronary artery disease is heavily influenced by the interaction of several risk factors such as dislipdaemia, high blood pressure, smoking, obesity, diabetes and physical inactivity (Niroumand *et al.,* 2015). Dyslipidemia which is described as increased plasma concentration of Triglyceride (TG), Total cholesterol (TC), Low Density Lipoprotein- Cholesterol (LDL-C) and low concentration of High Density Lipoprotein-Cholesterol (HDL-C) is the major risk factor and predictor for CVD (Nwagha *et al*., 2010). Cholesterol, triglycerides and phospholipids are the major plasma lipids, they are essentially water insoluble and are present together in lipoprotein particles in which they are water-miscible; they have been incriminated as an aetiological factor in cardiovascular disease. *S*everal studies have shown a strong link between high level of LDL-C, low level of HDL-C and incidence of CVD; as a result, the Castelli's risk index-II which is calculated as

(LDL-C/HDL-C), is often used to estimate cardiovascular risk, (Mudhaffar 2013). Increased cardiovascular risk have also been found to be associated with high levels of TG and increased LDL-C particles. (Nwagha *et al.,* 2005). Consequently, atherogenic dyslipidemia, defined as high LDL-C/HDL-C ratio and high TG, is associated with high cardiovascular risk (Rajab, 2012).

Cholesterol is an important compound and a major constituent present in all cellular membrane and is a precursor of steroid hormones such as testosterone. It is the hormone that stimulates sexual development in the male infants; bone and muscles growth in adult males and is responsible for the sexual drive and overall strength. Testosterone production increases rapidly with onset of puberty and after the age 40 years there is a slow decline of plasma testosterone level, 1-2 % per year. Low testosterone has been found to be associated with adverse lipid profile (Parinita, 2012, Kanthe *et al*., 2012).

According to Basaria and Dobs (2007), low plasma testosterone is associated with many health conditions and has unfavorable effects on men's health; many studies have suggested that it might be the underlying cause of various clinical conditions as diabetes, Endothelia Dysfunction (ED), the metabolic syndrome (MetS) and cardiovascular diseases in males. It is a known fact that the metabolic syndrome is a recognized risk factor for atherosclerosis and coronary morbidity and mortality.

Insulin resistance and androgen deficiency have been said to associate with vascular diseases and increase of pro inflammatory factors. Hypertension, abnormal lipid profiles, insulin resistance, and endothelial dysfunction are common features in men with low testosterone levels (Traish *et al.,* 2009).

There are controversial but considerable evidences suggesting that low testosterone levels are associated with atherogenic Lipids profile defined as (increased concentrations of triglycerides total cholesterol, low density lipoprotein cholesterol (LDL-C) and low concentrations of High density lipoprotein cholesterol (HDL-C). It was also suggested that low testosterone levels may contribute to the onset and/or progression of CVD. Arising from these findings, Traish *et al.,* (2009) in a review concluded that an early identification of patients with androgen deficiency might reduce the possibility of the progression of a variety of clinical conditions.

Several efforts have been made through clinical studies in recent years to identify better biomarkers that would beat the traditional risk factors and classical ratios that can predict cardiovascular diseases, but no major breakthrough has been made (Mudhaffar, 2013, Lars *et al.,* 2018). Classical ratios such as the Castelli's risk indices and atherogenic coefficient are strong indicators of the CVD risk; however, Atherogenic Index of Plasma (AIP) has recently been found to be a more favorable indicator of dyslipidaemia and associated diseases such as cardiovascular diseases.

It has been shown that compared with other atherogenic indices such as the Castelli's risk index-I (TC/HDL-C), Castelli's risk index-II (LDL-C/HDL-C), and atherogenic coefficient (TC-HDL-C/HDL-C), AIP (log10 (TG/HDL-C)) is the strongest and the most sensitive indicator of atherosclerosis and coronary heart disease (Nwagha *et al.,*2010, Bhardwaj *et al*., 2013). There is presently, no published study yet that have examined the association between plasma testosterone and lipid profile in our study area.

This study was therefore aimed to evaluate the relationship between plasma total testosterone and lipid profile in our study group in order to determine if testosterone could be used as a predictive biomarker for men at risk for cardiovascular disease in our study area.

1. 2 Statement of problem

Cardiovascular Disease is the leading cause of one third of deaths worldwide. Men are consistently more at risk of developing premature coronary events and dying from coronary artery disease than women. Early identification before the onset of cardiovascular events is an invaluable preventive measure. Lipid parameters are most often used for the prediction of cardiovascular risk. Traditional lipid parameters alone are inadequate for the prediction of cardiovascular disease (CVD) risk especially in some individuals with moderate risk. Several efforts have been

made through studies to identify better biomarkers that would surpass the established risk factors and classical indices that can predict CVD, but no major breakthrough have been made.

However, there is an increasing body of literature indicating that men with coronary artery disease (CAD) have significantly lower testosterone levels than men without CAD. Cross-sectional studies comparing men with and without CAD have repeatedly demonstrated significantly lower levels of both total and bioavailable testosterone in men with CAD than in controls with normal coronary arteries.

Furthermore, there has been a long time belief that testosterone have undesirable effects on lipid profile. Epidemiological evidences has shown that low testosterone in men is related to elevated total cholesterol (TC), triglycerides (TG), LDL-C and decreased HDL-C and an increased incidence of dyslipidemia.

This topic therefore seeks to evaluate the relationship between plasma total testosterone and atherogenic lipid profile so as to unearth the possibility of using testosterone as a single marker to predict CVD risk in male subjects in our study area.

1.3 Justification of Study

Relationship between low plasma testosterone and CVD is a developing area of study. Conflicting Studies suggesting that low testosterone levels are associated with atherogenic Lipids, have unfavorable effect on men's health and might be the underlying cause of a variety of conditions such as cardiovascular diseases exist (Traish *et al.,* 2009), however this is not in our study area. This study will therefore help to evaluate the relationship between testosterone and lipid profile in our study group, in order to determine its suitability as a predictive marker for the development of CVD in our study area.

1.4 Aim of Study

The aim of this study was to evaluate the relationship between plasma total testosterone and lipid profile levels of men in our study group.

1.5 Specific Objectives

The specific objectives of the study were:

i. to determine the levels of Plasma Total Testosterone in our study population.
ii. to determine the plasma lipid profile (Triglycerides, Total, HDL and LDL Cholesterol, levels) in our study population.
iii. to determine the atherogenic index of plasma (AIP) of our study population.

iv. to determine the BMI, DBP, SBP of our study population.

v. to determine the fasting blood glucose levels of our study population.

vi. to determine the correlation between testosterone and Lipids with some other cardiovascular risk factors in our study group.

1.6 Research Question

In this study the following research questions were asked

- How is plasma total Testosterone associated with atherogenic lipid profile?
- Can plasma total testosterone levels be used as predictive marker to the development of cardiovascular diseases?

1.7 Research Hypothesis

Null Hypothesis (H0): There is no association between Plasma Total Testosterone and atherogenic lipid profile.

Alternate Hypothesis (HA): There is an association between Plasma Total Testosterone and atherogenic lipid profile.

1.8 Scope of Study

This study focused on males within the age group of 18-75 years. They were assessed for the following biochemical parameters; plasma lipid profile (Triglycerides, Total, HDL and LDL Cholesterol, levels) and Fasting Blood Glucose. Atherogenic index of plasma (AIP), Body mass index (BMI) and Blood pressure (SBP and DPB) levels of our study population were also evaluated.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TESTOSTERONE PHYSIOLOGY

Androgen or male sex hormone is any group of hormones that primarily influences the development of the male reproductive system and maintains the male masculine characteristics. Commonly thought of only as male sex hormones, androgens are also present in the females but at a much lower concentrations than the males. The most potent and predominant androgen in the males is testosterone.

Testosterone is a major steroid hormone in males which regulates the differentiation of sex, spermatogenesis, production of male sexual characteristics, and fertility. It is the hormone responsible for the regulation of the primary male sexual development, such as testicular descent, enlargement of the testes and penis and increase in libido (Bozzola *et al.,* 2018).

Testosterone is also involved in regulating secondary male characteristics like male hair patterns, vocal changes, and voice deepening. It plays a major role in the metabolism of carbohydrates, fat and protein and also stimulates erythropoiesis- which accounts for a higher packed cell volume in males. Testosterone levels tend to decline with increasing age; consequently, men tend to experience a decrease in

testicular size, libido, muscle mass, erythropoiesis, lower bone density and increased fat production (Hauser *et al.,* 2018).

2.1.1. Synthesis of Testosterone

The synthesis of Testosterone in men involves an enzymatic sequence of steps from cholesterol within the Leydig cells located in the testes under the control of the gonadotrophins, predominantly, luteinizing hormone (LH). The preliminary step in the process of conversion of cholesterol to testosterone in the Leydig cells is regulated by LH; two intermediates hormones, dehydroepiandrosterone (DHEA) and androstenedione are formed this process. Androstenedione is converted to testosterone by the enzyme 17-beta-hydroxysteroid dehydrogenase. (Hauser *et al.,* 2018). This process is controlled by the hypothalamic pituitary gland (HPG) axis. Gonadotropin-releasing hormone (GnRH) which is secreted from the hypothalamus stimulates the pituitary gland to release luteinizing hormone (LH), which, in turn, acts on testicular Leydig's cells to produce testosterone. In the prostate, Testosterone is metabolized by the enzyme 5-alpha-reductase to dihydrotestosterone (DHT) which by the action of the enzyme, aromatase is metabolized to estradiol (Miller and Auchus, 2011).

During puberty, the hypothalamic-pituitary-gonadal axis plays a major role in regulating testosterone levels and gonadal function. The hypothalamus secretes

GnRH, which stimulates the anterior pituitary, to secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH). Both LH and FSH act on receptors in the gonads; however, the LH in particular acts on the Leydig cells to increase testosterone production.

The secretion of Testosterone is regulated through a negative feedback; high levels of testosterone in the blood feedback to the hypothalamus to suppress the secretion of GnRH which in turn feedback to the anterior pituitary, making it less responsive to the stimuli by GnRH. Other factors that may reduce the secretion of testosterone are fever and stress. GnRH is released by hypothalamus every 1 to 3 hours throughout the reproductive life of males and the levels of FSH and LH remain fairly constant from puberty, to about the fourth decade of life, where levels peak and slowly begin to decline. However, the secretion of GnRH and gonadotropins is low before puberty, which accounts for the low Testosterone levels during this phase of the male development. (Bozzola *et al.,* (2018).

The majority of testosterone is bound to plasma proteins such as sex-hormone-binding-globulin and albumin. The small amounts of free testosterone in the blood act at the level of the tissues, primarily the seminal vesicles, bone, muscle, and prostate gland. Testosterone is converted at the cellular level, to dihydrotestosterone by the enzyme 5-alpha-reductase; both Testosterone and

dihydrotestosterone can bind to cell receptors and regulate protein expression. Testosterone is bound (80%) to sex hormone-binding globulin (SHBG) and, to a lesser extent, to other proteins, like albumin. Approximately 2% of testosterone exists free in the serum. The non–SHBG-bound forms with the free testosterone make up the biologically active fraction of testosterone known as the bioavailable testosterone. (Morris and Kevin, 2012). The synthesis of testosterone increases rapidly with commencement of puberty and about age 40 years the plasma testosterone level gradually diminishes up to1-2 % per year. (Harman *et al.,* 2001).

2.1.2 Association between Testosterone and Aging

The secretion of testosterone occurs in the three phases of a male life. The first phase is a brief secretion during the first trimester of intrauterine life, next phase is during early neonatal life and lastly after puberty and this continues until the eventual gradual decline in about the fourth decade. Increase in testicular secretion of testosterone triggers the somatic changes that occur in the males at puberty. According to Kaufman and Vermeulen, (2005) and Wu *et al*, (2008), there are gradual decreases in circulating testosterone, increases in gonadotrophin and sex hormone–binding globulin (SHBG) levels, after middle age, and these trend continues till late old age among men who remain in good health, however this trend could become worsened by the existence of chronic illnesses and/or other

conditions such as diabetes. These age-related changes in testosterone levels from the increase of chronic disease states have been attributed to impaired hypothalamic regulation of testicular function, attrition and dysfunction of the Leydig cell and atherosclerosis of testicular vessels (Liu *et al.,*2006, Sartorius*., et al.,.* 2012, Perheentupa *et al.,*2013). Consequently, the ageing hypothalamic-pituitary-testicular axis over time increasingly operates with multi-level functional defects that, eventually lead to reduced circulating testosterone levels during male ageing (Veldhuis *et al.,* 2009, Travison *et al.,* 2009).

Although aging is associated with low levels of circulating Testosterone, however, unlike aging women, men do not experience the inevitable universal, predictable, and clinically obvious decrease in sex hormone levels with cessation of reproductive ability known as the menopause. Rather, these changes and their manifestations are subtle. These changes in the male are commonly known as the andropause. Also known as the male menopause, Andropause is used to describe the slow, steady decline of complex hormone, primarily testosterone, in the aging male .This phenomenon does not affect all men, at least not with the same intensity. The characteristic symptoms include fatigue, depression, lean body and bone mass, changes in body hair and skin, decrease in libido and erectile function. Due to the non-specific nature of andropausal symptoms, hypogonadism (androgen deficiency)

most often remains undiagnosed and untreated in many cases. In cases where it is diagnosed, it remains untreated owing to the alleged concern regarding unpleasant effects on the prostate and heart. (Morris and Kelvin, 2012).

Normal plasma levels of testosterone ranges from 300 to 1000 ng/dl. Lower concentrations of Testosterone have been associated with a number of clinical conditions such as the metabolic syndrome, type 2 diabetes, carotid intima media thickness, and aortic arterial disease (Laaksonen *et al.,*2004, Mäkinen, *et al.,*2005). Low Testosterone levels are also said to be associated with high levels of cardiovascular risk factors like higher body mass index and fat mass. Low levels of Testosterone is linked to increased fatty streak formation, Dandona and Rosenberg, (2010), Swerdloff, and Wang, (2012), showed that low plasma levels of testosterone are associated with an increased fat mass, insulin resistance, impaired glucose tolerance, high levels of triglycerides and cholesterol and a reduced HDL-C. All of these factors are found in the metabolic syndrome and type 2 diabetes and they contribute to cardiovascular risk. Other studies have also shown that obesity correlates with male hypogonadism (HG) in a twofold relationship: obesity increases the incidence of Hypogonadism and vice versa. Several studies also reported the effect of testosterone replacement on obesity and diabetes (Yassin *et al.,*2016, Salman *et al.,*2017).

2.2. METABOLISM OF LIPIDS AND LIPOTROTEIN

Lipids are water insoluble organic compounds, which are essential for many normal functions. They occur in micro- organisms, higher plants and animals where they function primarily in energy storage and also serve as important structural component of cells. They play a significant role as enzyme co-factors, hormones, and intracellular messengers (Xenoulis and Steiner, 2010). The functions of Lipids can be described conveniently as structural, storage and metabolic although individual lipids may have several roles at different times. They play important part in biological structures whose purposes are to provide barriers that protect organisms against their environment. Lipids also play therapeutic roles, they however play significant role in the aetiology of various diseases as evidenced in atherosclerosis; a cardiovascular disease characterized by a thickening of the arterial walls. The major lipids reported to be present in the plasma are fatty acids, triglycerides, cholesterol, cholesterol esters (compounds), and phospholipids. Lipids are bound to specific proteins (apoproteins) to form macromolecular complexes known as lipoproteins which constitute the functional transport unit in the circulatory system to support metabolism (Bonnie *et al*, 2007).

2.2.1 Lipoproteins

Lipoproteins are spherical lipid – protein complexes containing triglycerides, phospholipids, and cholesterol and amphipathic proteins called apolipoproteins. They differ in chemical composition, physical properties and metabolic functions, but have a common role of transporting lipids from one tissue to another. Lipid can be differentiated on the basis of their structure, density, function and types of lipoproteins they contain. Plasma lipoproteins differ in their physical and chemical characteristics such as size, density and composition. (Isam *et al*, 2017).

The compositional differences of lipoproteins influence the density of the particles and there is a strong relationship between biological function and the broad density classes they fall. The major types of lipoproteins based on density from lowest to highest are; chylomicrons, Very Low-Density Lipoprotein (VLDL), Low-Density Lipoprotein (LDL), and High-Density Lipoprotein (HDL). It is noteworthy that as density increases, particle size decreases and so does ratio of lipid to protein. Chylomicrons and VLDL lipoproteins are rich in triglycerides. While the Chylomicrons are synthesized by enterocytes from lipids absorbed in the small intestine, the VLDL is synthesized in the liver. The function of these two lipoproteins is to deliver energy-rich triglycerides to cells in the Triglyceride (TG) is striped from chylomicrons and VLDL by an enzyme - Lipoprotein lipase found

on the surface of endothelial cells, through the action of this enzyme, TG is digested to form fatty acids and glycerides, which then diffuse into the cell to be oxidized, or in the case of an adipose cell, to be re-synthesized into TG and stored in the cell. When VLDL particles are stripped of TG, they become denser and they are remodel at the liver and transformed into LDL. The function of LDL is to deliver cholesterol to cells, where it may be used in membranes, or for the synthesis of steroid hormones (Xenoulis and Steiner, 2010).

High density lipoprotein cholesterol (HDL-C) is believed to be synthesized in the liver and the intestine where it is involved in reverse cholesterol transport. Excess cholesterol is eliminated from the body through the liver which secretes cholesterol in bile or converts it to bile salts.

Numerous studies have suggested that HDL-C may act to prevent atherosclerosis by facilitating the mobilization of cholesterol from peripheral tissue and transporting it to the liver for catabolism and excretion. Presently, there is no known clinical disorder that has been attributed to abnormally elevated HDLC rather increased levels of HDLC has a protective effect. However, low levels are found to be associated with highest risk of coronary artery disease and atherosclerosis and these effects are independent of other risk factors.

LDL and other lipoproteins are eliminated from the circulation by receptor-mediated endocytosis while excess cholesterol from cells are transported back to the liver by HDL in a process known as reverse cholesterol transport. The liver and small intestine synthesizes and secretes HDL precursor where it travels in the circulation, accumulates more cholesterol to form mature HDL, which then returns the cholesterol to the liver. (Xenoulis and Steiner, 2010).

2.2.2 Cholesterol Metabolism

Cholesterol is the main sterol in animal tissues, the major source of cholesterol is dietary intake, but it can also be synthesized endogenously by the liver and other tissues. It is an important compound present in all cellular membranes and is a precursor of bile acids and of the steroid, adrenal and sex hormones. It is widely distributed especially in nervous tissues, blood and bile. It occurs both in the free and esterified form with fatty acids. Humans obtain cholesterol from foods of animal origin and synthesized by nearly all cells. Acetic acid and acetoacetic are the precursor of the sterol molecule. They combine with Coenzyme A and through a long biosynthetic process produce cholesterol. Active synthesis also occurs in the skin, muscle, heart, lungs, spleen, red cells, kidneys, gonads and adrenals. Cholesterol is absorbed from the intestine and transported to the liver by chylomicron remnants; hepatic cholesterol then enters into the circulation as very

low density lipoprotein (VLDL) which is metabolized through the action of the enzyme lipoprotein lipase to intermediate lipoprotein (IDL) and low density lipoprotein (LDL) which is then removed by the liver or peripheral tissues. Cholesterol plays a very critical role in the metabolism of bile acid and synthesis steroid hormone and vitamin D. In the peripheral tissues cholesterol is converted to steroid hormones or used to form the cell walls and membranes. The quantity of cholesterol transported from the liver to peripheral tissues greatly exceeds its catabolism, so the excess amount of cholesterol is returned back to the liver by high density lipoprotein (HDL). High cholesterol diet leading to hyperlipidemia is regarded as an important factor in the development of Ischemic heart disease. (Xenoulis and Steiner, 2010).

2. 2. 3 Hyperlipidemia

Hyperlipidemia is a disease that is characterize by an excess of blood lipids in the blood stream. It is a modifiable but a major risk factor for atherosclerosis and other cardiovascular diseases. Depending on cause, hyperlipidaemia may be classied either as primary or secondary type and the cause may be either be due to high intake of food rich in fat or as a result of other metabolic disturbances or diseases. (Karam *et al,* 2017). Hyperlipidemia is associated with increased levels of Low Density Lipoprotein cholesterol and triglyceride with low levels of HDL

cholesterol and cardiovascular disease. Increased circulating levels of Low Density Lipoprotein (LDL) underlie the development of atherosclerosis, coronary heart disease, hypertension, obesity and Type -II diabetes mellitus. Low Density Lipoprotein (LDLC) is pro-atherogenic therefore, high levels of LDLC increases Coronary Heart Disease risk. High density lipoprotein (HDL) is anti-atherogenic thus low levels of HDL also increases CHD risk (Xenoulis and Steiner, 2010).

Diagnosis of hyperlipidemia depends on laboratory measures for hyperlipidemia indices (lipid profile): a test that typically measures the levels of Triglycerides (TG), Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) and High Density Lipoprotein Cholesterol (HDL-C). Treatment of hyperlipidemia depends on reducing lipids on the blood stream. (Isam *et al.,* 2017.)

2.2.4 Relationship between Lipid Profile and Aging.

Plasma lipid levels are related to age and values of elderly persons seem to experience positive changes during the aging process. During pre pubertal age, Lipoproteins and Triglycerides show no gender difference, however, in boys, plasma levels of HDLC decline while plasma TG and LDL increases slightly. LDLC and TG levels increase slowly but progressively from late adolescent years to the mid 50s, at which point they become fairly constant (Schleich and Legros, 2004). Carroll *et al*, (2005) showed that, total cholesterol (TC) and low-density

lipoprotein cholesterol (LDL-c) gradually increase after adolescence until the age of 60–65 in men and 70–75 in women, and thereafter start to decline. (Upmeier *et al.,* 2011), suggested that the lipid profile of subjects 80-year-old and above seems to change for better even in the absence of some treatment. High-density lipoprotein cholesterol (HDL-c) and triglycerides change less during adulthood, however, some cross-sectional studies have reported that HDL-c tends to be higher in old age-groups. Existing data on the longitudinal changes of HDL-c in the elderly is rather inconsistent.

With advanced age, the role of serum lipids, in determining the risk of cardiovascular and total mortality becomes more complex due to multiple illnesses and frailty (Brescianini *et al* 2003). Previous cohort studies have reported an inverse relationship between TC and mortality (Schupf *et al.,* 2005). However, HDL-c has shown to be an important and independent predictor of cardiovascular risk and survival in the elderly (Packard *et al.,* 2005).

2.3 RELATIONSHIP BETWEEN TESTOSTERONE AND LIPIDS

Dyslipidemia is one of the most important threats to public health and it is a major risk factor for hypertension, metabolic syndrome (MetS), Type II Diabetes Mellitus (T2DM), and Coronary Heart Disease (CHD). There has been a long time belief that testosterone have undesirable effects on lipid profile. In a study carried

out by Morrison *et al.,* (2003) they reported that low concentration of HDL-C associated negatively with an increase in the plasma testosterone concentration in male adolescents. Epidemiological evidences has shown that low testosterone in men is related to elevated total cholesterol (TC), triglycerides (TG), LDL-C and decreased HDL-C and an increased incidence of dyslipidemia (Nan *et al.,* 2014).

Similar studies have also reported that testosterone levels are negatively associated with Total Cholesterol, LDL-C, and triglyceride (TG), they however observed that testosterone levels appear to have a complex and controversial relationship with HDL-C levels and cardiovascular risk. More reports indicated that men are more prone to coronary heart disease (CHD) and possess higher risks of mortality than women; this difference has been somewhat ascribed to Testosterone. (Aruojo *et al.,*(2011) and Corona *et al.,*(2011) reported that endogenous testosterone in men was found to be conversely related to the severity of carotid atherosclerosis as well as the incidence and severity of Coronary Heart Disease. Besides, low testosterone has also been reported to be related to a number of metabolic disorders, such as the metabolic syndrome, insulin resistance, and type 2 diabetes mellitus (Kalyani and Dods, 2007, Laughlin *et al.,* 2008). Consequently, Testosterone Replacement Therapy (TRT) has been recommended to ameliorate the symptoms of some of these metabolic and vascular disorders in the middle-aged and elderly men with

low serum testosterone (Bhasin *et al.,* 2009, Kang, 2013). Corona *et al.,* (2011) found that Testosterone Replacement Therapy (TRT) was associated with a significant reduction of TG and an increase of HDL-C was able to improve central obesity in subjects with MetS and glycometabolic control in patients with MetS and T2DM. Other studies observed no relationship between TRT and lipid profile, while some reported unfavorable effects of TRT on serum lipids. Report from the study by Zitzmann and Nieschlag, (2007) and Saad *et al.,*(2008) indicated a positive relationship between testosterone and HDL-C levels while Testosterone replacement therapy for androgen deficiency reduced TC and LDL-C and increased HDL-C level. This suggested that low testosterone contributes to increased TGs, TC, LDL-C and reduced HDL-C while testosterone treatment results in a favorable lipid profile, thus suggesting that testosterone may be protective against the development and/or progression of atherosclerosis.

2.3.1. Relationship between Testosterone and Cardiovascular Disease

It has been reported that men develop cardiovascular diseases earlier than women; this have led to the belief that testosterone exerts a detrimental influence upon the cardiovascular system. It is well established that total and bioavailable testosterone in men decreases with age and the age-associated decline may be related to the increased prevalence of cardiovascular disease (CVD) and

morbidities. In population based studies, low levels of Testosterone were reported to be associated with an increase in all-cause mortality and this was shown to be accounted for mainly by cardiovascular disease. (Khaw *et al.,* 2007, Araujo *et al.,* 2011).

Although the link between abnormal lipid profiles and risk of CVD has been generally accepted, Jones *et al.,*(2003) demonstrated that low total and bioavailable Testosterone levels were associated with increased risk of aortic atherosclerosis in elderly men. This association was independent of other cardiovascular risk factors like diabetes mellitus, BMI, smoking and alcohol intake. Saad *et al.,* (2012), Salam *et al.,* (2012) also provided evidences suggesting a link between low testosterone and CVD and that testosterone deficiency is an independent cardiovascular risk factor. A link between hypogonadism and increased Coronary artery disease (CAD) has also been suggested in several studies; it is also thought to contribute to the development of the metabolic syndrome (MetS), which increases CVD risk (Mäkinen *et al.,* 2008, Montaganana and Lippi, 2008 and Shanani *at al*., 2008). In a similar attempt to find a relationship between hypogonadism, MetS, T2DM and CVD, (Liu *et al*.; 2003), provided an exhaustive review on androgens and CVD and concluded that Testosterone levels are consistently lower in men with CVD.

Low plasma testosterone is manifested in the various components of the metabolic syndrome, including increased insulin resistance and glucotoxicity, increased visceral obesity and lipotoxicity as well as increased production of inflammatory factors all of which contribute to endothelial dysfunction (Kim *et al.,* 2006). Increased vasoconstriction, arterial sclerosis, oxidative stress, thrombosis, inflammatory cell adhesion, smooth muscle proliferation, and endothelial permeability all result from endothelial dysfunction. Endothelial dysfunction is associated with dyslipidemia, obesity, and diabetes and they all are cardiovascular risk factors. (Higashi *et al.,* 2009).

These data therefore suggests that Testosterone could have a direct effect on cardiovascular health in the absence of other risk factors.

2.3.2. Relationship between Testosterone and hypertension

The relationship between hypertension and androgen deficiency in relation to cardiovascular risk is complex because other risk factors are involved. It has been established in many studies that endogenous Testosterone levels lowers with age. According to Svartberg *et al.,*(2004) lower total testosterone levels were observed in men older than 25 years old who presented with hypertension. Similarly, an assessment of the effects of induced hypogonadism on hypertension in men with prostate cancer; the authors found diastolic blood pressure was elevated, along

with mean pulse pressure, after 3 months of treatment. Hypertensions, dyslipidemia, obesity with insulin resistance (IR) are manifest components of the metabolic syndrome and when they are present in the same subject, it often leads to hyperglycemia and visceral obesity. The relationship between the components of the MetS is complex, which makes it difficult to analyze each component alone without considering them comprehensively. In addition, the effects of androgen deficiency on hypertension, insulin resistance, and dyslipidemia call attention to the need to understand how testosterone influences each of these components.

A result, in which both the systolic and diastolic blood pressure were significantly lowered during treatment with intramuscular Testosterone in 66 hypogonadal men for up to 9.5 years has also been reported (Zitzmann and Nieschlag, 2007). The information in the above studies does suggest that Testosterone replacement therapy reduces blood pressure.

2.3.3. Association between Androgen Deprivation Therapy (ADT) and Lipid Profile

Lower Testosterone levels in aging men are associated with elevated triglycerides and reduced HDL-C levels .Testosterone suppression in men with prostate cancer resulted in elevated total cholesterol and LDL-C after 3 months of treatment compared with baseline. Also, a six months course of Testosterone deprivation in

men with prostate cancer which showed no significant effect on HDL-C and TG levels (Nishiyama *et al.,* 2005). Chen *et al.,*(2005), assessed a long-term anti androgenic treatment for 2.5 years, and revealed a distinctive lipid profile, showing significantly elevated TG and decreased HDL-C values. This suggests that anti androgenic therapy contributes to CAD through changes in lipoprotein values, TG levels, and an associated reduction in HDL-C levels. The above mentioned studies each revealed an increase in lipid concentrations and an unfavorable lipid profile in response to ADT.

2.3.4 Effects of Testosterone Replacement Therapy on Lipid Profiles

Several studies have shown that Testosterone treatment in hypogonadal men lowered serum cholesterol and TGs and total cholesterol and LDL-C. Analysis of total cholesterol, LDLC, HDLC, and TGs was carried out by Page *et al.,* (2005) in 171 hypogonodal men; 52 were aged between 60years and above, while 119 were aged below 60years. They all received Testosterone gel treatment for 6 months (180 days). They found that Treatment of men aged 60 and above resulted in a greater reduction of total cholesterol, LDLC, and TGs than that observed in men under the age of 60. Similarly, a study conducted by Zitzmann and Nieschlag, (2007) showed that regular treatment with intramuscular testosterone undecanoate kept LDLC levels low and HDLC levels high in hypogonadal individuals for up to

9.5years. The above studies suggest that Testosterone replacement has a favorable effect on the lipid profile in men with low Testosterone levels. ADT results in unfavorable lipid profiles, while androgen supplementation therapy improves lipid profiles and body composition in men with hypogonadism.

2.3.5 Testosterone Replacement Therapy: Concerns and Benefits

There are controversies surrounding testosterone replacement therapy in middle aged and older men. These controversies are based on two major concerns: the first being that testosterone might promote coronary heart disease while the second concern is provoked by the fears of an alleged link between Testosterone and the development of prostate cancer. However, researches, reviews and article have extensively dealt with the first concern with the provision of numerous evidences that have brought reassurance regarding the beneficial effect of physiological levels of testosterone and the male heart.

With regards to the second concern, epidemiological and clinical investigations have failed to demonstrate any association between testosterone levels and the risk of developing prostate cancer over the last decade. Similarly, no studies have demonstrated that lower than normal testosterone levels are protective against developing prostate cancer and that physiological levels of Testosterone increase

the risk of prostate cancer. (Travis *et al.,* 2007; Roddam *et al.,* 2008, Traish *et al.,*2009) .

Furthermore, Testosterone levels lowers with age, while prostate cancer incidence increases with age, this observation suggests that low Testosterone might contribute to the development of prostate cancer and normal physiological Testosterone levels could be protective against prostate cancer. Chronic Testosterone therapy has been shown to reduce arterial calcification, improve lipid profiles, and reduce inflammatory cytokine levels (Maggio *et al.,*2007).

The above studies are suggestive that androgen deficiency might contribute to a number of clinical conditions related to the cardiovascular system, and that Testosterone therapy might improve some of these conditions. The common effects of Testosterone therapy on various physiological processes were noted long ago in the area of increased energy, decreased fatigue, increased lean body and muscle mass, and decreased fat mass. More recently, Testosterone's effects on functional mobility and cognition were reported, although the subjects in that study did not have symptoms of androgen deficiency and most were not hypogonadal, it is promising that components of the MetS can be improved by Testosterone therapy in hypogonadal men (Emmelot-Vonk *et al.,*2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Area

This study was carried out in the Benin City metropolis, of Edo State. Benin City which is the capital and largest city of Edo State in southern Nigeria was the capital of the defunct Bendel State. It is situated approximately 40 kilometres north of the Benin River and 320 kilometres east of Lagos. It lies in latitude 06.34^{o}N and a longtitude 5.6^{o} E .Benin City has a tropical savanna or wet and dry climate with mean monthly temperature above 18^{o}C every month of the year. Benin City is the centre of Nigeria's rubber industry, and oil production is also a significant industry.

The indigenous people of Benin City are Edo or Bini, and they speak the Bini language and other Edo languages, (Bondarenko and *Roese* 1999). The people of the city have one of the richest dress cultures on the African continent and are known for their beads, body marks, bangles, anklets and raffia work (Okhakhu, 2016).

The Binis are also famous for bronze casting. The Benin Bronzes' - portrait figures created in iron, carved Ivory and especially in brass (conventionally called bronze)

were taken from the City by the British and are currently displayed in various museums around the world. The most prominent of these artifacts was the famous Queen Idia mask which was used as a mascot during the second festivals of Arts and Culture (FESTAC' 77) held in Nigeria in 1977 now known as Festac mask. (Okhakhu, 2016).

The Benin City metropolis is made up of three Local Government Areas namely, Oredo, Egor and Ikpoba - Okha.

3.2 Sample Size Determination

The sample size was calculated using the formula proposed by Araoye (2004).

Using the estimated prevalence of 5.6% (Araujo *et al.,* 2007); of symptomatic androgen deficiency in men, the minimum sample size (N) for the study was calculated as shown:

$$N = \frac{Z^2 \ X \ P \ X \ q}{d^2}$$

Where 'Z' is the critical value, and in a two- tailed tests this is equal to 1.96. 'P' is the estimated prevalence of prostate disorders. q is the probability which is 1-P,'d' is the absolute sampling error that can be tolerated. In this study it was fixed at 5%.

Therefore the minimum sample size 'N' is = $\frac{1.96^2 \text{ x } 0.056 \ (1- \ 0.056)}{0.05^2}$

$$N = 3.8416 \frac{\text{x } 0.056(0.944)}{0.0025}$$

$$N = \frac{0.2031}{0.0025}$$

N = 81

10% was added for contingency (N = 90)

The minimum sample size required was calculated to be 90; however, a total number of 188 subjects were selected.

3.3 Study Population

A study population of 188 apparently healthy male subjects resident in the Benin City metropolis were screened and selected for this cross- sectional study. The study population was further grouped into three:

Group A: Control group (n = 94), comprised male subjects aged 18-39 years

Group B: Test group (n = 47), comprised male subjects aged 40-59 years.

Group C: Test group (n = 47), comprised male subjects aged 60-75 years.

3.4 Inclusion Criteria

Apparently healthy Nigerian male subjects between the ages of 18-75 years who ar e resident in our study area selected were selected.

3.5 Exclusion Criteria

Subjects having previous history of heart disease, diabetes mellitus, hypertension a nd obesity were excluded from the study.

3.6 Ethical Clearance and Informed consent

Ethical clearance was obtained from the Ethics Committee of the Edo State Ministr y of Health Benin City. After the participants had been informed about the procedu re, they gave consent by signing an informed consent form.

3.7 Sample Collection

By taking aseptic precautions 10ml of fasting venous blood was collected from each subject and control. About 2mls of the blood sample was dispensed into a fluoride oxalate anticoagulant specimen container, spun and plasma separated for the estimation of blood glucose. The other portion of 8 ml was dispensed into lithium heparin container and centrifuged for separation of plasma. The aliquoted plasma samples were stored frozen for the analysis of Testosterone and lipids.

3.8 BIOCHEMICAL ANALYSIS AND OTHER MEASUREMENTS

3. 8.1 Estimation of Plasma Total Testosterone

Method: Enzyme Linked Immunosorbent Assay (ELISA) Technique

Principle:

Competition occurs between an unlabeled antigen present in the samples and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The unbound antigens are removed by washing and decanting procedures. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtitre plate reader. The intensity of the color formed is inversely proportional to the concentration of testosterone in the sample. Absorbance is measured spectrophotometrically at 450nm. A set of standards were used to plot a standard curve from which the amount of testosterone in samples and controls were directly read.

ASSAY PROCEDURE

All reagents and samples were brought to room temperature 25 µL of each Standard, Control and samples were dispensed into appropriate number of coated mouse monoclonal anti - Testosterone antibody Microtitre wells using new disposable pipette tips. 200 µL of Testosterone HRP Enzyme Conjugate was dispensed into each well. Content was mixed for 10 seconds. Incubation was done for 60 minutes at room temperature. Contents of the wells were briskly shaken out. The wells were rinsed 3 times with 400µL (per well) diluted Wash Solution (40x

concentrated). Residual droplets were removed by sharply striking the wells on absorbent paper. 200 µL of Tetramethylbenzidine (TMB) Substrate Solution was added to each well. Incubation was done for 15 minutes at room temperature. To stop enzymatic reaction, 100 µL of Stop Solution was added to each well and gently mixed for 20 seconds. The absorbance of each well was read at 450 with a microtiter plate reader within 10 minutes after adding the Stop Solution.

Calculation of Testosterone Concentration:

A calibration curve was constructed on a log-log graph using the absorbance and concentrations of standards. The concentration of samples and controls were read from the calibration curve.

(Tietz *et al.,* 2001)

3.8.2 Estimation of Plasma Total Cholesterol

Method: Enzymatic colorimetric method by Tinder, (1969) was used

Principle:

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reactions by - products, H2O2 is measured quantitatively

in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

cholesteryl ester hydrolase
Cholesteryl ester + H2O ----------->cholesterol + fatty acid

Cholesterol oxidase
Cholesterol + O2 ----------> cholest-4-en-3-one + H2O2

peroxidase
2H2O2 + 4-aminophenazone + phenol ----------> 4-(p-benzoquinone-monoimino)- phenazone + 4 H2O

Procedure

To 1000µl of reagent in tubes meant for samples, standard and blank, 10µl of samples, standard and distilled water were added respectively. The contents were mixed and incubated for 5mins at 37°C. The absorbance of the samples and standard was measured against reagent blank at 546nm

Calculation:

Plasma Cholesterol

concentration (mg/dl) = $\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}}$ x Conc. of Cholesterol Standard

3.8.3 Estimation of HDL-Cholesterol

Precipitation Method by Lopes-Virella *et al.,* (1977)

Principle:

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of acid phosphotungstic in the presence of magnesium ions. After centrifugation the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant is determined.

Procedure

Precipitation of HDL-C:

200µl of each of samples and standard were dispensed into appropriately labeled centrifuged tubes and 500µl of HDL-Cholesterol precipitant was added, mixed and allowed to stand for 10mins at room temperature respectively .The tubes were centrifuged at 4,000 rpm for 10minutes .100µl of supernatants were assayed for Cholesterol using the Enzymatic colorimetric method by Tinder shown above.

Calculation:

$$\text{Conc. Of HDL Cholesterol in Supernatant (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard}$$

3.8.4 Estimation of Triglycerides

Method: GPO - PAP Enzymatic colorimetric method by Tietz *et al.,* (1990)

Principle:

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H2O2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{glycerokinase}} \text{glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{glycerophosphate oxidase}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone-monoimino)- phenazone} + 4H_2O + HCl.$$

Procedure

Test tubes meant for samples, standard and blank were labeled, 10µl of samples and standard were dispensed into each of the corresponding tubes. 1000µl of reagent was added to each tube respectively. The contents were mixed and incubated for 5mins at 37°C. The absorbance of the samples and standard was measured against reagent blank at 546nm.

Calculation:

$$\text{Triglyceride Conc. (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Conc. of Triglyceride Standard}$$

3.8.5 Calculation of Low Density Lipoprotein Cholesterol (LDL-C)

LDL-Cholesterol was calculated using the Friedewald Equation.

$$\text{LDL-C (mg/dl)} = \text{Total Cholesterol (mg/dl)} - \text{HDLC} - \left(\frac{\text{Triglycerides (mg/dl)}}{5}\right)$$

(Friedewald *et al.,*1972).

3.8.6 Estimation of Blood Glucose:

Method: Glucose oxidase method by (Cole *et al.,* 1997).

PRINCIPLE

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red - violet quinoneimine dye as indicator.

Procedure:

To 1000µl of reagent in tubes meant for samples, standard and blank, 10µl of samples, standard and distilled water were added respectively. The contents were mixed and incubated for 5mins at 37°C. The absorbance of the samples and standard was measured against reagent blank at 500nm wave length.

Calculation:

$$\text{Plasma Glucose Conc. (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard}$$

3.8. 7 Calculation of Atherogenic index

Atherogenic index was calculated by using the following formula:

AIP = log10 (TG/HDL-C)

AIP is classified according to CVD risk as shown:

Low Risk −0.3 to 0.1

Medium risks 0.1 to 0.24

High risk ≥ 0.24

(Dobiášová, 2006),

3.8.8 Calculation of Body Mass Index

Procedure:

Heights and weights were measure using standard techniques.

Body Mass Index (BMI) was calculated using the formula, weight (kg) divided by the square of height (m^2)

BMI (kg/m2) = Weight (kg) /height (m^2).

BMI, Body mass index is classified as follows:

Below 18.5 Underweight

18.5–24.9 Normal weight

25.0–29.9 Over weight

30.0–34.9 Obesity class I

35.0–39.9 Obesity class II

Above 40 Obesity class III

(Dobiášová, 2006 and Lee *et al*., 2010).

3.8.9 Measurement of Blood pressure:

Blood pressure (composite of systolic blood pressure (SBP) and diastolic blood pressure (DBP) in mmHg) was measure with a sphygmomanometer using standard techniques.

Normal BP : ≤ 120mmHg Systolic and ≤ 80mmHg Diastolic.

Elevated BP: 120 to 129mmHg Systolic and ≤ 80mmHg Diastolic.

Pre hypertension: Systolic 120 to 139mmHg or Diastolic blood 80 to 89 mmHg.

High Blood Pressure (Hypertension): Systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg.

(Pickering *et al*., 2005)

3.10 Statistical Data Analysis

Data were expressed as mean ± standard deviation. Comparative analyses were done using the ANOVA. Correlation between variables was done using Pearson's linear regression analysis. Statistical significance was set at $P<0.05$. All statistical

analyses were done using statistical package for social sciences (SPSS) (version 21.0) software.

CHAPTER FOUR

4.0 RESULTS

The results of the analysis are as presented below:

Table 4.1 shows the mean levels of: BMI, SBP, DBP and FBG of the Control and Test Groups (A, B and C). The values of BMI (23.77 ±3.99, 26.75 ± 4.45, 28.23 ± 4.38), SBP (116.83 ± 15.27, 129.13 ± 18.54, 131.85 ± 18.26), FBG (84.26 ± 22.08, 89.66 ± 23.24, 108.58±62.98), were observed to be significantly higher among the test groups (Groups B & C) when compared with the control group (Group A) respectively ($P<0.05$). The values of DBP (76.91± 9.29, 86.32± 14.01, 84.32 ± 11.28 was seen to significantly increase with age, ($P<0.05$) but a value of 86.32 ± 14.01 was observed to be highest in Group B.

Table 4.2 shows the mean levels of Lipids profile (TC, TG, HDL-C, and LDL-C) AIP, and Total Testosterone (TT) of the control and test groups (A, B and C). The mean Plasma concentration of lipids: TC (139. 38 ±38.34, 157.36 ± 36.66, 184.81 ± 33.56) , TG (56.99 ±20.40, 77.92 ± 27.81, 90.55 ± 35.37) and LDL-C, (79.31 ± 33.24, 94.8 ±32.96, 119.40 ± 31.57) were all observed to be significantly higher in the test group when compared with the controls ($P < 0.05$). The values of AIP (0.05 ± 0.18, 0.20 ± 0.20, 0.28 ± 0.17) were also observed to be

significantly higher in the test groups than the controls ($P < 0.05$). HDL-C concentration of 49.09 ± 12.56, 47.26 ± 10.45, 45.53 ± 10.83 was however lower in the test groups but this was not significant ($p > 0.05$).The mean total testosterone (TT) levels of the control and test groups (A, B and C); 6.84 ± 2.69, 5.25 ± 2.27 ng/ml, 2.64 ±1.15 were observed to be significantly lower in the test group ($P < 0.05$).

Table 4.3 shows the correlations between TT and other parameters in the test group. TT correlated negatively and significantly ($P < 0.05$) with Age ($r = - 0.626$, $P = 0.000$), TC ($r = -0.250$, $P = 0.015$), LDLC ($r = - 0.247$, $P = 0.017$), but it was observed to correlate positively and significantly with DBP, ($r = 0.205$, $P = 0.047$).

Table 4.4 shows the correlations between AIP and other parameters of the test subjects. It was observed that AIP correlated positively and significantly with Age ($r = 0.0261$, $p = 0.011$), TC ($r = 0.404$, $p = 0.000$), TG ($r = 0.816$, $p = 0.000$), LDLC ($r = 0.473$, $p = 0.000$) but negatively and significantly with HDLC ($r = -0.492$, $p = 0.000$).

Table 4.1: Mean ± SD of Body Mass Index, Systolic Blood Pressure, Diastolic Blood Pressure and Fasting Blood Glucose of the various Age groups

PARA	GRP A	GRP B	GRP C	F VAL.	P VAL.	POST HOC TEST		
						AvB	AvC	BvC
BMI	23.77±3.99	26.74±4.45	28.23±4.38	19.905	0.000	0.000	0.000	0.201
SBP	116.83±15.27	129.13±18.54	131.85±18.26	15.658	0.000	0.000	0.000	0.715
DBP	76.91±9.29	86.32±14.01	84.32±11.28	13.794	0.000	0.000	0.000	0.659
FBG	84.26±22.08	89.66±23.24	108.57±62.98	6.880	0.001	0.692	0.001	0.037

Table 4.2: Mean ± SD of Lipid profile, AIP and Total Testosterone of the various Age Groups

PARA	GRP A	GRP B	GRP C	F VAL.	P. VAL.	POST HOC TEST		
						AvB	AvC	BvC
TC	139.38±38.34	157.34±36.66	184.81±33.56	23.996	0.000	0.019	0.000	0.001
TG	56.99±20.40	77.91±27.81	90.55±35.37	27.118	0.000	0.000	0.000	0.059
HDL-C	49.09±12.56	47.26±10.45	45.53±10.83	1.513	0.223	0.654	0.205	0.753
LDLC	79.31±33.24	94.81±32.96	119.4±31.57	23.539	0.000	0.024	0.000	0.001
AIP	0.05±0.18	0.2±0.20	0.28±0.17	27.832	0.000	0.000	0.000	0.086
TT	6.84±2.69	5.25±2.27	2.64±1.15	52.813	0.000	0.000	0.000	0.000

Table 4. 3: Correlation between Plasma Total Testosterone and other Test parameters in the Test Group

Total Testosterone (TT)	**r**	**p-value**
Age	-.626**	.000
Body Mass Index	-.155	.137
Systolic Blood pressure (SBP)	.112	.281
Diastolic Blood Pressure (DBP)	.205*	.047
Fasting Blood Glucose (FBG)	-.139	.181
Total Cholesterol (TC)	-.250*	.015
Triglyceride (TG)	-.148	.155
High density lipoprotein cholesterol (HDL C)	.084	.419
Low density lipoprotein cholesterol (LDL C)	-.247*	.017
Atherogenic index of plasma (AIP)	-.163	.116

Key:

**. Correlation is significant at the 0.01 level

*. Correlation is significant at the 0.05 level

Table 4.4: Correlation between AIP and other Test parameters in the Test Group

Atherogenic Index of Plasma (AIP)	**r**	**p-value**
Age	.261*	.011
Body Mass Index	.103	.322
Systolic Blood pressure (SBP)	.131	.209
Diastolic Blood Pressure (DBP)	.094	.370
Fasting Blood Glucose (FBG)	.153	.141
Total Cholesterol (TC)	.404**	.000
Triglyceride (TG)	.816**	.000
High density lipoprotein cholesterol (HDL C)	-.492**	.000
Low density lipoprotein cholesterol (LDL C)	.473**	.000
	-.163	.116

Total Testosterone (TT)		

Key:

**. Correlation is significant at the 0.01 level

*. Correlation is significant at the 0.05 level

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The relationship between Total Testosterone and Body Mass Index, Diastolic blood Pressure, Systolic Blood Pressure, Fasting Blood Glucose, Atherogenic Index of Plasma and lipids in 188 apparently healthy male subjects in Benin City was determined in this cross - sectional study.

The study showed that the mean plasma Total Testosterone, Body Mass Index, Diastolic blood Pressure, Systolic Blood Pressure, Fasting Blood Glucose, Lipids (except High Density Lipoprotein Cholesterol) levels and Atherogenic Index of Plasma increased with age, while high density lipoprotein cholesterol levels lowered with age. It was also observed that Body mass index, Total Cholesterol, Triglycerides, Low density Lipoprotein Cholesterol and Atherogenic Index of Plasma were not only significantly higher in the Test group, but also correlated positively with age.

The mean plasma testosterone concentration was observed to be lower among the test group compared to the controls. Low serum total Testosterone is common in the aging population and has a prevalence of 30% in men over the age of 60 years; it is also said to be associated with several CVD risk factors including an atherogenoic lipid profile, insulin resistance obesity and prothrombotic fibrinolytic profile (Kapoor *et al*., 2007, Chris *et al*., 2010). Studies in male animals have

shown that induced hypogonadism increases atherosclerosis but testosterone replacement reverses it. (Neetleship *et al*., 2007). Therefore, the finding also of a significant negative correlation between Testosterone and age in the test group is in agreement with previous studies by Kaufman and Vermeulen, (2005) which shows that male total testosterone concentrations lowers gradually after the age of 40 years.

Furthermore, a significant negative correlation of TT with TC and LDLC was also observed in this study and it is in agreement with the findings of reports from previous clinical, epidemiological, cross-sectional and case -control studies which confirmed that total and LDL cholesterol inversely correlate with testosterone levels and are associated with atherogenic lipid profile (Akishita *et al.,* 2010, Haring *et al.,* 2011, Nan *et al.,* 2014). Also, Testosterone treatment resulted in a decrease in total and LDL cholesterol (Ly *et al., 2007,* Zitzmann and Nieschlag, (2007), thereby further establishing a relationship between testosterone and atherogenic lipids.

According to a study by Chandima *et al*., (2013) healthy men with lower levels of plasma testosterone tend to have lower levels of HDL-Ch and lipoprotein lipase;

by contrast, our study showed no significant association of testosterone with HDL cholesterol even though the levels were observed to be lower in the test group.

The negative correlation between testosterone and diastolic blood pressure in our study agrees with the report of Al-Chalubi, and Al- Waeli, (2010). However, the physiological and clinical significance of this relationship needs to be further evaluated since.

Atherogenic Index of Plasma (AIP) associated positively with Age, Total Cholesterol, Triglycerides, Low density lipoprotein Cholesterol but negatively with HDLC levels; this finding is in agreement with the report of Niroumand *et al*., (2015) and Myat *et al*, (2018) who found a significant positive correlation between AIP and increasing levels of cardiovascular risk factors. Bhrdwaj *et al*., (2013) had earlier reported the role of AIP in predicting atherosclerosis. He reported that AIP was the highest and the most sensitive index for assessing cardiovascular risk factors and predicting acute coronary events when compared with other indices. Furthermore, Mudhaffar, (2013) suggested that AIP may be used as an alternative screening tool in assessing cardiovascular risk in an event where all atherogenic variables are normal.

Numerous studies have shown that low testosterone levels are associated with hypertension, dyslipdaemia, obesity and insulin resistance all of which contributes

to cardiovascular disease risk (Somani *et al.,* 2010). In the Tromsø study, the cross-sectional analysis of 1274 men without known cardiovascular diseases showed that TT was inversely and independently associated with TG and positively and independently associated with HDL-C (Agledahl *et al.,* 2008). In a review on the relationship between androgens and CVD by Liu *et al.,* (2003), the author concluded that Testosterone levels were consistently lower in men with cardiovascular disease.

The negative effects of Androgen deprivation therapy (ADT) on lipids and the favourable effects of Testosterone Replacement therapy (TRT) on lipids have been exhaustibly discussed in different literatures; therefore, the finding in our study of an association between low testosterone and atherogenic lipids as well as with other individual cardiovascular risk factors (DBP, AIP and Age) is an indication of a possible link between Testosterone and Cardiovascular disease in our study area and this agrees with the finding of Saad *et al.,* (2012), Salam *et al.,* (2012) amongst others.

Consequent on these findings, our study seems to have provided reasonable justifications for the possible use of Testosterone as a predictive marker for assessment of men who may be at risk of cardiovascular disease.

5.1 CONCLUSION

This study showed that plasma testosterone is associated atherogenic lipids; Age and Diastolic blood pressure and some other independent cardiovascular risk factors. This may be an indication that a low total plasma testosterone could be associated with the development of cardiovascular diseases in the aging males in our study population. These findings are also suggestive that Total Testosterone could have a direct effect on cardiovascular health.

Conclusively, this study showed that Testosterone is associated with atherogenic lipid; it may therefore be considered a risk factor and/or a predictive marker for men at risk for cardiovascular disease.

RECOMMENDATION

The following are recommended with regards to the relationship between Testosterone and lipids:

1. Testosterone levels should be checked routinely in aging males and attention paid to the lipid profile of subjects with relatively low plasma Total Testosterone.

2. Further studies are needed to clarify the relationships between Total Testosterone and plasma lipids to confirm its clinical implications by using the active forms of Testosterone (Free and Bioavailable).

3. Further studies on subjects with cardiovascular diseases should be carried out to better establish the relationship between Total Testosterone and cardiovascular diseases in our study area.

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INFORMED CONSENT FORM

TITLE OF STUDY: Androgen Level, Atherogenic Lipid index and Profile Among Aging Men in Benin City.

INVESTIGATORS: IRUOBE, LAURETTA[1] OSADOLOR, HUMPHREY[2]

1 Department of Medical Laboratory Services, Ministry Of Health, Benin City, Nigeria and

2 Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

FINANCIAL SPONSORSHIP: This research study is self-sponsored.

PURPOSE OF RESEARCH: The purpose of this study is to evaluate the relationship between plasma total testosterone and lipid profile of men in the Benin City Metropolis, Nigeria

PROCEDURES INVOLVED IN THE STUDY: 1. An Early morning venous blood sample will be obtained from each male for the measurement TT, FBG, TG, Total, HDL and LDL Cholesterol, levels)

4. Weight, Height and Blood pressure of each participant will also be measured for the research purpose.

COMPENSATION: There will no financial compensation for participating in this study.

VOLUNTARY PARTICIPATION: Please note, that your participation in this study is entirely voluntary and that you will neither be coerce to take part nor will any form of discrimination against you should you opt out. In the event that you decided to stop participating, you are very free to withdraw even if you had earlier given your consent. Your refusal or withdrawal from participating will not in any way affect your relationship with the Department.

RISK: Blood will be collected from you, and there shall be minimal painful discomfort associated with the blood collection. No other adverse effect or risk is expected to be associated with participation in this study.

BENEFITS: Findings from this study will provide information on the relationship between plasma total testosterone and atherogenic lipid profile of men in the Benin City Metropolis, Nigeria

CONFIDENTIALITY: Information obtained will be treated with utmost confidentiality. Your names will not be used. The blood samples will not be given out to other investigators for any other study.

CONTACT INFORMATION

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CERTIFICATE OF CONSENT

I have read the above information. I had the opportunity to ask questions about it and I have been answered to my satisfaction.

(A) I consent voluntary to take part as the participant in this study.

(B) I do not consent to participate in this study.

Name of Participant: ……

Signature of Participant: ……..……

Date: ……..……

QUESTIONNAIRE

This questionnaire is part of a study to evaluate the Androgen Level, Atherogenic Lipid index and Profile among Aging Men in Benin City". Information about you will be needed. Please we solicit your cooperation in answering the questions below. The information obtained will be treated with utmost confidentiality.

1. Identification number ……
2. Age:……..(years)
3. Gender: Male [ ]
4. Marital Status: Single [ ] Married [ ] Divorced [ ]
5. Weight: ……
6. Height: ……..
7. Occupation: ……..
8. Tribe: ……
9. Academic qualification: Pry [ ]Secondary [ ]Tertiary [ ] Non formal [ ]
10. How often do you go for routine Medical Checkup? Bi annually [ ] Annually [ ] Never [ ]
11. Do you smoke? Yes [ ] No [ ]
12. Do you take alcohol? Yes [ ] No [ ]
13. Are you diabetic? Yes [ ] No [ ] Not Sure [ ]
14. Are you hypertensive Yes [ ] No [ ] Not Sure [ ]

15. Do you have a heart condition? Yes [ ] No [ ] Not Sure [ ]
16. Have you have had issues with infertility? Yes [ ] No [ ] Not Sure [ ]
17. Have you ever had hormonal therapy: Yes [ ] No [ ]. Not Sure [ ]
18. If yes, how long? 3 Months [ ] 6 Months [ ] 9 months [ ] over 12 months [ ].
19. Do you have a decrease in your sex drive (libido)? Yes [ ] No [ ] Not Sure [ ]
20. Are your erections less strong? Yes [ ] No [ ].
21. Do you have a decrease in strength and/or endurance? Yes [ ] No [ ] Not Sure [ ]
22. Do you have a lack of energy? Yes [ ] No [ ] Not Sure [ ]
23. Have you noticed a lost in your height? Yes [ ] No [ ] Not Sure [ ]
24. Are you more sad and/or grumpy than usual? Yes [ ] No [ ] Not Sure [ ]
25. Have you noticed a decreased enjoyment in life? Yes [ ] No [ ] Not Sure [ ]
26. Do you play any sport (s)? Yes [ ] No [ ] Not Sure [ ]
27. What kind of sport(s) do you play? …….
28. Have you noticed a recent decrease in your ability to play your sport (s)? Yes [ ] No [ ] Not Sure [ ]
29. Are you happy with your kind of work/Job? Yes [ ] No [ ] Not Sure [ ]
30. If yes, have you noticed a recent decrease in your work performance? Yes [ ] No [ ] Not Sure [ ]