SOME BIOCHEMICAL CHANGES AND METABOLIC SYNDROME IN PERIMENOPAUSAL IGBO WOMEN IN ENUGU METROPOLIS

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AUGUST, 2018.

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A thesis presented to the Department of Medical Laboratory Science, Faculty of Health Sciences and Technology submitted to the School of Post Graduate Studies in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D) in Chemical Pathology of Nnamdi Azikiwe University, Nnewi Campus.

AUGUST, 2018.

CERTIFICATION

This is to certify that I am responsible for the work submitted in this thesis. The original work is mine except as specified in the acknowledgements and references; the thesis has not been submitted to this University or any other Institution for the award of a degree.

Signature…… **Date**……

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APPROVAL PAGE

This project work titled "Some biochemical changes and metabolic syndrome in perimenopausal Igbo women in Enugu metropolis" carried out by Ikegwuonu Ifeoma Chinwe (Reg. no : 2011347006F) has been approved for the award of ph.D in Medical Laboratory Sciences, Chemical Pathology specialty, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus. Anambra State.

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DEDICATION

This work is dedicated to the Holy Spirit my Advocate and Inspirator.

ACKNOWLEDGEMENT

I wish to appreciate God who gave me life; direction and the requisite providence that made this work a reality.

My profound gratitude goes to my supervisor Prof. C.C. Onyenekwe for his direction, commitment and relentless effort to see to the completion of this work.

I express my gratitude to Dr. I.C. Maduka, for his guidance and assistance in my laboratory analysis. I cannot forget my colleague, Dr. S. Ogbodo, Dr. Emeka Neboh and Mr. Okey Onwukwe who provided assistance in my literature search and data analysis.

I am grateful to Dr. P. O. Manafa, Dr. N. R. Ukibe, Dr. Nancy Ibeh, (Head of the department), Prof P. O. Ezenduka (Dean Faculty of Health Science), Prof. N. R. Agbakoba and other lecturers in the Department for their encouragement and corrections to fine tune this work.

This work would not have been possible without the special contributions of the Clinicians and other staff of Gynecology unit of Enugu State University Teaching Hospital and Mother of Christ Specialist Hospital Enugu. I am especially grateful to Prof. Ezeugwu, the Dean of Faculty of Clinical Sciences in ESUTH and Rev. Sr. (Dr). T. Ezechukwu, the Chief Gynecologist in Mother of Christ Specialist Hospital Enugu, who made their patients available and assisted me with sample collection. I am also indebted to Mr. Hamilton Chukwuma who helped me greatly with sample collection. I would also like to appreciate all the participants who gave their consent to participate in this study especially from Holy Spirit Catholic Church, G.R.A Enugu and Methodist Church Ndiagu Enugu.

My special appreciation also goes to my darling husband Chief (Hon) Ifenna Patrick Ikegwuonu for his supports (financial and moral) and our adorable children, Pharm. Adanna Perpetua Ikebudu, Mr. Tobenna Patrick Ikegwuonu, Mr. Chidera Michael Ikegwuonu, Miss Ifeoma Christopheen Ikegwuonu, Master Ifechukwu Geoffrey Ikegwuonu, Miss kosisochukwu Joan Ikegwuonu and Pharm. Adichie Ikebudu (my son in-law), whose understanding and assistance are unquantifiable. Finally to my late mum, H.R.H Lady Nnonyem Achufusi, my family members, friends and colleagues who never relented in praying for my success.

I love you all and God bless you all.

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DEFINITION OF TERMS

ALP - - - - -	Alkaline Phosphatase
BMD - - - - -	Bone Mineral Density
BMI - - - - -	Body Mass Index
DBP - - - - -	Diastolic Blood Pressure
DM - - - - -	Diabetes Mellitus
E_2 - - - - -	Estradiol
FBG - - - - -	Fasting Blood Glucose
FSH - - - - -	Follicle Stimulating Hormone
HDL-C - - - -	High Density Lipoprotein cholesterol
HTN - - - - -	Hypertension
IDF - - - - -	International Diabetes Federation
IUCD - - - - -	Intrauterine Contraceptive Device
LDL-C- - - - -	Low Density Lipoprotein Cholesterol
LH - - - - -	Luteinizing Hormone
MetS - - - - -	Metabolic syndrome
NCEP-ATP 111 - - -	National Cholesterol Education Panel 111
Non-MetS - - - -	No Metabolic syndrome
SBP - - - - -	Systolic Blood Pressure
TC - - - - -	Total Cholesterol
TG - - - - -	Triglyceride
VLDL-C - - - -	Very Low Density Lipoprotein cholesterol
WC - - - - -	Waist Circumference
WHO - - - - -	World Health Organization

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ABSTRACT

Perimenopause is associated with a lot of hormonal changes. These hormonal changes also influence metabolic processes accounting for some biochemical changes which possibly give rise to metabolic syndrome (MetS) in perimenopausal women. There is insufficient information on the association between hormonal changes, MetS and perimenopause hence this study investigated some biochemical changes and possible metabolic syndrome in perimenopausal women. Two hundred subjects (one hundred and twenty perimenopausal women mean age 50 years and eighty premenopausal women mean age 35 years) who were living in Enugu were recruited for this study. Anthropometric indices (blood pressure, waist circumference, weight and height) of these women were measured. Ten milliliters of fasting blood samples collected from the participants were used for measurement of hormonal and biochemical parameters. Hormone profile (luteinizing hormone(LH), estradiol(E_2) and follicle stimulating hormone(FSH) were determined using Enzyme immunoassay (ELISA), fasting plasma glucose (FPG), lipid profile, uric acid, and alkaline phosphatase levels were assayed using enzymatic colorimetric methods while levels of inorganic phosphate and calcium were determined using phenolphthalein monophosphate substrate and o-cresolphthalein methods respectively. All data were analysed using the Statistical Package for the Social Sciences (SPSS) version 20 computer software at 95% confidence level, and results were expressed as mean ± standard deviation (SD). A comparison of the mean ± SD of the perimenopausal and the premenopausal women showed significantly ($p< 0.05$) higher values of blood pressure (systolic and diastolic), fasting plasma glucose, waist circumference, luteinizing hormone, follicle stimulating hormone, total cholesterol, low density lipoprotein cholesterol, uric-acid and calcium; while there was a significantly ($p<0.05$) lower value of estradiol in perimenopausal women compared to the premenopausal women. There were no significant difference ($p>0.05$) in the mean values of other parameters between perimenopausal and premenopausal women. The metabolic syndrome (MetS) of the perimenopausal and premenopausal women were observed using three different criteria the National Cholesterol Education Program- Adult Treatment Panel 111(NCEP-ATP 111), the World Health Organization (WHO) and International Diabetes Federation (IDF). The incidence of MetS was higher in the perimenopausal women compared to the premenopausal women in all the three criteria studied. The prevalence of metabolic syndrome was different in all the three criteria studied. The levels of calcium, uric-acid, inorganic phosphate and alkaline phosphatase were determined in the perimenopausal and premenopausal women with MetS in the three groups using the different cut-off points of the four parameters. Calcium was predominantly higher in the three criteria. The relationships between the parameters were observed, LH and FSH levels showed positive correlation with FPG while levels of E_2 was negatively associated with FPG. Similarly levels of LH showed positive association with inorganic phosphate while E_2 levels were negatively associated with alkaline phosphatase. The significantly higher biochemical changes in the perimenopausal women in comparison with their premenopausal counterparts which gave rise to higher metabolic syndrome in all the three different criteria assessed in this study showed that perimenopausal women in this study had higher risk of metabolic syndrome than the premenopausal women. The study demonstrated that central obesity and hypertension were the predominant components and also risk factors for the development of metabolic syndrome associated diseases among the Igbo women with metabolic syndrome in Enugu Metropolis. This study also suggests that calcium may be included in the panel of components of metabolic syndrome in Igbo women in Enugu metropolis.

CHAPTER ONE

INTRODUCTION

1.1 Background

Metabolic syndrome (MetS) is an increasingly important public health issue which deserves more attention. MetS is the name for a group of risk factors that raise the risk for heart disease and other health problems, such as diabetes and stroke (Adeoye et al 2015). The term "metabolic" refers to the biochemical processes involved in the body's normal functioning while risk factors are traits, conditions or habits that increase the chance of developing a disease (Carr, 2003). Metabolic risk factors are the biochemical conditions, traits or habits that increase the chance of developing a disease, such as a large waist line which is also known as abdominal obesity, a high triglyceride (TG) level, a low high density lipoprotein cholesterol (HDL-C) level, high blood pressure (BP) and high fasting blood glucose (FBG) (Carr, 2003). Metabolic syndrome is diagnosed in the presence of three or more of the above listed risk factors in an individual (Wahab et al, 2008).

Metabolic syndrome was first described by Reaven in the late 1940s but became clearly defined as a clinical entity in the 1980s (Wanngmethe et al, 2005). In 1998, the World Health Organization (WHO) was the first to provide a definition of metabolic syndrome. The criterion for MetS was based on the evidence that insulin resistance is central to the pathophysiology of MetS. Therefore, they state that insulin resistance must be present in an individual plus any two of the following; obesity, dyslipidemia and hypertension for the person to be diagnosed as having MetS. The Insulin resistance was defined as fasting plasma glucose greater than 100mg/dl or impaired glucose tolerance (IGT) defined as a glucose level above 140mg/dl for 120 minutes after ingestion of 75g of glucose load during an oral glucose tolerance test (Alberti and Zimmet, 1998). The definition also allows patients with type 2 diabetes to be diagnosed with MetS if they met the other criteria (obesity, dyslipidemia and hypertension). Obesity was defined as body mass index (BMI > 30kg/m^2) or waist to hip ratio, insulin resistance as fasting plasma glucose (FPG >100mg/dl), dyslipidemia as fasting Triglyceride (TG ≥1.7mmol/l) or high density lipoprotein cholesterol (HDL-C <1.0mmol/l) and hypertension as blood pressure (systolic ≥ 140mmHg, diastolic ≥90mmHg). In response to this finding, the European Group for the study of Insulin Resistance (EGIR) proposed a modification of the WHO definition (Balkau and Charles, 1999). Like the WHO, the EGIR considered that insulin resistance was central to the pathophysiology of the MetS, so insulin

resistance was also required as a pivotal criterion but in this case, insulin resistance was defined by a fasting plasma insulin value that is greater than the 75thpercentile. The use of elevated fasting insulin alone as a reflection of insulin resistance simplifies the definition but it also means that patients with type 2 diabetes cannot be diagnosed as having MetS, since fasting insulin may not be a useful measure of insulin resistance in such patients. Similar to the WHO definition, European Group for Insulin Resistance definition requires two additional criteria, obesity, hypertension and dyslipidemia. But obesity in EGIR definition was simplified to waist circumference while WHO definition used a choice of waist to hip ratio or body mass index. The National Cholesterol Education Program Adult Treatment Panel 111 (NCEP ATP 111) released its definition in 2001, which was updated by the American Heart Association and the National Heart Lung and Blood Institute in 2005 (Grundy et al, 2005). According to the NCEP ATP111 definition, metabolic syndrome is present if three or more of the following five criteria are met. Elevated FBG (≥ 110mg/dl), TG (≥ 1.7mmol/l), BP (systolic ≥ 130mmHg, diastolic ≥ 85mmHg), Waist circumference (WC ≥ 88cm for women) and HDL-C < 1.29mmol/l). This definition incorporates the key features of hyperglycemia/insulin resistance, visceral obesity, atherogenic dyslipidemia and hypertension. It uses measurements and laboratory results that are readily available to physicians, facilitating its clinical and epidemiological application. It is also simple and easy to remember; importantly it does not require any specific criterion to be met.

The proliferation of definitions suggested that a single unifying definition was desirable, in the hope of accomplishing this task; the International Diabetes Federation (IDF) proposed a new definition of MetS in April, 2005. (Zimmet et al, 2005). Although it includes the same general criteria as the other definitions, it requires central adiposity defined on the basis of waist circumference as a mandatory criterion for MetS diagnosis. The obesity requirement is met by population specific cut points. This accounts for the fact that different populations, ethnicities and nationalities have different distributions of norms for body weight and waist circumference. It also recognizes that the relationship between these values and the risk for type 2 diabetes or cardiovascular disease differs in different populations. International Diabetes Federation criterion states that for a person to be classified as having metabolic syndrome, such a person must have waist circumference ≥ 80cm for women (sub-sahara African) and any two or more of the following four conditions: elevated triglyceride concentration (≥ 1.7mmol/l), blood pressure (systolic ≥130mmHg, diastolic ≥85mmHg), fasting blood glucose (≥ 100mg/dl) and reduced high density lipoprotein-cholesterol (<

1.29mmol/l). Insulin resistance was the pivotal feature in the WHO criterion, waist circumference rather than body mass index was the differentiating factor of the MetS definition in the NCEP ATP-111 panel while waist circumference remain a mandatory feature for metabolic syndrome in the IDF criterion.

The worldwide prevalence of metabolic syndrome varies from 13.6% to 46% (Adegoke *et al,* 2008) depending on the diagnostic criteria used and the population studied. The prevalence of metabolic syndrome in Nigeria has been documented in recent studies as ranging between 12.1% in post menopause to 54.3% in individuals with diabetes mellitus (Adeoye *et al,* 2015). The prevalence of metabolic syndrome has been found to be affected by factors such as age, ethnicity, sex and the criterion used (Fezeu *et al,* 2007). It was estimated that around 20-25 percent of the world's adult population have metabolic syndrome and they are twice as likely to die from cardiovascular diseases and three times as likely to have a heart attack or stroke compared to those without the metabolic syndrome (UKPDS, 1996).

The predisposing factors of metabolic syndrome are being associated with Perimenopause (Achie *et al*, 2012). Perimenopause also known as menopausal transition is the time when the ovaries gradually begin to make less oestrogen leading to increases in luteinizing hormone and follicle stimulating hormone due to negative feed-back mechanism (Harlow *et al*, 2012). A female is born with about two million eggs in her ovaries, each in its own follicle. These follicles become fewer as she ages and climacteric begins when only about 1000 follicles are left, which are less responsive to gonadotropin, so less oestrogen and progesterone are secreted (Manson and Kaunitz, 2016). During climacteric (mean age 50 years) also known as Perimenopause there are changes in the woman's body, the uterus, vagina and breasts atrophy, intercourse becomes uncomfortable and vaginal infections more common. The skin becomes thinner, cholesterol levels rise increasing the risk of cardiovascular disease and bone mass declines increasing the risk of osteoporosis (Sullivan, 2017). Other changes like hot flashes, mood changes, irritability and skipped periods are seen as a result of sudden dilation of cutaneous arteries as the blood vessels constrict and dilate in response to shifting hormone balances (Dienye *et al,* 2013).

Perimenopause continues until the woman reaches menopause which is the cessation of menstrual cycles. Perimenopause usually occur between the ages of 45 and 55 years in Caucasians (Harlow *et al*, 2012) and early 40's to late 50's in Nigeria (Ameh *et al,*

2016).These biochemical changes during perimenopause tend to increase the risk of cardiovascular diseases. The factors associated with increased cardiovascular disease tend to cluster in peculiar biological traits such as increased blood pressure, fasting plasma glucose, triglycerides, low density lipoprotein cholesterol, abdominal obesity and decreased high density lipoprotein cholesterol (Oguoma *et al,* 2017). The constellation of these factors is identified as metabolic syndrome and is associated with an approximate doubling of the risk of cardiovascular morbidity and mortality. The more components of metabolic syndrome that are present in an individual, the higher the cardiovascular mortality risk (Hu et al., 2004), Despite these reports there is paucity of information on metabolic syndrome in Enugu Southern Nigeria and no data on perimenopausal women. Enugu State is central in position in Southern Nigeria and inhabits most of Igbo indigenes. This study is aimed to understanding the biochemical changes and metabolic syndrome of perimenopausal Igbo women in Enugu metropolis and thereby proffers preventive approaches.

1.2 Statement of problem

The prevalence of metabolic syndrome in most sub-Saharan African countries has been under reported. A prevalence of 5.9 percent was reported in urban women in Cameroon using WHO diagnostic criteria (Fezeu et al., 2007), while a prevalence rate of metabolic syndrome in Nigeria is on the increase daily (Ghazali and Sanusi, 2010). Perimenopause is accompanied with a number of hormonal changes which result in many biochemical alterations in the body. These biochemical changes affect the blood sugar levels, blood pressure, visceral adiposity and lipid status of the body; these constitute some of the risk factors of metabolic syndrome. The re-emerging prevalence of obesity in Nigeria and increasing prevalence of type-2 diabetes mellitus (Okafor, 2012) may be some of the propelling factors to increasing rate of metabolic syndrome yet perimenopause and MetS have been understudied in Nigeria. Therefore, there is need for continued investigation of metabolic syndrome and perimenopause in different population in Nigeria. Hence this study investigated some biochemical changes and metabolic syndrome of perimenopausal and premenopausal women in Enugu metropolis.

1.3 Justification for the study.

Metabolic syndrome is an independent risk factor for coronary heart disease and is associated with an approximate doubling of the risk of cardiovascular morbidity and mortality (Adeoye *et al*, 2015). Perimenopause phase has been understudied, most researchers have worked on

post menopause and premenopause yet perimenopause phase is a period when a number of biochemical changes as well as many derangements in health conditions start developing as a result of hormonal changes (Manson and Kaunitz, 2016). These derangements in health conditions and biochemical changes lead to higher incidence of MetS and cardiovascular occurrences (Okafor, 2012). The prevalence rate of metabolic syndrome is increasing in Nigeria (Ghazali and Sanusi, 2010), yet there is paucity of information on the metabolic syndrome and perimenopause amongst Igbo women in Enugu South-east Nigeria.

1.4 Significance of study

Metabolic syndrome is an important public health problem with high rates of cardiovascular morbidity and mortality (Fezeu et al, 2007). The outcome of this study may reveal some biochemical alterations in perimenopause and the relationship between the components of metabolic syndrome and perimenopause. The findings of this study may also help to identify women who are at high risk of developing metabolic syndrome associated diseases and its burden amongst these sets of Igbo women.

1.5 Aim

The principal aim of this study was to investigate the risk of metabolic syndrome by examining some biochemical changes in perimenopausal Igbo women in Enugu metropolis.

Specific objectives

1. To determine luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E_2), lipid profile, fasting plasma glucose, calcium, inorganic phosphate, uric acid, alkaline phosphatase levels and also measure the blood pressure, body mass index and waist circumference in perimenopausal and premenopausal Igbo women.

2. To determine prevalence of metabolic syndrome amongst Igbo women using NCEP-ATP 111, WHO and IDF criteria.

3. To determine if calcium, alkaline phosphatase, inorganic phosphate and uric-acid could be added to the panel of metabolic syndrome in objective (2) above.

4. To deduce if incidence of metabolic syndrome is same amongst premenopausal and perimenopausal Igbo women in Enugu metropolis.

1.6 Research questions

1. Are there some biochemical changes present in perimenopausal Igbo women in Enugu metropolis?

2. Are there differences in prevalence of metabolic syndrome using the three criteria (WHO, NCEP-ATP 111 and IDF)?

3. Are there other biochemical parameters that could be included in the panel of metabolic syndrome (MetS)?

4. Is the incidence of MetS in premenopausal same as in perimenopausal Igbo women?

1.7 Hypothesis

Ho: There is no change in biochemical parameters as well as metabolic syndrome among perimenopausal Igbo women in Enugu metropolis.

Hi: There is a significant change in biochemical parameters as well as metabolic syndrome among perimenopausal Igbo women in Enugu metropolis.

Ho: There are no differences in prevalence of metabolic syndrome using the three criteria.

Hi: There are differences in prevalence of metabolic syndrome using the three criteria.

Ho: There are no other biochemical parameters that could be added to the panels of metabolic syndrome.

Hi: There are some other biochemical parameters that could be added to the panels of metabolic syndrome.

Ho: There are no differences in the incidence of metabolic syndrome in both premenopause and perimenopause in Igbo women.

Hi: There are differences in the incidence of metabolic syndrome in both premenopause and perimenopause in Igbo women.

1.8 Scope of the study

This study examined some biochemical changes and metabolic syndrome in perimenopausal and premenopausal Igbo women in Enugu metropolis. The study involved perimenopausal women between 45-55years (mean age= 50years) and premenopausal women between 30-40

years (mean age =35years) in Enugu metropolis. The biophysical/anthropometric parameters measured were blood pressure, waist circumference and body mass index while the biochemical parameters determined were luteinizing hormone, follicle stimulating hormone, estradiol, fasting plasma glucose, lipid profile (TG, TC, HDL-C, LDL-C, and VLDL-C), uric acid, calcium, inorganic phosphate and alkaline phosphatase. Metabolic syndrome was studied using the three criteria National Cholesterol Education Program Adult Treatment Panel 111 (NCEP-ATP 111), World Health Organization (WHO) and International Diabetes Federation (IDF).

CHAPTER TWO

LITERATURE REVIEW

2.1. Menopause

Menopause is an inevitable milestone in the reproductive life of women. It refers to a women's last menstrual period and a woman can be said to have reached menopause when she has had one year without menstruating (Adegoke *et al*, 2008). The quality of life of perimenopausal and menopausal women are strongly influenced by social, cultural and economical settings in which they live (Dimkpa, 2011). They face various challenges from coping with hot flushes and night sweats to dealing with the discomfort of vaginal dryness. Every woman's experience of menopause is unique; she may experience all of the symptoms or none of them, some women find menopause transition barely noticeable while others find it life altering (Henn, 2010). Menopause is seen as a new phase in their life cycle, characterized by several symptoms brought about by decreased hormonal activity in the body system. Hence, women at this stage need to make suitable adjustments that will enable them cope with the new challenges successfully. The period is similar to retirement from active service, whereby the retiree feels a sense of loss because job has been taken out of her hands. Thus, women transiting into menopause feel that their youthful attractions are fading away. Menopause which is a natural phenomenon among women was not considered a problem in Africa many years ago until recently. This could be attributed to the simple life style which mothers of those days lived as well as the low level of education, whereby women were only meant to play the roles of child bearing and housekeeping.

Menopause has in recent times become a cause for concern due to the sophisticated life styles of the modern day African and indeed Nigerian women who attach importance to aesthetics now, than hitherto (Dimkpa, 2011). To this end, some women perceive it as 'the end of the road' to their ability to remain attractive to their spouses, which is a major need for counseling women who have attained menopause.

2.2 Human gonadotropins

Follicle stimulating hormone is a member of the gonadotropin family, which includes also luteinizing hormone and human chorionic gonadotropin (hCG). Gonadotropins are complex heterodimeric glycoproteins which consist of two linked protein components designated as

the α- and β-subunits. The α-subunit is common to the three gonadotropins, whereas the β-subunit confers specificity and biological activity.

According to the "two cell, two gonadotropin" theory of both FSH and LH are necessary for ovarian follicular maturation and the syntheses of ovarian steroid hormones. Luteinizing hormone promotes the production of androgens (dehydroepiandrosterone, androstenedione, and testosterone) from cholesterol and pregnenolone, by stimulating 17α-hydroxylase activity in the thecal cells. The androgens then diffuse to the granulosa cells where FSH stimulates the expression of the cytochrome P450 aromatase, which converts the androgens to oestrogens (Janssen *et al*, 2008).

Follicle-stimulating hormone (FSH) plays a key role in the development and function of the reproductive system and is widely used both in clinical and research settings. The accurate and reliable measurement of FSH levels is essential for safe and successful treatment in developmental and reproductive medicine (Isong *et al*, 2016), as well as for research studies examining the association between FSH levels and various disease outcomes.

The measurement of FSH in circulation is employed in the diagnosis of disorders of reproduction and development, whereas therapeutic preparations of FSH are widely used for induction of ovulation in women and stimulation of spermatogenesis in men. The effects of gonadotropins may not be limited to endocrine and reproductive functions. Before starting complex epidemiological studies examining the associations between FSH and various diseases, it is important to assess the extent of the hormone's underlying fluctuations in circulation. FSH levels peak during the menstruation and ovulatory phase and are lower during the late follicular and luteal phases of the menstrual cycle (Manson and Kaunitz, 2016). After menopause, FSH levels gradually increase through negative biofeedback as a result of ovarian function cessation. As a woman approaches perimenopause the number of small antral follicles recruited in each cycle diminishes and consequently insufficient inhibin B is produced to fully lower FSH and the serum level of FSH begins to rise. Eventually FSH level becomes so high that down regulation of FSH receptors occurs and by postmenopause any remaining small secondary follicles no longer have FSH nor LH receptors (Isong *et al*, 2016).

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are called gonadotropins because they stimulate the gonads - in males, the testes, and in females, the ovaries. They are not necessary for life, but are essential for reproduction. These two

hormones are secreted from cells in the anterior pituitary called gonadotrophs. Most gonadotrophs secrete only LH or FSH, but some appear to secrete both hormones. Physiologic effects of the gonadotrophins are known only in the ovaries and testes. Together, they regulate many aspects of gonadal function in both males and females.

The pituitary hormones influence the development and maturation of several ovarian follicles in each menstrual cycle. Usually only one follicle develops fully while the others recede, the dominant follicle produces an egg which will be released and can be fertilized. The growing follicle secretes increasing amounts of oestrogen, following the peak of oestrogen production; there is a surge of luteinizing hormone. The luteinizing hormone surge triggers the release of the mature egg from its follicle (Saladin, 2012).

Luteinizing hormone (LH) is an important hormone both men and women produce. LH plays a role in puberty, menstruation, and fertility. The amount of LH in the blood can indicate underlying problems associated with a variety of reproductive health issues (Manson and Kaunitz, 2016). LH is an important part of the menstrual cycle. It works with follicle-stimulating hormone (FSH), which stimulates the ovarian follicle, causing an egg to grow. It also triggers the production of oestrogen in the follicle. The rise in oestrogen tells the pituitary gland to stop producing FSH and to start making more LH. The shift to LH causes the egg to be released from the ovary, a process called ovulation. In the empty follicle, cells proliferate, turning it into a corpus luteum. This structure releases progesterone, a hormone necessary to maintain pregnancy. If pregnancy doesn't occur, the levels of progesterone drop off and the cycle begins again. In both sexes, LH stimulates secretion of sex steroids from the gonads. In the testes, LH binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone. Theca cells in the ovary respond to LH stimulation by secretion of testosterone, which is converted into oestrogen by adjacent granulosa cells.

2.3 Oestrogens

Oestrogens are hormones that are important for sexual and reproductive development, mainly in women. They are also referred to as female sex hormones. The term "oestrogen" refers to all of the chemically similar hormones in this group, which are estrone, estradiol (primary in women of reproductive age) and estriol. In women, oestrogen is produced mainly in the ovaries. Ovaries are grape-sized glands located by the uterus and are part of the endocrine

system. Oestrogen is also produced by fat cells and the adrenal gland. At the onset of puberty, oestrogen plays a role in the development of female secondary sex characteristics, such as breasts, wider hips, pubic hair and armpit hair (Saladin, 2012). Oestrogen also helps regulate the menstrual cycle, controlling the growth of the uterine lining during the first part of the cycle. If the woman's egg is not fertilized, oestrogen levels decrease sharply and menstruation begins. If the egg is fertilized, oestrogen works with progesterone, another hormone, to stop ovulation during pregnancy. During pregnancy, the placenta produces oestrogen, specifically the hormone estriol. Estradiol a form oestrogen primary in women of reproductive age is produced by the ovaries in response to signals from the pituitary gland at puberty. The primary function of estradiol is to modulate the course of the menstrual cycle; its secretion gradually increases over the first two weeks reaching its peak during ovulation and drops sharply right before the menstrual period (Manson and Kaunitz, 2016). Oestrogen controls lactation and other changes in the breasts, including at adolescence and during pregnancy.

Oestrogen is instrumental in bone formation, working with vitamin D, calcium and other hormones to effectively break down and rebuild bones according to the body's natural processes. As oestrogen levels start to decline in middle age, the process of rebuilding bones slows, with postmenopausal women eventually breaking down more bone than they produce. This is why postmenopausal women are four times more likely to suffer from osteoporosis than men, (Sullivan, 2017).

Oestrogen also plays a role in blood clotting, maintaining the strength and thickness of the vaginal wall and the urethral lining, vaginal lubrication and a host of other bodily functions. It even affects the skin, hair, mucous membranes and the pelvic muscles (Isong *et al*, 2016).The hormone also affects the brain, and studies also show that chronically low oestrogen levels are linked with a reduced mood (Sullivan, 2017).

2.3.1 Changes in oestrogen levels

There are many times throughout a person's life when oestrogen levels may change. For example, oestrogen levels naturally increase during puberty and during pregnancy. Oestrogen levels fall during transition into menopause and after menopause, or when a woman stops menstruating. This reduction in oestrogen production can cause symptoms such as hot

flashes, vaginal dryness and loss of sex drive. Oestrogen levels also decrease after childbirth (Manson *et al,* 2016).

Other conditions that can cause oestrogen levels to drop include hypogonadism (or diminished function of the ovaries) and polycystic ovarian syndrome. Extreme exercise and anorexia can also cause a decrease in oestrogen levels because women with low body fat may not be able to produce adequate amounts of oestrogen (Suzuki *et al,* 2006).

Oestrogen is found in most oral birth control pills (along with the hormone progestin.) Oestrogen helps stop ovulation during pregnancy, and birth control pills mimic this effect by regulating the levels of oestrogen and thereby preventing ovulation from occurring.

2.4 Characteristics of menopause

Estimates of the median or mean age at menopause have been inconsistent, but they generally range from 48 to 52 years. Factors that may contribute to the timing of menopause include cancer chemotherapy (Richards *et al*., 1990), cigarette smoking, (NAMS Consensus Opinion, 2000) and surgical trauma to ovarian blood supply (Ravn *et al*., 1995). Link between hereditary factors and age at menopause also has been suggested. The specific role for each of these factors, however, has not been established conclusively (NAMS Consensus Opinion, 2000).Menopause is associated with physiological and psychological changes that influence sexuality. During menopause, the primary biological change is a decrease in circulating oestrogen levels (Graziottin and Leiblum, 2005). Oestrogen deficiency initially accounts for irregular periods and diminished vaginal lubrication. Some of the most commonly reported symptoms associated with menopause include hot flushes, headaches, irritability, insomnia and depression (Adegoke *et al*, 2008). A precise understanding of the symptoms an individual may display at menopause is often difficult to achieve. Some patients will show severe multiple reactions that may be disabling while others will show no reactions or minimal reactions. It has been reported that most women in developed countries will live a third of their lives after the menopause (Henn, 2010) and vasomotor as well as psychosomatic symptoms occur frequently during this period of life although their severity and duration may vary widely between individuals (Adegoke *et al*., 2008). Continual oestrogen loss is associated with changes in the vascular, muscular and urogenital systems, as well as

alterations in mood, sleep, and cognitive functioning; these influence sexual function through both direct and indirect mechanisms (Graziottin and Leiblum, 2005).

2.4.1 Physiological changes associated with menopause

Menopausal transition is associated with a decline in oestrogen, GH, IGF-1, and DHEA, a decrease in muscle protein synthesis, and an increase in catabolic factors such as inflammation (Maltais *et al*., 2009). More importantly, low physical activity, protein intake and elevated oxidative stress are the greatest contributors of sarcopenia in postmenopausal women. The low concentration of oestrogen seems to be related with a decline of muscle mass and muscle strength, but the conflicting results among studies make it difficult to establish a formal relationship (Maltais *et al*, 2009). The characteristic trigger of natural menopause is a decline in ovarian function, leading to a cessation of ovulation and a reduction in circulating levels of estradiol, from a range of 50 to 300 pg/mL (at the early follicular and late follicular phases of the menstrual cycle, respectively) to less than 30 pg/mL (Table 2.1). The small amount of circulating estradiol in postmenopausal women is thought to be the result of local tissue conversion of testosterone via aromatase activity (Alexander *et a*l, 2003).The quantity of total oestrogen still available depends on two factors: the intensity and rate of ovarian exhaustion (the degree and extent of oestrogen depletion varies extensively between individuals); and the amount of adipose tissue, which functions as an endocrine gland. A higher body mass index is associated with increased production of estrone, via conversion of adrenal and residual ovarian androgens by aromatases in adipose tissue (Graziottin and Leiblum, 2005). The study by Adegoke *et al*, (2008) indicates that among Nigerian women psychological (psychosomatic) symptoms do present as part of postmenopausal symptoms in about a quarter of affected women. Age of the women at the onset of menstruation (menarche) seems to play a role on the experience of some psychosomatic symptoms at menopause with those experiencing them having started menstruation at a significantly lower age than those who did not experience the symptoms.

Table: 2.1.

Typical Premenopausal and Postmenopausal serum steroid hormones concentration.

	Typical Serum Level, pg/mL		
Steroid Hormone	Reproductive Age	Natural Menopause	Iatrogenic Menopause
Estradiol	100–150	10–15	10
Testosterone	400	290	110
Androstenedione	1900	1000	700
DHEA	5000	2000	1800
DHEAS	3,000,000	1,000,000	1,000,000

DHEA: dehydroepiandrosterone; DHEAS= dehydroepiandrosterone sulfate

(Lobo, 1999).

As shown in Table 2.1, the serum levels of testosterone and of proandrogens exceed that of estradiol, even during peak reproductive years, by several-fold to several thousand-fold (Lobo, 1999). In women, about half of circulating testosterone is secreted directly by the ovarian stroma and adrenal zona fasciculata in roughly equal quantities; the other half is derived from conversion of the proandrogen androstenedione, which is secreted by the adrenal zona fasciculata tissues (Graziottin and Leiblum, 2005). The proandrogen dehydroepiandrosterone sulfate (DHEAS) is produced entirely in the adrenal zona reticularis; conversion of DHEAS accounts for about 30% of circulating dehydroepiandrosterone (DHEA), with the remaining DHEA secreted by the adrenal zona reticularis and the ovarian theca (Burger, 2002). During menopause, a changing hormone profile in the body causes important shifts in the levels of oestrogen present in the female body. Overall, this change is primarily a large drop in the average amount of circulating oestrogen. The falling level of oestrogen is the primary cause of familiar menopause symptoms such as hot flushes, mood swings, and appetite changes. As levels of oestrogen decrease; a woman's risk of developing high blood pressure increases dramatically (Usoro and Nsonwu, 2006; Ebeigbe *et al.,* 2011). Due to the interplay of other hormones and the effect that oestrogen has on other important risk factors, post-menopausal women are actually at higher risk for developing high blood pressure than are men (Ebeigbe *et al.,* 2011). Investigations to determine the effect of menopause on visual function, cardiovascular and ocular hemodynamics showed that menopausal women had significantly higher IOP in both eyes as compared to premenopausal women (Onakoya et al., 2009). Other studies have reported a positive correlation between intraocular pressure and systemic blood pressure.

2.4.2 Menopausal transition

Menopausal transition is the period of time when the endocrinological, biological, and clinical features of the approaching menopause commence (Santoro, 2002). The menopausal transition has been identified as the nexus of a variety of hormonal, physiological, emotional, psychosocial and relational changes that are associated with an increased risk for sexual dysfunction (Dennerstein *et al*., 2002).

Menopausal transition usually begins approximately 4 years prior to menopause and is characterized by menstrual cycle irregularity caused by increased frequency of ovulatory cycles. Natural menopause occurs at a median age of 51 years, with the average life span of a woman in post-menopausal status extending up to 30-35 years (Bechlioulis *et al.,* 2009). Adegoke *et al* (2008) reported that among Nigerian women psychological (psychosomatic)

symptoms do present as part of postmenopausal symptoms in about a quarter of affected women. Age of the women at the on start of menstruation (menarche) seems to play a role on the experience of some psychosomatic symptoms at menopause with those experiencing them having started menstruation at a significantly lower age than those who did not experience the symptoms (Adegoke *et al*., 2008).

Women commonly report a variety of symptoms associated with menopausal transition, including more frequent vasomotor symptoms (hot flushes and night sweats), vaginal symptoms and trouble sleeping (Grady, 2006). Major hormonal changes that occur in menopause are; a decrease in estradiol levels with concomitant increases in follicular stimulating and luteinizing hormone levels (Bechlioulis *et al.,* 2009).

2.5. Physiology of female lower urinary and genital tracts as related to menopause.

The LUT and genital tracts develop in very close proximity in the female embryo. They both arise from the primitive urogenital sinus in the first trimester (Henn, 2010). Oestrogen receptors are expressed in the squamous epithelium of the urethra and vagina and in areas of the trigone of the bladder that have undergone squamous metaplasia (Blakeman *et al*., 2000). These receptors are however not present in the urothelium of the bladder dome, reflecting the different embryological origin of this tissue. The levator ani muscle of the pelvic floor is also oestrogen sensitive (Lachowsky and Nappi, 2009).

Oestrogen increases cell cycle activity in all of these tissues, and a lack of oestrogen results in a decrease in the number of epithelial cells in the urethra, bladder and vagina (Blakeman *et al.*, 2001). Cyclical variation in oestrogen levels during the menstrual cycle may lead to both symptomatic as well as urodynamic changes, with the premenstrual period being the most bothersome (Blakeman *et al.,* 2001).

2.6. The perimenopausal period

The World Health Organization defines perimenopause as the 2–8 years preceding menopause (WHO, 1996). The period of Perimenopause defers from women to women (Kenneth Saladin, 2012). In some women it may take shorter period like one to two years while in others it may last for up to four years (Burger *et al,* 1999). Typically, perimenopause begins in a woman's 4^{th} decade of life. Subtle hormonal changes usually commence in a woman's late 3^{rd} decade of life in some cases; however, the clinical significance of these changes is not known (NAMS Concensus Opinion, 2000).

The complete passage from the reproductive years to postmenopause has been described, by the Stages of Reproductive Aging Workshop (STRAW), in terms of stages defined by menstrual cycle length and frequency (Dennerstein *et al.*, 2002). Much current research and literature on postmenopausal sexual dysfunction has focused on comparing postmenopausal women to premenopausal women. However, it is important to emphasize that change, in the hormonal milieu, including reduction in circulating estradiol levels, as well as the resulting physiological responses and tissue effects, are most pronounced during the perimenopausal stage, comprising the early and late menopausal transition periods (Burger *et al*., 1999).

During perimenopause, oocytes undergo accelerated depletion, which leads to eventual cessation of ovulation and significant changes in serum and hormonal levels, especially oestrogen (Richardson *et al.*, 1987). As ovarian oestrogen production decreases, the pituitary gland increases follicle-stimulating hormone (FSH) production to stimulate the ovary to secrete oestrogen (NAMS Concensus Opinion, 2000). Several hormonal systems manifest age-related changes that may or may not have their onset during the perimenopausal years. Conditions that are not related to perimenopause, such as obesity, diabetes, thyroid disorders, or hypertension, often develop during midlife (NAMS Concensus Opinion, 2000).

As a result, confirmation of perimenopause usually relies on the woman's medical history and the symptoms that she experiences (e.g., irregular menses, hot flashes, mood changes irritability), as well as ruling out other causes for those changes (NAMS Concensus Opinion, 2000). The physical symptoms that occur and the pattern of menstrual cycles during perimenopause differ markedly from woman to woman. Thus, Irregular menstrual cycles or bleeding do not indicate the onset of perimenopause without ruling out other causes, including local uterine pathology, pregnancy, and thyroid abnormalities (NAMS Concensus Opinion, 2000).

The 5- to 10-fold reduction in circulating estradiol that occurs during perimenopause has profound structural and functional consequences for the reproductive tract and surrounding tissues. Loss of oestrogen (in addition to a concurrent decline in circulating androgens) contributes to reduced overall blood flow (Graziottin and Leiblum, 2005). Typical changes in external (labia minora, labia majora, clitoris) and internal (vagina, uterus) reproductive components include reduction in size, thinning of skin and mucous membranes, parallel involution of the corpus cavernosa, and loss of subcutaneous fat (Alexander et al., 2003).

These changes are accompanied by significant alterations in the urinary tract, including reductions in intraurethral pressure, bladder size, and thickness of the mucous membranes lining the bladder and urethra. In addition, there is significant reduction in pelvic muscle tone

and in the resilience of connective-tissue support for urogenital structures (such as the uterosacral ligament), (Alexander et al., 2003).An increasing degree of urogynecologic and sexual comorbidity with increasing age is an important feature in women, in addition to comorbidity with a range of metabolic, neurologic, and immunologic disorders. In the epidemiological survey by Laumann and colleagues, the presence of urinary tract symptoms strongly increased the risk of both arousal disorders (RR=4.02; 95% CI 2.75 to 5.89) and sexual pain disorders (RR=7.61, 95% CI 4.06 to 14.26) (Graziottin and Leiblum, 2005).

Nonetheless, it is possible to describe broad changes in biological structure and function that are driven by hormonal influences during the menopausal transition. Figure 2.1 illustrates the characteristic time course of menopausal signs and symptoms; the most prominent features of the perimenopause (which is also characterized by the most significant drop in mean estradiol levels) include hot flushes/night sweats, urogenital symptoms (especially vaginal dryness) and sleep disturbances. The prevalence and severity of these symptoms vary somewhat across cultural and national boundaries, with Asian women reporting somewhat lower prevalence than Caucasian women (Chim *et al.,* 2002). However, across all groups, perimenopause has been consistently identified as the stage involving the highest incidence and greatest intensity of menopausal symptoms (Pan *et al,* 2002).

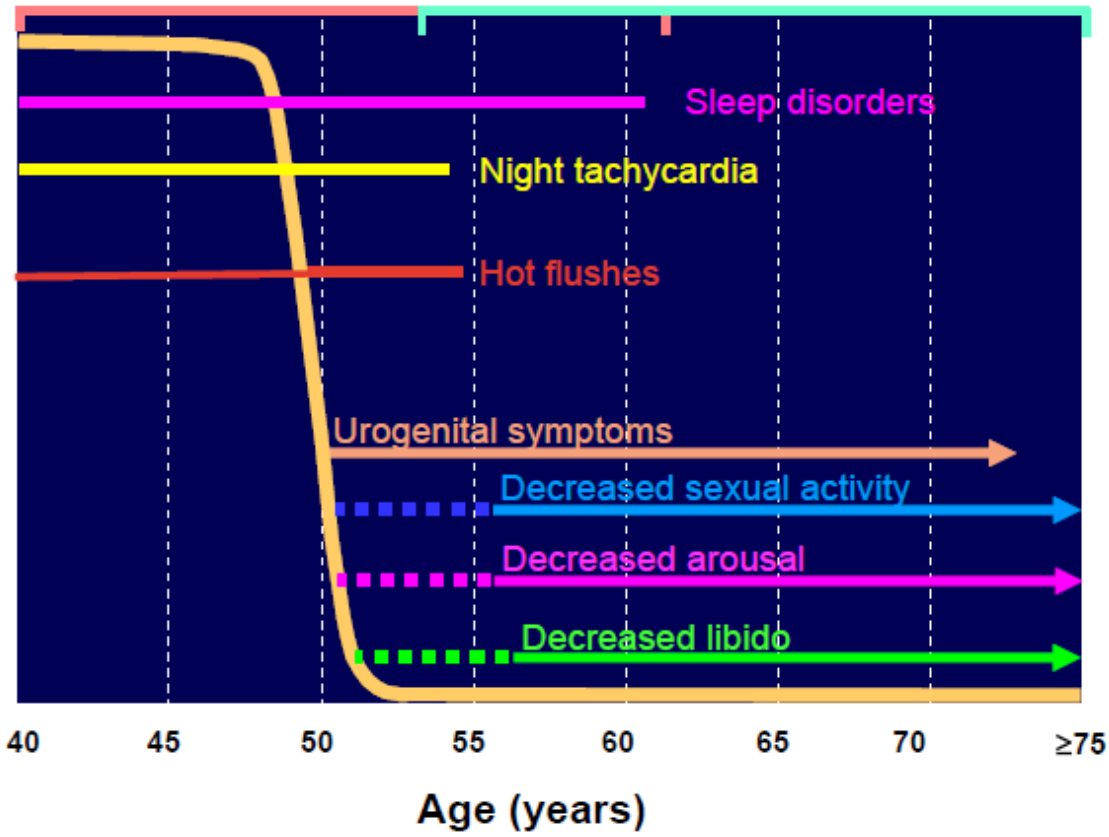

Fig. 2.1 Temporal pattern of menopause related symptoms.

(Graziottin and Leiblum, 2005)

For example, the prevalence of vaginal dryness increases dramatically from early to late perimenopause (Figure 2.2) and only gradually during postmenopause; vaginal tissue integrity and lubrication is affected by both oestrogens and androgens (Dennerstein *et al.*, 2000).The prevalence of dyspareunia (painful intercourse) also increases dramatically during this period and is significantly associated with vaginal dryness and low estradiol levels (Graziottin and Leiblum, 2005).

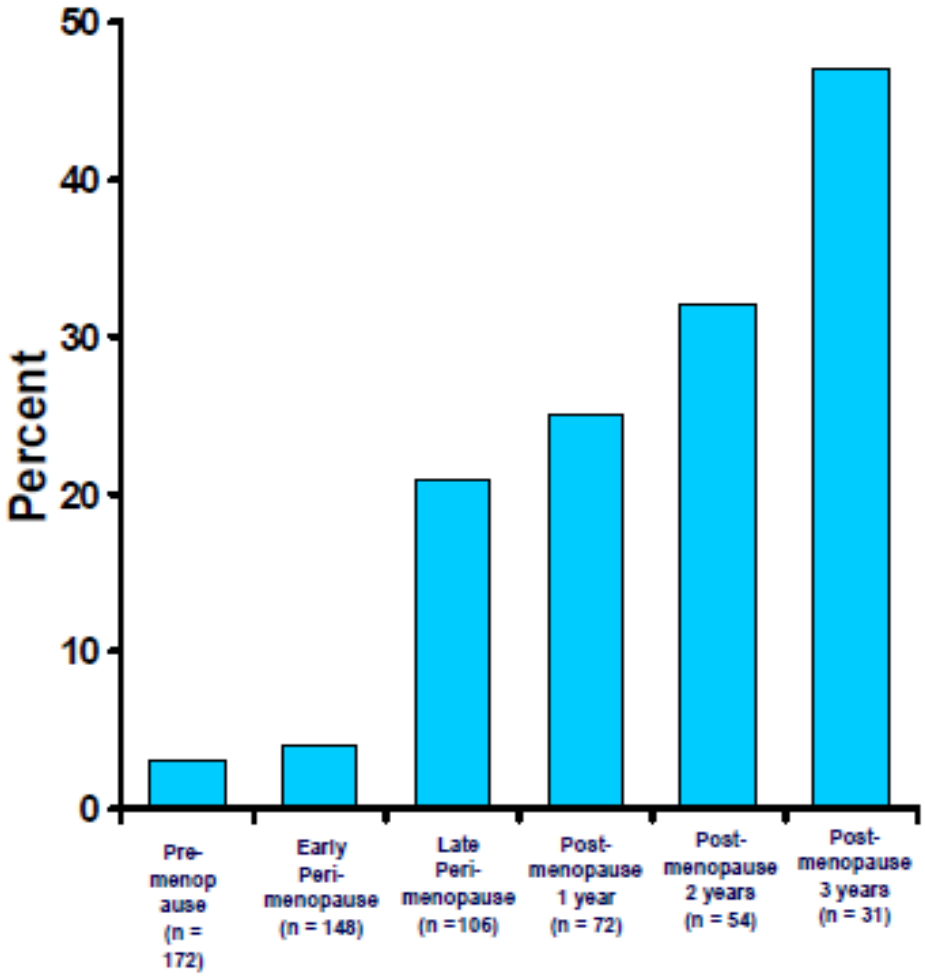

FIG. 2.2. Prevalence of vaginal dryness during Pre-, Peri- and Postmenopause.
(Graziottin and Leiblum, 2005)

2.6.1 Menstrual changes in perimenopausal women

Menstrual cycle changes that occur in perimenopause are usually marked by elevated FSH levels and elevated levels of luteinizing hormone, whereas levels of estradiol keep decreasing (Santoro *et al.,* 1996). However, FSH levels can fluctuate from month to month and from woman to woman during perimenopause, which limits their utility as a predictor (NAMS Concensus Opinion, 2000). Moreover, finding elevated FSH levels only does not predict when menopause will start. Oral contraceptive (OC) use lowers FSH levels, and women may need to stop taking them temporarily (and use a non-hormonal form of birth control) before FSH levels can be measured to help substantiate a presumptive diagnosis of menopause. Estradiol levels usually remain in the normal range until follicular growth and development begin to cease. However, oestrogen levels have been reported to decrease occasionally before menopause (Santoro et al., 1996). Fluctuations of oestrogen can become extreme during perimenopause. Many clinicians regard the appearance of menstrual cycle irregularity in a previously regularly menstruating woman as confirmation of perimenopause. Menstrual cycle patterns, however, differ widely during perimenopause (Manson and Kaunitz., 2016). Studies have shown that intermenstrual intervals often shorten significantly during perimenopause, and menstrual cycles may become irregular as well (Greendale and Sowers, 1997). Another study reported that long intermenstrual intervals may be interspersed with very short cycles (NAMS Concensus Opinion, 2000). Studies have shown that the menstrual cycle may shorten by as much as 3–7 days, perhaps as a result of ovulation occurring earlier than day 14 of the cycle (NAMS Concensus Opinion, 2000).

Some women may skip several cycles and then return to regular cycles. Others may have irregular spotting or regular menstrual cycles until the onset of menopause. Because any menstrual pattern is possible, the perimenopausal woman is not totally protected from an

Unplanned pregnancy until amenorrhea greater than 1 year occurs or consistently elevated levels of FSH (greater than 30 MIU/mL) can be demonstrated (NAMS Concensus Opinion, 2000).

2.6.2 Abnormal uterine bleeding in perimenopausal women

Prolonged intervals of amenorrhea are common among perimenopausal women, and no therapy is usually needed if the woman ovulates periodically. Abnormal uterine bleeding is a more serious concern and requires further investigation. Abnormal uterine bleeding is generally defined as any of the following:

Heavier uterine bleeding than usual, Prolonged uterine bleeding, Menstrual periods occurring more often than every 3 weeks, spotting between menstrual periods, bleeding after sexual intercourse.

The possible causes of abnormal uterine bleeding in perimenopausal women include an ovulation, uterine fibroids, uterine lining abnormalities, cancer, and blood clotting problems. Specific organic causes (neoplasia, complications of unexpected pregnancy, or bleeding from extra uterine sites) must be ruled out (Award et al., 1993). Hormonal contraceptives, particularly progestin-only products and intrauterine devices, can result in abnormal uterine bleeding and should be considered in the differential diagnosis.

Women who bleed fewer than nine times each year and have no molimina warrant evaluation as well. An evaluation of abnormal uterine bleeding should include a history and physical examination plus one or more of the following procedures: endometrial biopsy, office aspiration curettage, dilation and curettage, saline sonohysterography, hysteroscopy, or trans vaginal ultrasound. As with all invasive procedures, the potential benefits and risks need to be discussed with the patient (NAMS Concensus Opinion, 2000).

2.6.3 Acute perimenopause symptoms

Data support the association of various acute symptoms with perimenopause. In addition, data confirm that perimenopausal physiological changes may be associated with long-term problems. Acute symptoms in perimenopause initiated by altered secretion of ovarian hormones include menstrual irregularities, vasomotor symptoms, and sleep disturbances. Behavioral changes have been variously ascribed to psychosocial/ cultural factors and may possibly be affected by endocrine factors (Dienye et al, 2013). It is an accepted fact that some menopausal symptoms overlap with some neuropsychological conditions (Award *et al*., 1993). It therefore becomes important that women need to be enlightened on this development so they can accept the psychological conditions as part of the unavoidable period of menopause and not as an abnormal period of life (Adegoke et al., 2008).

2.6.4 Vasomotor symptoms

The hot flash or flush is the most frequent perimenopausal vasomotor symptom, experienced by up to 85% of women (NAMS Concensus Opinion, 2000). A few women will have hot flashes years before menopause; others experience them for years after menopause (Award et al, 1993). Hot flashes that occur with perspiration during sleep are termed night sweats.

The perimenopause experience is perceived differently among women of different cultures (NAMS Concensus Opinion, 2000). For example, between 75% and 85% of perimenopausal

women in North America and northern Europe have reported hot flashes, in contrast to 25% of women in Japan (Chim et al, 2002). In the United States, the prevalence of vasomotor complaints did not differ in a survey of African American and Caucasian women (Pham et al., 1997).

2.6.5 Thyroid abnormalities

Thyroid dysfunction can affect the menstrual cycle. Hypothyroidism is generally associated with menorrhagia but may result in amenorrhea; hyperthyroidism may be associated with amenorrhea (Williams *et al*, 2001). Although the signs and symptoms of these conditions may be subtle, the functional impairment can be great. Perimenopausal women should be screened for thyroid dysfunction. A thyroid-stimulating hormone (TSH) level using a "sensitive" TSH assay is the initial screening test. If the TSH level is abnormal, then thyroid function should be evaluated further (Williams *et al*, 2001).

2.7. Effects of menopause on the body

2.7.1 Effects of menopause on blood pressure

Hypertension is associated with alterations in calcium metabolism, leading to increased calcium loss, compensatory activation of the parathyroid gland, and increased movement of calcium from the bones (Cappuccio *et al.,* 1999).The link between high blood pressure and menopause is complicated. While there is great indication that blood pressure increases with menopause, there is not a clear understanding of why this happens. There are many factors that are being considered such as age, and weight gain which happen as women get older. However, studies suggest that declining oestrogen levels may be the main contributing factor for elevated blood pressure in menopausal women (Usoro *et al.,* 2006). Pre-menopausal women tend to have lower diastolic and systolic pressure than men, but as women go into menopause, their systolic pressure increases to become slightly higher than that of men (Ebeigbe *et al*., 2011). The long-lasting impairment of hypertension in calcium homeostasis may constitute one of the mechanisms involved in the pathophysiology of age-related excessive reduction of bone mineral density (BMD). Previous study on Nigerian women showed that there was a significant positive correlation between intraocular pressure (IOP) and systemic blood pressure in hypertensive postmenopausal women (Ebeigbe *et al.,* 2011). The study also showed a statistically significant positive correlation between systolic blood pressure and IOP in the hypertensive premenopausal women. Moreover, it has been reported that calcium loss associated with high blood pressure may be due to lack of ability of the

kidneys to handle this mineral (Cakmak *et al*., 2015). Recent study has also suggested that raised angiotensin II levels in hypertensive settings have a harmful effect by increasing bone resorption and decreasing mineralization (Pérez-Castrillón *et al*., 2003).

Some studies report an increase in alcohol consumption and smoking among menopausal and postmenopausal women, both of which are also known risk factors for high blood pressure, heart disease, and stroke. All of these factors may, of course, work singly or in combination to increase the risk (Ebeigbe *et al.,* 2011). Exposure to lead and lead poisoning may be responsible for an elevated risk of hypertension as well. Bone loss, which takes place at a much higher rate during and after menopause, releases lead stored in the skeletal system. Lead exposure and lead poisoning have long been known to increase hypertension risks in men. The link between lead exposure and hypertension is strongest among post-menopausal women (Onakoya *et al*., 2009; Ebeigbe *et al.,* 2011).

2.7.1.1 Hypertension and bone mineral density

One of the mechanisms explaining the effect of hypertension on BMD is the increase in gene polymorphism in angiotensin-converting enzyme and in angiotensin II levels (Pérez-Castrillón *et al.,* 2003).This may cause an increase in bone resorption and may inhibit mineralization. Pérez-Castrillón *et al* showed that angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers might also be useful in the treatment of osteoporosis in hypertensive women. Further, it is known that thiazides and angiotensin-converting enzyme inhibitors are related to increased calcium resorption (Pérez-Castrillón *et al*., 2003).

2.7.2 Effects of menopause on blood glucose

Metabolic alterations, which may be seen in type 2 diabetes mellitus (DM), can trigger impairments of calcium homeostasis, skeletal metabolism, and bone mass (Carnevale *et al.,* 2004).Some recent studies have demonstrated an increased fracture risk related to type 2 diabetes; in type 2 diabetes complicated by osteoporosis, a larger decrease in bone formation than in bone resorption may be seen compared to the setting of postmenopausal osteoporosis, which mainly influences the indexes of bone formation and may be a lower turnover ratio type (Carnevale *et al*., 2004). Although type 1 DM has been associated with decreased BMD (Suzuki *et al.,* 2006), there have been conflicting reports about BMD in type 2 DM; some authors have reported elevated BMD, some have reported decreased BMD, and others have reported that BMD did not change (Suzuki *et al.,* 2006).

2.7.2.1 Insulin resistance changes with menopause

Two of the most important pathophysiological components of the metabolic syndrome are increased visceral fat accumulation and insulin resistance (Carr, 2003). Abdominal obesity is closely associated with increased insulin resistance, compensatory hyperinsulinemia, and increased risk of type 2 diabetes, independent of an individual's total body fat content (Carr,2003). The pathophysiology underlying the insulin-resistant state is complex. Insulin resistance, with inadequate compensatory hyperinsulinemia, diminishes the normal suppression of FFA arising from adipose tissue by insulin. The increased levels of FFA may impair peripheral glucose uptake, increase hepatic gluconeogenesis, and reduce hepatic clearance of insulin (Despres, 1993).

The literature to date is not clear as to whether menopause is associated with increased insulin resistance. What little data there are remain contradictory. Several groups have shown increased fasting insulin and increased fasting glucose levels (Poehlman *et al*, 1997), in postmenopausal compared with premenopausal women, which would imply worsened insulin resistance with the menopause. However, insulin sensitivity is known to worsen with advancing age and increasing central obesity, making it difficult to tease out the effect of menopause from these processes (Carr, 2003). Studies using accurate measures of insulin resistance, such as the euglycemic hyperinsulinemic clamp or the frequently sampled intravenous glucose tolerance test, are scarce (Toth *et al.*, 2000).

De Nino *et al.* (2001) showed reduced insulin sensitivity (*i.e.* higher insulin resistance) in postmenopausal women compared with BMI-matched premenopausal women. However, others have shown no differences in insulin sensitivity in postmenopausal compared with premenopausal women (Toth *et al.*, 2000). DeNino *et al.* (2001) compared measures of insulin resistance and visceral adipose tissue in age-grouped women ranging from 20–78 year. They found that reduced insulin sensitivity did not appear until women were older than 60 year and had accumulated levels of visceral fat that approximated the levels seen in men, suggesting a possible threshold effect of abdominal fat on insulin resistance (DeNino *et al.*, 2001). Guthrie *et al.* (2001) reported prospective data on 265 healthy perimenopausal women with normal fasting glucose. The group of women (16%) who developed impaired fasting glucose (6.1 mmol/l) over the 5-year period had higher baseline BMI, fasting glucose and insulin, waist circumference, TG and lower HDL levels, as well as greater increases in BMI and insulin over the study period compared with women who maintained normal fasting glucose. There was no difference in menopausal status between the two groups; this implies

that weight gain had a stronger influence on the development of impaired fasting glucose than menopause itself (Gutherie *et al.,* 2001).

2.7.3 Effects of menopause on the urinary system

Increased prevalence of lower urinary tract symptoms (LUTS) and urinary incontinence is associated with both systemic aging and with menopause (Graziottin and Leiblum 2005).Women with LUTS have more than a 7-fold greater risk for sexual pain disorders, and a 4-fold greater risk for sexual arousal disorders, than women without such symptoms. Hypotonia and hypertonia of the pelvic floor muscles, which increase in prevalence with age, can also contribute to sexual pain (Graziottin, and Leiblum 2005).

2.7.4 Effects of menopause on the nervous system

Central and peripheral nervous system function is strongly affected by reduction in ovarian hormones; these changes may both drive and be driven by urogenital structural and functional alterations. Hormonal fluctuations during the menstrual cycle have been linked with significant variation in sensory capabilities and response, and studies in oophorectomized rats have revealed that the size of the pudendal-nerve response increases with exogenous oestrogen. In addition, multiple neurotransmitter systems in the brain, especially the areas known to regulate mood and desire (including the amygdala, hippocampus and hypothalamus) are heavily influenced by sex hormones (Graziottin and Leiblum, 2005). The decline in oestrogens at menopause occurs concurrently with an increased risk for depression, which is associated with high risk of sexual dysfunction (Bromberger *et al.,* 2003). Although several studies have demonstrated that this risk is driven by symptoms, especially vasomotor instability, and not by a direct effect of reduced oestrogen on mood, some studies have also suggested that menopause may be associated with reduced endorphin levels (Avis *et al.,* 2001).The positive effects of tibolone on mood may in part derive from restoration of endorphin levels (Graziottin and Leiblum, 2005).

2.7.5 Effects of menopause on reproductive hormones

The role of androgens in maintaining urogenital health and sexual function during and after menopause (in addition to their importance in overall health, mood and sexual desire) is the subject of much current research, although interest in androgens as a component of gynecological care dates to the 1940s (Graziottin and Leiblum 2005). In contrast to the

relatively sharp decline in circulating oestrogens during natural menopause, androgen levels tend to peak when women are in their 20s and drop gradually with age; typical serum levels of testosterone and androstenedione at age 60 are about half those at age 40 (Sarrel, 2002).

Oestrogens and androgens act at times in opposition, and at other times in conjunction. An excellent example is in the vagina: both oestrogens and androgens appear to be important in maintenance of vaginal blood flow, but androgens promote nonvascular smooth muscle relaxation while oestrogens attenuate it (Sarrel, 2002). Significant hormonal changes occur at the time of menopause and this has an impact on all ooestrogen sensitive tissue (Blakeman *et al.,* 2000). The female lower urinary tract (LUT) is no exception. Ooestrogen deficiency becomes clinically more overt over time and is associated with a number of LUT symptoms (Henn, 2010). These include frequency, nocturia, urinary incontinence (UI), urinary tract infections (UTIs), and urgency. These symptoms often coexist with those of vulvovaginal atrophy, such as vaginal dryness, pruritus, burning, and dyspareunia (Henn, 2010). Oestrogen is known to have vasodilatory effects in the systemic circulation. Hence, decreased oestrogen levels during menopause may therefore complicate or contribute to ocular pathologies as oestrogen receptors are found in both retinal and choroidal tissue (Onakoya *et al.,* 2009). Menopause-related hormonal changes can lead to weight gain and make blood pressure more reactive to salt in the diet which in turn, can lead to higher blood pressure (Ebeigbe *et al.,* 2011). Another factor which may lead to weight gain is a loss of energy, thereby increasing the risk of high blood pressure. Depression is yet another risk factor for hypertension, which afflicts some menopausal women (Ebeigbe *et al*., 2011).

2.7.6 Menopause and plasma Lipids

Significant increases in triglycerides, total and low-density lipoprotein (LDL) cholesterol occur within 3-5 years of natural menopause, while in ovariectomized women an increase in total cholesterol, triglycerides and lipoprotein a [Lp(a)] occurs within the first 6 weeks after ovariectomy (Graff-Iversen *et al.,* 2008). Menopause is associated with an androidal body shape and deposition of abdominal fat, a body “profile” that is associated with an increased risk for CAD in women (Sharma et al., 2008). More women than men develop hypertension at an older age, particularly after menopause (Sharma et al., 2008). Although the association between abdominal adiposity and the constellation of lipid abnormalities is well known; but the underlying pathophysiology is not clear (Carr, 2003). High amounts of abdominal fat are associated with increased insulin resistance, free fatty acid (FFA) levels, and decreased

adiponectin (Carr, 2003). These factors contribute to increased secretion of apolipoprotein B (apo B)-containing particles, leading to hypertriglyceridemia and increased hepatic lipase (HL) activity resulting in a predominance of small dense LDL particles and a reduction in large antiatherogenicHDL2 particles. A similar pattern of lipid abnormalities emerges with menopause (Carr, 2003).

2.7.6.1 Changes in LDL with menopause

Postmenopausal women have higher total cholesterol, LDL cholesterol, triglycerides (TG), and lipoprotein (a) [Lp(a)] levels and lower HDL cholesterol levels than premenopausal women (Li *et al.*, 1996). Although elevated LDL is not a component of the metabolic syndrome, LDL levels increase by 10–20% (Matthews *et al.*,2001) with menopause, and the greatest change in LDL concentration appears to occur early in the transition from premenopause to postmenopause (Matthews *et al.*, 2001). Apo B, the primary apolipoprotein of LDL particles, and other apo B-containing particles are also higher in postmenopausal compared with premenopausal women (Li *et al.*, 1996).

LDL particle composition also changes with menopause. The prevalence of small, dense LDL is low in premenopausal women (10–13%), but increases to 30–49% in postmenopausal women (Carr *et al.*, 2000). LDL is comprised of a spectrum of particles that vary in size, density, chemical composition, and atherogenic potential. A preponderance of small, dense LDL is associated with an increased risk of myocardial infarction (Austin et al., 1988) as well as the severity of CVD (Campos *et al*., 1992).

The risk of CVD is 3-fold higher in women with small, dense LDL than in those with large, buoyant LDL (Austin *et al.*, 1988). Campos e*t al.* (1992) recently showed by electron beam CT that postmenopausal women with high coronary calcium scores had smaller LDL particle size, higher LDL levels, and fewer large HDL2 particles than postmenopausal women with low coronary calcium scores.

2.7.6.2 Changes in TG with menopause

Many longitudinal studies have shown that TG levels increase with the transition through the menopause (Carr, 2003), and the increase in TG also appears early in the postmenopausal period (Matthews et al., 2001). Poehlman *et al.* (1997) found that the prospective transition to postmenopause was associated with a 16% increase in TG. Although men generally have higher TG levels than women, TG increases in middle-age (between 40–69 year) in women, but not in men (Razay *et al.*, 1992), and TG appears to be a better predictor of CVD risk in women than in men (Atsma *et al*, 2006). Carr2003 reported a prospective increase in TG

levels in women who became postmenopausal during a 6-year period, whereas there was no change in TG in the similarly aged women who remained either premenopausal or perimenopausal. Increasing TG with menopause may be related to the fact that TG levels are highly correlated with increasing abdominal fat content and insulin resistance (Carr, 2003).

2.7.6.3 Changes in HDL with menopause

Most studies show that total HDL levels fall slightly with menopause (Do *et al.,* 2000), whereas others reveal no changes (Kannel *et al.,* 1976). Menopausal changes in HDL metabolism are more complex than the measurement of total HDL reveals, because the more anti-atherogenic HDL2 levels decrease (by 25%), whereas HDL3 levels increase (Carr, 2003). HDL2 particles are the large, buoyant, and more cardio protective subspecies of total HDL. The strong inverse relationship between HDL cholesterol and abdominal adiposity appears to be largely dependent on variations in HDL2 levels (Lamarche *et al.,* 1997).

2.7.6.4 Changes in LP (a) with menopause

Lp(a), an LDL-like particle with structural homology to plasminogen, is not frequently measured in clinical practice, but has been shown to predict cardiovascular events in women independent of LDL levels (Shlipak et al., 2000). Lp (a) levels are primarily genetically determined, but several studies have now shown significant increases in Lp (a) levels (by 25–50%) with menopause (Bruschi et al., 1996). This rise in Lp (a) levels with menopause may reflect the fact that Lp (a) levels are sensitive to sex steroid hormones and return to premenopausal levels with oestrogen replacement (Bruschi *et al.*, 1996).

2.7.6.5 Changes in proteins of lipid metabolism with menopause

Proteins of lipid metabolism underlying the menopausal change in lipids have been evaluated in few studies (Carr, 2003). The increased prevalence of small, dense LDL with menopause may be explained by higher HL activity in postmenopausal women (Berg *et al.,* 2001). Endogenous oestrogen levels are inversely associated with HL activity (Tikkanen *et al.,* 1986). HL hydrolyzes the TG and phospholipid in LDL and HDL and is one factor that determines the size and density of LDL and HDL particles (Carr, 2003).

The higher the HL activity, the more TG and phospholipid hydrolyzed, resulting in smaller, denser more atherogenic lipoprotein particles. Lipoprotein lipase hydrolyzes TG in triglyceride-rich lipoproteins, generating FFA that can serve as an energy source or can be stored in adipocytes. We have recently shown a small, but significant, rise in lipoprotein lipase activity with the transition through menopause (Carr, 2003). Cholesteryl ester transfer protein (CETP) catalyzes the exchange of cholesterol ester in HDL and LDL particles for TG

in VLDL, and high CETP concentrations are associated with reduced HDL levels. Menopausal status does not appear to affect CETP activity (Lewis-Barned et al., 1999).

The perimenopausal changes in lipid metabolism reveal an overall shift toward a more atherogenic lipid profile with increased LDL and TG levels, reduced HDL2 concentration, and smaller, denser LDL particles, similar to the metabolic syndrome (Carr, 2003). This classic dyslipidemia is closely associated with increasing amounts of visceral fat, which may explain why these features emerge with the menopause. It is likely that these adverse changes in lipid metabolism during the menopausal transition will contribute to future CVD risk (Carr, 2003).

2.7.7 Effects of menopause on the Muscles

It is well established that aging is associated with a decline in muscle mass called sarcopenia (Lindle *et al*, 1997). Sarcopenia is clinically defined as 2 standard deviations below the mean appendicular muscle mass of young healthy adults of a reference population (Gallagher *et al.*, 1997).It is related to limited functional performance and physical disability (Baumgartner *et al.*, 1998) and women are more susceptible to present these health problems, as compared to men, because they live longer (Baumgartner *et al*, 1998). This muscle loss is primarily due to an imbalance between muscle protein synthesis, muscle protein breakdown and the increase of catabolic factors such as oxidative stress and inflammation (Lindle *et al*, 1997). In addition, other factors such as menopause-associated decline in hormonal levels are thought to be implicated in this process (Maltais *et al*., 2009).

A good body of evidence supports that the decline in muscle mass may be in line with the decrease in oestrogen that characterizes menopausal years (Maltais *et al.*, 2009). The decrease of oestrogen contributes to the loss of BMD, the redistribution of subcutaneous fat to the visceral area, the increased risk of cardiovascular disease and the decrease in quality of life (Carr, 2003). To exacerbate the negative impact of menopause on women's health, the drop in oestrogen may also have a direct effect on muscle tissue (Carville *et al.*, 2006).

Women tend to lose muscle strength around the 5th and 6th decades of age (Samson *et al.*, 2000). As such, some studies showed that women experience a 21% decrease in strength between the age of 25 and 55 years (Maltais *et al*, 2009). As with muscle mass, the loss of muscle strength appears to be concurrent with the occurrence of menopause (Carville *et al.*, 2006).

IGF-1 is a protein that activates muscle protein synthesis and inhibits muscle protein degradation. It is mediated by growth-hormone releasing hormone (GH-RH) and works in pair with GH. Serum IGF-1 is produced primarily by the liver and its levels are more stable than GH. The effect of IGF-1 is mainly on the PI3K/AKT pathway which is also activated by exercise (Maltais *et al.*, 2009). Noteworthy, the PI3K/AKT pathway holds two types of receptors; insulin receptors (IR) and IGF-1 receptors, explaining why both insulin and IGF-1 can promote muscle protein synthesis. IGF-1 and oestrogen has been shown to decrease with menopause, thereby augmenting pro-inflammatory cytokine levels such as IL-6 and TNF-α (Maltais *et al*., 2009). Some authors suggested that the loss of muscle strength coincides with the oestrogen deficit of menopause (Carville *et al.*, 2006). Although the mechanisms that lie beneath are not clearly understood, some studies show a correlation between muscle strength and circulating oestrogen levels (Sitnick *et al*., 2006). It is proposed that oestrogen has an anabolic effect on muscle by the stimulation of IGF-1 receptors (Sitnick *et al.*, 2006).

In addition, there is some evidence that oestrogen receptors (ER) are present in human muscles (expressed at mRNA level), under the form of ERα and ERβ in the nuclei of muscle fibers and capillaries (Wiik *et al.*, 2009). Interestingly, it has been shown that the number of ERs on muscle fibers is greater in men, women and children, as compared to postmenopausal women (Wiik *et al.*, 2009). Noteworthy, oestrogen receptors are not only dependent on circulating oestrogen to be activated but IGF-1 can also activate the transcriptional activity of oestrogen receptors (Ciana *et al.*, 2003). Hence, oestrogen receptors could play their role on muscle strength through the action by both oestrogen and IGF-1. Nonetheless, both oestrogen and IGF-1 drop at menopause, which is likely to affect muscle mass and strength (Maltais *et al.*, 2009).

Among biological markers associated with muscle mass loss, DHEA is a pro-hormone that can transform into sex steroids, such as androgens and oestrogen. DHEA has many important roles in the human body as it may contribute to the increase in muscle mass, the improvement in glucose and insulin levels, the decrease in fat mass and reduce the risk of breast cancer (Labrie *et al.*, 2005). Circulating DHEA seems to decline with age, especially at menopause for women. As such, a steep decline seems to occur between perimenopausal women and postmenopausal status (Yen *et al.*, 1995).

Vitamin D supplementation seems to be important not only to maintain BMD, but also muscle function and strength, therefore attenuating the risk of falls and the loss of balance.

2.7.7.1 Osteopenia in menopause

Osteopenia defines as BMD that is not normal, but that is not as low as the density in osteoporosis. It is defined by bone densitometry with a T-score of -1 to -2.5 based on the definition of the World Health Organization (WHO) (Cakmak *et al.,* 2015). Osteopenic decreased BMD leads to bone fragility and an increased risk of bone fractures. The major causes for osteopenia include calcium deficiency, vitamin D deficiency, genetic factors, and physical inactivity (Cakmak *et al.,* 2015). A variety of pharmaceutical agents have been recommended for the treatment of osteopenia, including hormone replacement therapy, selective oestrogen receptor modulator therapy, and antiresorptive therapy (Karaguzel and Holick, 2010).The difference between osteopenia and osteoporosis is that in osteopenia, the bone loss is not as severe as in osteoporosis, which means that someone with osteopenia is more likely to fracture a bone than someone with normal bone density, but less likely to do so than someone with osteoporosis (Karaguzel and Holick, 2010).

2.7.8 Effects of menopause on oxidative stress.

With menopausal transition, there is an increase in oxidative stress marked by the imbalance between the production of free radicals and the destruction of these free radicals through an inadequate antioxidant system (Signorelli *et al.,* 2006). Oxidative stress is related to a higher reactive oxygen species production from the mitochondria, which in turn provokes cell apoptosis (Hiona and Leeuwenburgh, 2008). The vicious cycle theory (Bandy and Davidson, 1990) proposes that mitochondrial DNA is damaged by oxidative stress, affecting the capacity of producing energy from the electron transport chain. The reduced capacity of the mitochondria to produce energy makes it more susceptible of apoptosis which finally provokes muscle fiber atrophy or death, eventually leading to sarcopenia (Maltais *et al.,* 2009). Noteworthy, the decline in IL- 6 and TNF-α have also been shown to increase the risk of physical disability (Visser *et al.,* 2002).

2.8 Endothelial function and dysfunction

Endothelium, the innermost cell layer in the vascular wall, is a very important regulator of vascular homeostasis, maintaining the balance between vasodilation and vasoconstriction, inhibition and stimulation of vascular smooth muscle cell proliferation and migration, thrombogenesis and fibrinolysis (Karatzis, 2005). Endothelium regulates vascular tone by releasing vasodilators, such as nitric oxide (NO), prostacyclin and bradykinin, and

vasoconstrictors, such as endothelin and angiotensin II, in response to physical and chemical stimuli. Endothelium-derived NO is the principal mediator of all vasoprotective effects; apart from being the most potent vasodilator, NO also has anti-inflammatory, antiproliferative, and antithrombotic properties (Karatzis, 2005). Reduced NO bio-availability, due to reduced production and/or increased inactivation of NO by reactive oxygen species, leads to endothelial dysfunction, initiating a series of processes that promote atherosclerosis. Endothelial dysfunction is present in the pre-clinical stages of atherosclerosis and can be detected long before structural changes in vessel wall are evident on angiography or intravascular ultrasound; its assessment could therefore serve as an integrating index of CV risk factor burden (Bechlioulis *et al.,* 2009). Endothelial function can be assessed noninvasively using high-resolution ultrasound in the brachial artery to monitor changes in arterial diameter in response to increased blood flow, an important physiological stimulus for endothelial NO production. This endothelium-dependent, NO-mediated process is known as flow-mediated dilation (FMD) (Bechlioulis *et al.,* 2009).

Endothelial dysfunction, demonstrated as reduced FMD, has been associated with most of the established CV risk factors (dyslipidaemia, hypertension, smoking, diabetes mellitus, family history of premature CAD, elevated plasma homocysteine) (Brevetti *et al*, 2003) and has been shown to be a reversible process (Yeboah *et al.,* 2007). Recently, its prognostic importance has also been reported; FMD has been reported to predict long-term CV events in patients with CV diseases and in healthy subjects (Yeboah *et al.,* 2007). However, the relation of endothelial dysfunction with clinical outcome has not been established in large prospective clinical trials and only limited data so far suggest that improvement of impaired FMD with treatment may also lead to an amelioration of CV prognosis (Brevetti *et al*., 2003).

2.8.1 Effect of menopause on the endothelial function

Natural menopause has been associated with vascular endothelial dysfunction. Several studies have demonstrated impaired endothelium-dependent vasodilation in healthy post-menopausal women (aged between 53 and 58 years) compared to younger pre-menopausal women (aged 30-35 years) (Bechlioulis *et al*., 2009). Flow-mediated dilation (FMD) of the brachial artery has also been shown to provide additional prognostic information about the CV risk of post-menopausal women (Karatzis, 2005).

However, since no direct comparison between age-matched post-menopausal and pre-menopausal women has been performed, it is not clear yet whether the observed endothelial dysfunction at menopause is due to the oestrogen loss at menopause or merely ageing

(Bechlioulis *et al*., 2009). Indeed, age has been identified, along with vessel diameter, as an independent predictor of impaired endothelium- dependent vasodilation in post-menopausal women. A large amount of evidence has recently emerged to strengthen the role of oestrogen loss in the endothelial dysfunction observed at menopause. Acute oestrogen deprivation following ovariectomy is related to endothelial dysfunction (Virdis *et al.,* 2000), which occurs within as little as 1 week after surgery (Ohmichi *et al*., 2003). Even in young women with normal menses, endothelial function assessed using FMD has been found to vary cyclically during the menstrual cycle in relation to endogenous oestrogen levels; low levels are associated with a relative decrease in flow-mediated dilation (FMD) (Williams *et al.,* 2001). This observation has attracted much clinical attention, as an increased vulnerability to acute coronary events during and immediately after menses, when the levels of endogenous oestrogen are low, was demonstrated (Hamelin et al., 2003). Finally, endothelial dysfunction has been demonstrated in several groups of young women with low levels of endogenous oestrogen. Young women with premature ovarian failure, who are known to be at increased risk for CVD; present significant vascular endothelial dysfunction compared to age-matched women with normal ovarian function (Kalantaridou *et al.,* 2006). Other groups of young women with low levels of endogenous oestrogen, such as women with hypothalamic hypogonadism (Hamelin *et al.,* 2003) and athletic amenorrhoea, (Virdis *et al.,* 2000) also demonstrate impaired endothelial function. In these studies, endothelial dysfunction was attributed to low oestrogen levels, while androgens did not seem to play an important role (Hamelin *et al.,* 2003).

2.9 Menopause and CVD

CVD-related morbidity and mortality are low in women of reproductive age, but increase to a significant level in older women, especially after menopause (Atsma *et al.,* 2006); this increase in CVD risk has been attributed to the loss of oestrogen at menopause. However, it is difficult to distinguish the effect of age from that of menopause on CVD, as age and menopause are strongly related and the increase in CVD risk with menopause may be simply due to ageing (Bechlioulis *et al*., 2009). The overall epidemiological evidence on the relationship between menopause, rather than age, and CVD remains controversial. Most epidemiological studies suggest that post-menopausal compared to pre-menopausal women are at higher risk of CVD (Bechlioulis *et al.,* 2009). A recent meta-analysis of eighteen observational studies revealed no relationship between natural menopausal transition and CVD occurrence after controlling for study design, age and smoking status (Bechlioulis *et al.,*

2009). However, a significant modest effect of early age at menopause and a more pronounced effect of bilateral ovariectomy on CVD were reported (Atsma *et al.,* 2006). Several other studies have suggested that a younger age at menopause may be associated with increased risk of CV mortality (Mondul *et al.*, 2005). Furthermore, the Nurses' Health Study demonstrated that, besides a younger age at natural menopause, bilateral ovariectomy is associated with a higher risk of CVD in women who have never used HT (Hu *et al*., 2004).

2.10 Menopause and urinary continence

LUT symptoms due to oestrogen deficiency tend to develop over time and may only present many years after the menopause. Urogenital complaints increase with age, and although nearly half of elderly women will be symptomatic, they often delay seeking treatment for several years (Ho *et al.,* 2010). Also, two thirds of women do not relate their vaginal or urinary complaints to the menopause. The prevalence of postmenopausal UI is between 16 and 29%, (Hsieh *et al.,* 2008) and urge urinary incontinence (UUI) in particular occurs more frequently after the menopause (Botlero *et al.,* 2009). Aging is clearly a significant factor in the pathogenesis of UI, but evidence seems to indicate that menopause and oestrogen deficiency are also implicated (Zhu *et a*l., 2009). Most studies show that many women develop UI at least 10 years prior to menopause and that stress urinary incontinence (SUI) actually starts to become less after the menopause (Norton and Brubaker, 2006). These findings emphasize that UI is a multifactorial process, and that menopause is basically one of a number of aetiological factors (Henn, 2010).

The majority of women perceive the development of urinary symptoms and specifically UI as a normal part of aging, rather than a pathological process (Norton and Brubaker, 2006). It is important to note that the aging population is at risk for a number of systemic illnesses that may present with LUT symptoms, including diabetes mellitus, congestive cardiac failure, and renal disease (Table 2.2) (Henn, 2010). Symptomatic changes in the LUT do occur as part of aging, and it is often difficult to distinguish these from those due to oestrogen deficiency. Nocturia increases in prevalence from 10% at the age of 50 to 50% at the age of 80 years (Lin *et al.,* 2005). The bladder also becomes less efficient with age. Older women experience a reduced flow rate, incomplete emptying of the bladder, a higher first sensation to void, and decreased detrusor pressures (O'Donnell, 2008). Histology reveals an increase in fibrosis and a reduction in muscle fibres and density in the aging bladder (Lin *et al.,* 2005). A similar picture is seen in the muscles of the pelvic floor (Henn, 2010).

Table 2.2: Transitional causes of urinary symptoms in the elderly.

- UTI
- Faecal impaction
- Oestrogen deficiency
- Restricted mobility
- Drug therapy
- Depression
- Mental impairment/confusional state

(Henn, 2010)

2.10.1 Sex hormones and urinary continence

Continence requires that the urethral pressure exceeds the intravesical pressure at all times except during micturition (Henn, 2010). Sex steroids influence the central nervous system control of the continence mechanism and these hormones also seem to have a direct effect on the detrusor muscle (Suzuki *et al.,* 2006). Animal studies have shown that oophorectomy alters the pressure flow characteristics of micturition (Botlero *et al*, 2009).This effect may only be partly reversed by oestrogen supplementation (Botlero *et al.*, 2009). Oestrogen also targets the functional layers of the urethra (epithelium, vasculature, connective tissue and muscle) which are integral for maintaining continence. The addition of progestogens might negate any positive (inhibitory) effect which oestrogen has on the detrusor muscle. Progestogen is associated with an increase in irritative bladder symptoms and UI in women taking combined hormonal therapy (Zhu *et al*, 2009).

2.11 Menopause changes in sleep Architecture

Menopause is associated with an increased amount of sleep related complaints but there have been few documented polysomnographic changes associated with menopause, despite widespread symptoms. In the Wisconsin cohort of individuals followed with polysomnograms over time, there were no significant polysomnographic changes associated with transition to menopause but there was documented an increase in obstructive apnea (Young *et al*., 2003). The polysomnograms of young women and men are similar but women tend to maintain delta sleep longer than do men with aging.

In spectral analysis of quantitative EEG measures, women have higher power density than men in delta, theta, lower alpha and higher spindle frequency range, however the effect of aging was the same for men and women with attenuation of slower frequencies and increased power in the beta range, implying advancing age has a bigger impact rather than gender on the sleep EEG (Young *et al.,* 2003). A study of younger ovulatory cycling women vs older women (either cycling or menopausal) showed worsening sleep efficiency with age, but not with oestrogen level, implying that loss of sleep efficiency was an age related effect (Lukacs *et al.*, 2004). Hollander et al (2001), on the other hand, in a study of 436 healthy women reported independent associations between poor sleep and both hot flashes and estradiol levels in ages 45-49 years.

Vasomotor symptoms have been associated with arousals and disruption of sleep architecture. It may be that the primary menopausal problem is vasomotor instability in a subset of women followed by consequences of disturbed sleep. Not all women experience sleep problems with

menopause, nor do all women experience hot flashes. In Hollander's study of healthy women (Hollander *et al*., 2001), only 17% reported poor sleep which is similar to a recent national survey (Lukacs *et al*., 2004). Those with poor sleep, however, were the subset with vasomotor symptoms and low oestrogen (Hollander *et al*., 2001).

2.11.1 Sleep disordered breathing and menopause

Prior to menopause, women have approximately 1/3 the frequency of sleep disordered breathing than men. Shortly after menopause this disparity drops for unclear reasons (Vorona *et al*, 2005). In the Wisconsin Cohort, the odds ratios compared to premenopause for >15 apnea hypopnea index (AHI) goes from 1.1 at perimenopause to 3.5 at postmenopause. In a Canadian study the prevalence of apnea in a relatively heavy (BMI=30) population of 1315 women went from 21% to 47% after menopause. Differences persisted despite adjustments for body mass index (Dancey *et al.,* 2001). Weight gain associated with menopause with concomitant increase in neck circumference potentially adds to the total development of postmenopausal OSA. Weight gain is common during menopause. There have been recent studies emphasizing the interaction between sleep duration and weight gain (Vorona *et al.,* 2005). Sleep deprivation due to decreasing sleep efficiency might be expected to create more hunger, causing more weight gain and SDB. Thus a cycle of hunger, gain and worsening SDB could be postulated.

The gender difference does not seem to be biased by the fact that men may be more likely to come to a sleep clinic, but rather a true reflection of a biologic change. The reasons are unclear and may have to do with gender differences in upper airway anatomy, distribution of fat tissue, sex hormone effects and gender differences in leptin. Leptin, a hunger suppressing adipokine stimulates breathing. Women with higher leptin levels are more resistant to airway collapse (Eichling and Sahni, 2005). Loss of leptin with development of abdominal obesity, insulin resistance and leptin resistance may partially explain the association between diabetes and disordered breathing.

Men have higher testosterone levels than women and testosterone increases disordered breathing. Presumably, this is due to its effect on oropharyngeal muscle mass. Exogenous testosterone has been shown to increase sleep disordered breathing (Bellipanni *et al.,* 2001). This might explain the gender difference, but does not completely explain why women develop more disordered breathing after menopause. However, loss of progesterone and a change in hypercapneic drive may also explain some of the change (Vorona *et al*, 2005).

Oestrogen, testosterone and progesterone all have been reported to affect sleep disordered breathing. Some small studies have been reported in which ERT has improved sleep disordered breathing (Eichling and Sahni, 2005). In one study of 5 patients the AHI was reduced from 30 to 25 one month after initiating oestrogen and to 17 after ten days of oestrogen/progesterone (Kefe *et al.,* 1999). In another study of 4 patients the AHI was reduced by 75% (Bellipanni *et al*., 2001). A third study of 6 patients showed a reduction of AHI from 22 to 12 with oestrogen alone (Dancey *et al.,* 2001). A larger crossover study of 62 healthy patients showed a reduction in AHI but not in upper airway partial obstruction (Eichling and Sahni, 2005). In another treatment group of 51 insomnia patients treated with oestrogen, the AHIs decreased with treatment with corresponding cognitive and subjective improvements (Vorona *et al.,* 2005). Finally, women with sleep apnea have had lower measured oestrogen levels. In this study of 53 consecutive women of all ages, there was a significant correlation between low oestrogen and progesterone levels and OSA (AHI>10) (Netzer *et al.,* 2003).

The significance of these observations relates to the consistent under-assessment of sleep apnea in the general population. In the Wisconsin prevalence study 93% of women and 92% of men with moderate to severe sleep apnea were not clinically diagnosed (Eichling and Sahni, 2005). It is a challenge to the sleep medicine community to alert the primary providers of post-menopausal patients to the possibility of disordered breathing. A primary physician may note a change in sleep complaints and attribute it to menopausal insomnia. Given that OSA can develop during menopause without any significant change in the BMI, it would be easy to not think of disordered breathing. It is also significant since menopause marks a change in cardiovascular risk in general. Thus, OSA is another cardiac risk to add to the lipid, blood pressure and other risk factors occurring at that same time (Eichling and Sahni, 2005).

2.11.2 Insomnia and depression associated with menopause

Mood disorders, specifically depression and anxiety, are associated with menopause (Harlow *et al.,* 2003). The reason for this is unclear and may be multifactorial. Several large longitudinal studies have documented this as reviewed by Lukacs *et al*., (2004). There is an associated increase in the incidence of insomnia following menopause. Separate, however, from the effect of menopause, there is a “chicken and egg” discussion regarding insomnia and depression in general. In longitudinal studies insomnia appears to precede depression in cyclic mood disorders (Baker *et al,* 1997). Sleep disruption has been associated with

depression (Baker *et al.*, 1997). Whether simple sleep deprivation can produce depression in susceptible individuals is unclear, but insomnia is closely intertwined with depression.

Presumably multiple arousals permit the development of intrusive anxious thoughts at multiple times during the night (Eichling and Sahni, 2005). Obviously, pre-existing anxiety or depression will exacerbate these intrusive thoughts that prevent re-initiation of sleep. Insomnia has a performance anxiety quality to it as well. Thus, waking up repeatedly gives the mind the "opportunity" to be anxious during the night. If the anxious thoughts are about the inability to sleep, a self-perpetuating process of insomnia has been created. Increased wake after sleep onset (WASO) is created and sleep efficiency drops. Thus, the "domino" theory of sleep disruption has long been thought to be at least one explanation for menopausal depression. It proposes that sleep is disturbed by hot flashes or other menopause related reasons. Insomnia follows sleep disruption and depression follows insomnia (Eichling and Sahni, 2005).

Insomnia has been associated with depression in women with a 4.1 odds ratio. The odds ratio of developing depression in patients with unremitting insomnia for one year was 39.8 in one study vs. an odds ratio of only 1.6 if insomnia resolves. This would imply that treating or preventing insomnia is a major method for preventing the onset of depression. Since depression has multiple well-documented increased health risks associated with it, treatment of insomnia would be expected to have major long-term health benefits (Eichling and Sahni, 2005). Lukacs *et al, (*2004) points out the difficulty in studying menopause related depression. There are problems with the variety of settings in which it has been studied and the lack of consistent definitions of menopause and depression in the various studies. This is pertinent because there have been a wide variety of responses to hormone therapy. If oestrogen treats or prevents depression, it becomes an appropriate and perhaps major indication for HRT.

The connection between menopause and depression may be mediated through vasomotor changes. In one study of 476 women, the perimenopausal women with vasomotor symptoms were 4.39 times more likely to have depression. This did not change with adjustment for prior depression history (Joffe *et al.*, 2002). A longitudinal study of mood and menopause has also recently reported the same association (Freeman *et al.*, 2004).

Another study of 309 women, using a standard depression scale and estradiol levels, reported hot flashes with disturbed sleep. There was no direct correlation between oestrogen levels and depression, but there was between hot flashes and disturbed sleep, and depression. This data supports the "domino" theory of disturbed sleep in menopause which proposes that

vasomotor symptoms caused by fluctuations in oestrogen levels results in depression rather than low oestrogen levels directly leading to depression (Avis *et al*., 2001).

Sleep disruption may be related to oestrogen deficiency in ways other than simple hot flashes. Moe performed a stress challenge study in which oestrogen replacement (ERT) and non ERT patients were stressed by having blood drawn during the night (Moe *et al.,* 2001). Although there was no difference at baseline between the 2 groups in polysomnography (PSG) parameters, following a stress challenge, the non ERT patients had more total wake time, less slow wave sleep and rapid eye movement (REM) sleep than the ERT group. This might imply a direct oestrogen effect on sleep quality and maintenance. Thus other disturbances such as personal stress factors or adverse environment would have less of a negative impact on the sleep of ERT patients. This perhaps expands the group of patients at-risk for depression from those with only hot flashes to those with other factors that could cause sleep disruptions.

2.12. Menopause and Aging

Aging is associated with a natural decline in physiological functions, including a loss of bone mass density (BMD), muscle mass and strength (Lindle *et al*., 1997). Overall, the decline in muscle mass averages 0.4 to 0.8 kg per decade, starting at the age of 20 years old (Gallagher *et al.,* 1997). However, this diminution is not linear and does not occur at the same rate and age in both sexes. In fact, it has been proposed that, in women, an accelerated loss of muscle mass and strength occurs at an earlier age than in men, around the time of menopause, possibly making them weaker at 65-69 years old than men aged 85-89 years old (Calmels et *al.,* 1995). It is well established that aging is related with the loss of muscle strength, and this loss is partly related to sarcopenia (Lindle *et al*, 1997). The decrease in muscle strength can play a detrimental role in physical function impairments, such as rising from a chair (Alexander *et al*., 1997), walking speed (Gallagher *et al.,* 1997), climbing stairs and the capacity to recuperate after a loss of balance (Jette and Jette, 1997).

2.13 Effect of weight gain during perimenopausal stage over menopausal syndrome

The prevalence and severity of menopausal symptoms depend on several factors. These include not only the hormonal changes imposed by the transition, but also psychosocial factors (Davis *et al.,* 2012). During the menopausal transition, as weight increases so do menopausal symptoms. Obesity is an independent risk factor for more severe menopausal symptoms (Fernandez-Alonso *et al*., 2010).

In a study in Zaria, Kaduna State of Nigeria, only 10.23% of the postmenopausal women were of normal BMI, 15.91% were underweight while 31.82%, 39.77% and 2.27%, respectively were overweight, obese and morbidly obese respectively. Implying that, 73.86% of the women had an above normal BMI (Achie *et al.,* 2012). The higher BMI in menopause is associated with increased levels of estrone and an increased risk of breast cancer (with larger tumours) and a higher age at natural menopause (Davis *et al*., 2012). Despite these risks, in most Nigerian cultures, the matronly or overweight figure is commonly considered as more befitting for older aged ladies. Concerted efforts need to be made to sensitize individuals to control the prevalence of obesity considering its role as a risk factor for cardiovascular diseases, diabetes mellitus and metabolic syndrome (Achie *et al.,* 2012).

2.13.1 Obesity and menopause

For women aged 55 – 65 years, weight gain is one of their major health concerns (Nappi and Kokot-Kierepa, 2012). This is understandable as obesity is one of the most common nutrition-related disorders globally, and its prevalence is increasing (Davis *et al.,* 2012). World-wide, the prevalence of obesity has more than doubled since 1980. In 2008, 1.5 billion adults, 20 years and older, were overweight (body mass index (BMI) 25 – 29.9 kg/m^2), including both developed and developing countries. Of these, over 200 million men and nearly 300 million women were obese (BMI $\geq$ 30 kg/m ^{2}) (WHO, 2012).

Moreover, the rates of obesity have increased notably in developing countries adopting a Western lifestyle (decreased physical activity and overconsumption of cheap, energy-dense food). The sharp increase in overweight and obesity rates observed in the last 20 years is dependent on, or controlled by, several factors and is only in part attributable to changes in lifestyle (Davis *et al.,* 2012). Obesity has substantial psychosocial consequences. Depression and depressive symptoms are common among obese patients. As increasingly evidenced in the literature, obesity substantially affects health-related quality of life.

It affects physical competence, appearance, self-esteem and social functioning. There are no clear differences between gender and ethnicity in these outcomes. In general, obesity is characteristically more prevalent in females than in males (Davis *et al.,* 2012). Fluctuations in sex hormones at different stages of reproductive life, such as menarche, pregnancy, and menopause transition, may play a role in the adipose tissue expansion. The menopause transition begins with the onset of menstrual irregularities and ends with the last menstrual period.

The prevalence of abdominal obesity is almost double that of general obesity, with rates in the US in 2008 of 65.5% in women aged 40 – 59 years and 73.8% in women aged 60 years or more (Davis *et al*., 2012). It has been suggested that BMI but not menopausal status determines central adiposity in postmenopausal women. However, there is substantial evidence that the perimenopause is associated with a more rapid increase in fat mass and redistribution of fat to the abdomen, resulting in a transition from a gynoid to an android pattern of fat distribution and an increase in total body fat (Poehlman *et al*., 1997). Studies using a range of radiological modalities have shown that postmenopausal women have greater amounts of intra-abdominal fat compared to premenopausal women (Toth *et al.,* 2000). Waist circumference represents both subcutaneous and visceral adipose tissue depot size and correlates closely with cardiovascular disease risk. In women, it is also closely associated with dyslipidemia (Toth *et al*., 2000). The waist-to hip ratio is another indicator of accumulation of visceral fat which can also be quantitated by CT scanning (Janssen *et al.,* 2010). In the study by (Achie *et al*., (2012) in Zaria, Nigeria, the identification of individuals at risk based on the waist circumference (79%) being higher than that defined by the BMI (73.86%) corroborates the findings of the study by Famodu and Awodu (2009), where the waist circumference was found to be a more accurate index of identifying individuals with a cardiovascular risk than the BMI. Previous studies have shown the BMI to have a positive correlation with the diastolic blood pressure (Okosun *et al*., 2000; Olatunbosun *et al*., 2000). The body mass index of the menopausal women has also been positively correlated with the waist circumference as observed in other studies which was due to increased visceral and subcutaneous fat in menopause (Achie *et al.,* 2012). Amongst obese postmenopausal women, the percentage of women with sexual problems is greatest in those with abdominal obesity (Lianeza *et al.,* 2007). Sexual well-being is adversely affected by insulin-resistance and the metabolic syndrome (Lianeza *et al.,* 2009), and sexual dysfunction is more prevalent in postmenopausal women with metabolic syndrome in comparison with healthy controls (Martelli *et al.,* 2012).

2.13.2 Obesity and menopausal bone loss

Obese women appear to lose bone at a lower rate than non-obese women across the menopause transition (Sowers *et al.,* 2010). However, the relationship between osteoporosis, fracture risk and excessive BMI is complex. Low BMI has been associated with osteoporosis and women with long-standing obesity have been observed to be less at risk for osteoporosis and fracture (van der Voort *et al*., 2001).These views have recently been challenged by the

results provided by the Global Longitudinal study of Osteoporosis in Women, which included 60,393 women ≥ 55 years from ten countries and assessed patient characteristics, fracture history, fracture risk factors, and anti-osteoporosis medications (Compston *et al.,* 2011). Using fracture as the endpoint, the risk of incident ankle and upper leg fractures was significantly higher in obese women, while the risk of wrist fracture was significantly lower. Obese women with fracture were more likely to have experienced early menopause and to report two or more falls in the past year (Davis *et al.,* 2012). In this population, self-reported co-morbid conditions were highly prevalent, including asthma, emphysema, and type 1 diabetes, and more common in obese women with incident fracture. These data clearly suggest that obesity is not protective against fracture in postmenopausal women (Compston *et al.,* 2011).

Given the evidence that mood disorders are one of the most important co-morbid conditions of sexual dysfunction in postmenopausal women, it is plausible that weight gain and obesity at menopause may be risk factors for poor sexual functioning (Davis *et al*., 2012).

However, little is known of the specific impact of weight gain on sexual function at menopause as a consequence of the 'domino' effect of other menopausal symptoms, especially psychological symptoms. Indeed, loss of fitness and weight gain were not the sole factors influencing the intensity of sexual complaints in a clinical sample of menopausal women (Nappi *et al.*, 2002). In peri- and postmenopausal women with urinary incontinence, increased BMI early in menopause represents a risk not only for urinary incontinence, but also for sexual dysfunction. Arousal, orgasm, lubrication and satisfaction are inversely correlated with BMI (Pace *et al*., 2009).

2.14. Hormone therapy in menopause

2.14.1 Effect of menopausal hormone therapy on weight and body composition

A Cochrane Review published in 2000 reported no evidence of an adverse effect of oestrogen-only or oestrogen – progestin therapy on body weight or BMI (Bonds *et al,* 2006). There has been no subsequent research that would challenge this conclusion. The effects of oestrogen therapy in postmenopausal women on body composition vary, with most randomized, controlled trials showing a reduction in central adiposity (Davis *et al*., 2012).

Although, overall, the effects of exogenous oestrogen appear to be favorable in terms of body composition, the route of oestrogen delivery may have subtle, but differing effects (Bonds *et al*, 2006).

Oral oestrogen has been associated with a small but significant increase in fat mass and a decrease in lean mass; whereas lean body mass and fat mass do not change significantly with transdermal estradiol. Neither route appears to alter visceral fat mass (Bonds *et al.,* 2006). The differing effect of oral versus transdermal oestrogen therapy may relate to the differing effects of oral versus transdermal oestrogen on growth factors and substrate oxidation (Davis *et al.,* 2012). Oral oestrogen, but not transdermal oestrogen, is associated with a significant decline in circulating insulin-like growth factor 1 (IGF-1) (Maltais *et a*l., 2009). This appears to be due to oral oestrogen impairing hepatic IGF-I production, which then causes increased secretion of growth hormone through reduced feedback inhibition. Divergent effects on fat mass have also been seen for oral raloxifene and transdermal estradiol. In growth hormone-replaced hypopituitary women, treatment with transdermal estradiol was associated with a reduction in fat mass. This effect was attenuated when the women were treated with raloxifene (Davis *et al.,* 2012).

Despite the divergent effects of oral and transdermal oestrogen noted above, improved insulin sensitivity has been observed with oral oestrogen-progestin therapy (EPT), and both oral oestrogen-alone and EPT may reduce the incidence of type 2 diabetes (Bonds *et a*l, 2006).

Hence, menopausal hormone therapy is not associated with increased weight or increased visceral adiposity. Studies mostly indicate a reduction in overall fat mass with oestrogen and oestrogen – progestin therapy, improved insulin sensitivity and a lower rate of development of type 2 diabetes (Davis *et al.,* 2012).

2.14.2 Effect of hormone therapy on endothelial function in postmenopausal women

The effect of HT on peripheral vascular endothelial function, assessed by flow-mediated dilation (FMD), in post-menopausal women has been extensively studied. Most of these studies have shown a beneficial effect of HT that seems to be preserved with various formulations (per os or transdermal, oestrogen alone or combined therapy) or dosages, both in healthy post-menopausal women (Kalantaridou *et al*., 2006) and women with few CV risk factors (Schrott et al., 1997). Young women with premature ovarian failure have also been shown to benefit; HT for 6 months completely reversed significant endothelial dysfunction in this group of women (Kalantaridou *et al.,* 2006). However, there have been some studies that showed no or partial improvement of endothelial dysfunction with HT administration in

several groups of women, including some healthy post-menopausal women (Vitale *et al.*, 2008). Oestrogen use in women with diabetes mellitus has been shown to be less effective in ameliorating endothelial function and arterial stiffness indices (Schrott *et al*., 1997). Furthermore, elderly women with many CV risk factors, with or without established CVD (Sharma *et al*., 2008), have been reported to be non-responsive to HT. Time since menopause has recently been demonstrated as a predictor of FMD improvement with HT; the improvement in endothelial function following oestrogen administration was greater in women within 5 years from menopause compared to those with more than 5 years in menopause (Vitale *et al*., 2008). It has to be noted that, apart from endothelial function, which is the focus of the current review, arterial stiffness has also been studied in relation to menopause and HT. Increased arterial stiffness has been demonstrated in post-menopausal women (Bechlioulis *et al*., 2009), while the effect of HT on arterial stiffness does not appear to be very clear, with various studies reporting conflicting results (Schrott *et al*, 1997). The differences in the effect of HT on endothelial function in different groups of women presented above are also reflected in the divergent results of clinical studies and have thus led to interesting discussions about the underlying potential pathophysiological mechanisms involved in the effects of HT. Several recent reports indicate that the effects of HT on vascular pathophysiology are very complex; the effects of oestrogen on the evolution of the atherosclerotic process appear to depend largely on the state of vascular pathology (Bechlioulis *et al*, 2009).

2.14.3 Hormonal therapy as prophylaxis against the development of UI

It is not clear whether oestrogen supplementation is beneficial when used as prophylaxis against the development of UI in perimenopausal and postmenopausal women, and also not whether the effect lasts past the treatment period (Henn, 2010). Data from a few studies suggest that combined hormonal therapy can, in fact, be associated with worsening stress and urge UI (Grodstein *et al.,* 2004). The relative risk of developing UI with oral oestrogen is 1.54, transdermal oestrogen is 1.68, combined oral therapy is 1.34, and combined transdermal therapy is 1.46. At 10 years after cessation of therapy the risk is identical to those women who have never used hormonal therapy (Hendrix et al., 2005).

2.14.4 Effect of hormonal changes on sleep

2.14.4.1 Progesterone

While oestrogen is a common focus of menopause discussions, progesterone has very profound effects on sleep and is somewhat more straightforward in its effects on sleep than

oestrogen (Eichling and Sahni, 2005). Progesterone, when given intravenously, has direct sedative qualities, stimulating benzodiazepine receptors that in turn stimulate the production of the non-rapid eye movement (NREM) associated gamma-aminobutyric acid (GABA) receptors (Bellipanni *et al*, 2001).During a normal menstrual cycle, there is a rapid peak during the mid-luteal phase and a drop off premenstrually, which are associated with sleep difficulties and increased arousals (Eichling and Sahni, 2005).

A second impact of progesterone is its effect on breathing. Progesterone is a respiratory stimulant and has been used to treat mild obstructive sleep apnea (Bellipanni *et al*, 2001). During pregnancy, there is remarkably little obstructive sleep apnea, given the amount of weight gain that typically occurs, and it is felt that the high progesterone levels characteristic of pregnancy function as a respiratory stimulant. Similarly, prior to menopause, when there are naturally occurring higher progesterone levels, less sleep apnea is seen.

2.14.4.2 Oestrogen

The effect of oestrogen on sleep is more complicated than that of progesterone. In animals, oestrogen suppresses REM sleep, but in humans it increases REM cycles. Oestrogen is involved in norepinephrine (NE), serotonin (5HT) and acetylcholine metabolism (Lachowsky and Nappi, 2009).

Oestrogen has been shown to decrease sleep latency, decrease the number of awakenings after sleep occurs, increase total sleep time and decrease the number of cyclic spontaneous arousals (Sarrel, 2002). During the luteal (low oestrogen) phase in premenopausal women, a two-fold increase in the number of arousals occurs, particularly when both oestrogen and progesterone levels are low (Irafox, 2002). Oestrogen is also related to temperature regulation in the body. An obvious effect of low oestrogen levels is the classic hot flash characterized by increases in both peripheral and central temperature. Hot flashes also are characterized by bursts of catecholamines and surges in luteinizing hormone (LH) (Antonijevic *et al*, 2000). A wide range in the severity of vasomotor symptoms is seen clinically at menopause. Obviously, hot flashes can be associated with increased arousals.

Oestrogen replacement is associated with increasing the amount of both slow wave sleep and REM (Antonijevic *et al.*, 2000). These changes would be associated with better quality sleep. In addition to its obvious prevention of hot flashes, oestrogen has a significant effect on core body temperature during sleep (Eichling and Sahni, 2005). Oestrogen in mammals is a thermoregulatory hormone that helps regulate the time of lowest body temperature during the night. Stopping oestrogen shifts this time forward and changes the depth of the temperature

drop (Saladin, 2011). Both of these changes result in more arousability and lighter sleep. It is hypothesized that one of the reasons for deeper sleep during the follicular phase in premenopause is the temperature regulating effect of high oestrogen during this time.

Oestrogen may have a direct impact on mood through its central nervous system (CNS) receptors in the modulation of neurotransmitters. It is involved in both 5 HT and NE regulation centrally. Oestrogen increases 5 HT post synaptic responsivity as well as increasing both the number of 5 HT receptors and their uptake (Eichling and Sahni, 2005). Oestrogen tends to be a 5 HT agonist. It also selectively increases NE activity in the brain, due to decreasing monoamine oxidase activity. It has other mixed effects on NE. It may also have dopaminergic effects as well. Finally it is a GABA agonist. All these effects imply that it would have an antidepressant effect centrally (Eichling and Sahni,2005).

2.14.4.3 Cortisol

Oestrogen also affects sleep via its effect on cortisol. Menopause is associated with higher levels occurring earlier in the sleep period than the usual morning cortisol spike (Moe *et al.,* 2001). Menopausal women are more susceptible to nocturnal rises in cortisol associated with mild stressors (Moe *et al.,* 2001). Oestrogen helps regulate the normal a.m. cortisol peak and therefore helps stabilize nighttime sleep. The same effect could be postulated to underlie the improvement of depression with oestrogen replacement therapy (Eichling and Sahni, 2005).

2.14.4.4 Melatonin

Melatonin and oestrogen are somewhat inversely related and have a very complicated interaction. Melatonin is a reproductive hormone in animals with seasonal reproductive habits, and it functions to suppress estrus in these animals (Eichling and Sahni, 2005). In very high pharmacologic doses, melatonin can be used as a birth control pill. In males it can cause regression of testicular tissue. Melatonin is linked to LH production, such that disruption of melatonin will disturb LH surges and therefore cause fertility difficulties (Eichling and Sahni, 2005). It seems that the predominant direction in which melatonin and oestrogen are related is melatonin driving reproductive hormones rather than vice versa. This may be related to mammalian estrus cycles that are less pronounced in humans (Bellipanni *et al*., 2001).

Melatonin primarily has a well-known circadian effect at sleep onset and may also be involved in sleep maintenance by blocking arousal mechanisms, thereby tending to keep humans asleep in the dark at night. In general, melatonin levels decrease with the aging process, but paradoxically, decreases in total melatonin levels are not necessarily associated

with menopause. Prior to menopause, there is an age related drop in melatonin but immediately afterward melatonin increases for several years (Eichling and Sahni, 2005). Oestrogen deficiency at menopause may stimulate production of melatonin or menopause marks the loss of a pineal and pituitary control of ovarian cyclicity (Bellipanni *et al.*, 2001). Post-menopausal women with insomnia generally have been shown to have lower melatonin levels than their cohorts (Bellipanni *et al,* 2001). Additionally, oestrogen may have a reciprocal melatonin-supportive function. Both tamoxifen, an anti-oestrogen, and oophorectomy cause decreases in melatonin (Eichling and Sahni, 2005).

2.14.4.5 Testosterone

Testosterone has been less well studied than the other sex hormones in relation to sleep. Testosterone tends to decrease REM sleep in animals, and significant gender differences have been seen in REM sleep in animals' studies (Eichling and Sahni, 2005). Testosterone seems to have a minor effect on sleep in humans except testosterone is related to developing or worsening obstructive sleep apnea (OSA). Higher testosterone levels seem to be associated with increased apnea. It has been shown that exogenous testosterone replacement can and does worsen obstructive sleep apnea (Eichling and Sahni, 2005). The reason for this is unclear. This has implications regarding testosterone replacement therapy for both men and women and may be one of the reasons for the gender difference in OSA.

2.15 Metabolic syndrome.

The metabolic syndrome has received more focus as the updated Adult Treatment Panel III guidelines emphasize treatment of the metabolic syndrome in addition to lowering of low density lipoprotein (LDL) levels (NCEP, 2001). Metabolic syndrome was initially recognized as an adult disorder because its early descriptions were made in adults and its constituent components are disorders which are seen commonly in adults or are associated with aging. The currently emerging data in Africa have mainly been from adult populations (>20 years) though some studies had involved subjects that were <20 years (van der Sande *et al.*, 2001). Several workers have observed that the prevalence of metabolic syndrome increases with age (Ogbera, 2010). Prevalence increased from 11% in subjects aged 20-29 years to 89% in those aged 70-79 years in Nigeria (Okafor, 2012).The metabolic syndrome may not be a single disease entity, but, rather, a constellation of closely related risk factors that together convey substantially increased cardiovascular risk after accounting for traditional CVD risk factors.

Metabolic syndrome today is not only seen in adults but it is now also beginning to occur in children and adolescents due to the growing obesity epidemic within this young population (Okafor, 2012). The features of the metabolic syndrome include the accumulation of visceral (abdominal) adiposity, insulin resistance, hypertension, and dyslipidemia (hypertriglyceridemia, reduced high density lipoprotein (HDL), and small dense LDL particles; Table 2.3) (Despres, 1993).

Table 2.3 Features of the metabolic syndrome

1. Central obesity
2. Insulin resistance
3. Dyslipidemia
a. Elevated TG
b. Small dense LDL particles
c. Reduced HDL
4. High blood pressure
5. Hypercoaguable state
6. Proinflammatory state

(Despres, 1993)

The metabolic syndrome is estimated to affect approximately 20–30% of the middle-aged population (Park *et al.,* 2003), and prevalence appears to be increasing in the U.S. population with increasing obesity and sedentary lifestyle (Park *et al,* 2003). Postmenopausal status is associated with a 60% increased risk of the metabolic syndrome, even after adjusting for confounding variables, such as age, body mass index (BMI), household income, and physical inactivity (Park *et al*., 2003). The risk of CVD attributed to the metabolic syndrome appears to be especially high in women, and it is estimated that half of all cardiovascular events in women are related to the metabolic syndrome (Wilson *et al.,* 1999).

Although syndrome X was initially coined by Gerald Reaven in 1988 (Reaven, 1988), the features of the metabolic syndrome were first described by Vague (1956) and have subsequently been called the insulin resistance syndrome, the central obesity syndrome, and the deadly quartet (Carr, 2003). Diagnostic criteria for the metabolic syndrome, as defined by the National Cholesterol Education Program Adult Treatment Panel III, are shown in Table 2.4; these easily obtained measures are useful for classifying patients (NCEP, 2001).

Table: 2.4. Diagnostic criteria for the metabolic syndrome (requiring three or more risk factors

Risk factor	Defining level
Waist circumference	
Men	>102 cm (>40 inches)
Women	>88 cm (>35 inches)
Triglyceride	≥1.7 mmol/liter (≥150 mg/dl)
HDL	
Men	<1.0 mmol/liter (<40 mg/dl)
Women	<1.3 mmol/liter (<50 mg/dl)
Blood pressure	≥130/≥85 mm Hg
Fasting glucose	≥6.1 mmol/liter (≥110 mg/dl)

(NCEP, 2001)

The etiology of the metabolic syndrome is unknown, but is thought to be a combination of factors (Bouchard, 1995). Studied 1028 male twins and found greater concordance of dyslipidemic hypertension in monozygotic than dizygotic twins. Within the discordant monozygotic twin pairs, the twin with dyslipidemic hypertension weighed significantly more as an adult, implying an interaction between genetic and environmental influences on the manifestation of the metabolic syndrome (Bouchard, 1995). Many believe that the underlying pathophysiology of the metabolic syndrome is related to increased visceral obesity and insulin resistance (Despres, 1993).

Studies on insulin resistance in indigenous African populations are quite few. In south-western Nigeria, a prevalence of 35% was reported among 500 healthy elderly subjects (Ezenwaka *et al.*, 1997). Among Ghanaians, Amoah *et al.* (2003) evaluated 200 randomly selected subjects who were urban residents with no previous history of hypertension or diabetes mellitus and reported that mean homeostasis model assessment for insulin resistance (HOMA-IR) index was significantly higher in 26.5% of the subjects who were found to be hypertensive (Okafor, 2012).

2.15.1 Risk factors of metabolic syndrome

2.15.1.1 Abdominal fat:

Abdominal fat can be considered an endocrine organ due to its capacity to secrete adipokines and other substances that are closely associated with metabolic diseases such as insulin resistance, type 2 diabetes and the metabolic syndrome (Wajchenberg, 2000). Aging and the menopause transition are each associated with changes in adipose tissue metabolism, which may contribute to the accumulation of body fat after menopause (Misso *et al*, 2005).

Deleterious changes in inflammatory markers and adipokines correlate strongly with increased visceral adiposity at menopause (Peter *et al.*, 2010). The transport protein, serum sex hormone binding globulin (SHBG), is a strong independent marker of insulin resistance (Jayagopal *et al.*, 2004) and type 2 diabetes risk (Peter *et al*., 2010) and has been increasingly implicated in the pathogenesis of type 2 diabetes and cardiovascular disease (Peter *et al.*, 2010). SHBG levels in postmenopausal women are negatively correlated with visceral fat (Janssen *et al.*, 2010) and an adverse adipokine profile (Peter *et al*, 2010). Importantly, the relationship between SHBG and insulin resistance in postmenopausal women is independent of both endogenous oestrogens and androgens (Davis *et al.*, 2011).

2.15.1.2 Waist circumference:

A high waist circumference, indicating accumulation of excessive central abdominal fat, and a low SHBG level are independent predictors of metabolic disease risk in postmenopausal women (Davis *et al.,* 2012).

A significant change in waist circumference in relation to final menstrual period has been observed (Janssen *et al.,* 2008) and significant increases in central abdominal fat have been reported from longitudinal studies of Caucasian and Asian women (Ho *et al*., 2010). Significant increases in total fat mass, percentage fat mass, truncal fat mass and visceral fat have been seen in non-obese premenopausal women followed over several years (Abdulnour *et al.,* 2012). The women who became perimenopausal or postmenopausal by the third follow-up year showed a significant increase in visceral fat ($p < 0.01$) compared with baseline. Weight circumference and fat mass (measured by bio-electrical impedance) have also been observed to increase in relation to the final menstrual period (Sowers *et al.,* 2010). These changes occurred similarly in both African-American and Caucasian women.

Within Asia, different ethnic groups exhibit different levels of insulin resistance. Ethnicity modifies the relationship between insulin resistance and type 2 diabetes that is related to an increase in central adiposity (Ho *et al.,* 2010).

In the study by Achie *et al*., (2012) in Zaria, Northern Nigeria, waist circumference (WC) of the study group (93.04±1.60 cm) was higher than that of the control (78.87±1.30 cm) ($p<0.01$). Waist circumference cut- offs vary with age, sex and race. Factors implicated include oestrogen deficiency in menopause which was found to be associated with a change in fat distribution. More fat is deposited around the abdomen than at the thighs or hips as seen in women in the reproductive age which presents as greater increases in fat mass and waist hip ratios (Achie *et al.,* 2012). Women of Indian origin have a significantly elevated risk of type-2 diabetes but the impact of menopause itself on this risk is unclear (Davis *et al*., 2012). Studies of the menopause transition and changes in body composition in Chinese women suggest that the menopause has an independent effect on the increase in fat mass as well as an increase in central adiposity (Ho *et al.,* 2010).

2.15.1.3 Obesity

The deleterious effects of obesity are diverse, ranging from an increased risk of premature death to several non-fatal diseases with an adverse impact on quality of life. Obesity is a major risk factor for diabetes mellitus and the cardiovascular diseases, coronary heart disease, infarction, stroke, and hypertension (WHO, 2012). However, the relationship between obesity and metabolic disease is complex (Davis *et al.,* 2012).

There is increasing recognition of a metabolically healthy but obese phenotype, observed in about 9% of obese men and 16% of obese women (Wahab *et al.,* 2008). The lower rate of cardio metabolic abnormalities in metabolically healthy obese individuals is not explained by diet composition or level of physical activity, highlighting the importance of a genetic contribution to the predisposition to the co-morbidities of obesity (Pischon *et al*, 2008). Obesity is also a major risk factor for urinary incontinence, dementia, some cancers (endometrial, breast and colon) and musculoskeletal disorders, especially osteoarthritis, a highly disabling degenerative disease of the joints (WHO, 2012) .

Age-adjusted prevalence of central obesity (using NCEP-ATP III and IDF definitions, based on waist circumference) was found to be higher in women compared to men and were lower in the rural than the urban areas (Okafor, 2012). Among Cameroonians, considering those with two components of metabolic syndrome, the most frequent combination was central obesity and high blood pressure, which was more predominant in women than men (81% vs. 52%). Combination of high blood pressure and hypercholesterolemia (24% vs. 6%) and high blood pressure and hyperglycemia (12% vs. 6%) demonstrated male predominance (Okafor, 2012). Though the resting energy expenditure of African-Americans, Caucasian and Nigerian population (postmenopausal women) was found to be indistinguishable there was however a greater incidence of obesity in the former two groups than in the Nigerian postmenopausal woman (Achie *et al.,* 2012). In South Africa, greater incidence of risk factors for the metabolic syndrome occurred in males but obesity was more common in females (25% vs. 14%). Both genders had abnormally high mean TG but male predominance appeared to be observed for dyslipidemia (Wahab *et al*., 2008). Metabolic syndrome was seen to be more common in males in Jos, Nigeria (Puepet *et a*l., 2009).

2.15.2. Metabolic syndrome and gender

Gender-specific differences have been demonstrated by different workers. Metabolic syndrome appears to be more common in females like obesity whereas hypertension appears

to be more common in males (Okafor, 2012). The prevalence of metabolic syndrome was only observed to be higher among males from the Jos plateau of Nigeria where the authors noted that the high activity profile of women may have contributed to this observation (Puepet *et al.*, 2009). This pattern is at variance with the findings from the north-western Nigeria (Sokoto) where the religious practice of putting the women in *Purdah* makes them sedentary.

2.15.3 Management of the metabolic syndrome in women

Postmenopausal women who develop features of the metabolic syndrome should be aggressively treated to reduce CVD risk. The successful management of metabolic syndrome hinges basically on lifestyle modification and pharmacological intervention. While attempts are ongoing in search for an approach that can simultaneously affect all the components, the current approach remains to treat each component as it becomes manifest (Okafor, 2012). Since it is believed that a major driving force in Africa is epidemiologic transition, reverting back to African traditional lifestyles is a potential point of action to prevent the development of metabolic syndrome in Africans. Management guidelines suggest a combination of lifestyle modification and drug therapy (Carr, 2003). Until recently, hormone replacement therapy (HRT) was an option for treatment of the postmenopausal metabolic syndrome, because it improved many of the metabolic abnormalities (Carr, 2003). However, with the recent release of data from the oestrogen progestin arm of the Women's Health Initiative demonstrating increased CVD risk in HRT users, HRT is no longer recommended for preventative therapy of CVD (Carr, 2003).

2.15.4 Lifestyle modification

Weight loss and physical exercise are both mainstays of therapy, as they address the underlying etiology of the metabolic syndrome (visceral obesity and insulin resistance) (Carr, 2003). Even modest weight loss has been shown to improve visceral adiposity and insulin resistance. There is a preferential loss of abdominal fat with aerobic exercise, as visceral adipocytes appear to respond more quickly to exercise-induced weight loss than subcutaneous adipocytes (Despres *et al.*, 1993). Regular endurance exercise may improve insulin sensitivity independent of total weight loss. Therefore, the aim of lifestyle modification therapy is to promote regular prolonged low intensity exercise (*i.e.* walking) to maintain weight and reduce visceral adipose tissue, rather than to set unobtainable weight loss goals (Carr, 2003).

2.15.5 Lipid lowering

Lifestyle changes may be inadequate to treat the dyslipidemia of the metabolic syndrome (increased TG, reduced HDL, and small dense LDL particles). Although LDL cholesterol has remained the primary target of lipid-lowering therapy, triglyceride lowering is an important secondary target to reduce CVD risk (NCEP, 2001). Nicotinic acid and fibric acid derivatives both act to reduce TG and increase HDL cholesterol. They are frequently used with statin medications, but caution should be used in combining these drugs. Although niacin is an inexpensive monotherapeutic agent that corrects the combined dyslipidemia of the metabolic syndrome, it has the disadvantage of increasing glucose levels in some patients (Carr, 2003).

Recent evidence has suggested an underutilization of lipid-lowering therapy in women. Baseline data from the Heart and Oestrogen/Progestin Replacement Study revealed that more than 60% of women with proven CVD did not meet the National Cholesterol Education Program goals for LDL lowering (Schrott et al., 1997). It is also important to note that lipid abnormalities associated with the metabolic syndrome can be subtle. The metabolic syndrome is associated with small dense LDL particles, moderately elevated TG (_1.7 mmol/liter or 150 mg/dl), and reduced HDL (1.3 mmol/l or 50 mg/ dl), but not elevated LDL cholesterol levels (Table 2) (NCEP, 2001). There has been increasing interest in LDL and HDL particle size and composition as additional risk factors for atherosclerosis (Carr, 2003). Given that LDL levels may underestimate CVD risk in the presence of small dense LDL particles, practitioners must treat the dyslipidemia of the metabolic syndrome in addition to treating elevated LDL cholesterol levels (Carr, 2003).

Table 2.5 Shows four most commonly used definitions of metabolic syndrome

	NCEP ATP111 (2005) revision	WHO (1998)	EGIR(1999)	IDF(2005)
Absolutely required	None	Insulin resistance(IGT,IFG,T2D or other evidence of IR	Hyperinsulinemia (plasma insulin> 75th percentile).	Central obesity (WC)≥80cm(sub-Saharan Africans)
Criteria	Any three of the five criteria below.	Insulin resistance or diabetes, plus two of the four criteria below.	Hyperinsulinemia, plus two of the four criteria below.	Obesity, plus two of the four criteria below.
Hyperglycemia	FBG ≥ 110mg/dl	Insulin resistance already required	Insulin resistance already required	FBG ≥ 100mg/dl
‘ Obesity	WC:>35 inches (female)	Body Mass Index >30kg/m²	WC ≥80cm (female)	Central obesity already required
Dyslipidemia	TG ≥ 150mg/dl	TG ≥ 150mg/dl or HDL-C <39mg/dl(f)	TG ≥ 177mg/dl or HDL-C<39mg/dl	TG ≥ 150mg/dl
Dyslipidemia (second, separate criteria)	HDL-C < 50mg/dl (female)			HDL-C <50mg/dl(f)
Hypertension	Systolic ≥ 130mmHg or Diastolic ≥ 85mmHg	≥ 140/90mmHg	≥140/90mmHg	systolic ≥130mmHg,or Diastolic ≥85mm Hg

IGT – Impaired Glucose Tolerance, IFG- Impaired fasting glucose, T2D- Type 2 diabetes, IR- Insulin resistance, WC- waist Circumference, TG- Triglyceride, FBG- Fasting blood glucose, HDL-C – High density lipoprotein cholesterol

2.15.6 Definitions of metabolic syndrome

World health organization (WHO), first developed its definition in 1998 (Alberti and Zimmet, 1998). Because insulin resistance was felt to be central to the pathophysiology of metabolic syndrome, evidence of insulin resistance is an absolute requirement in the WHO definition. This insulin resistance could be impaired fasting glucose, defined as a fasting glucose level above a predetermined cutoff commonly 100mg/dl or impaired glucose tolerance, defined as a glucose level above a predetermined cutoff, commonly 140mg/dl for 120 minutes after ingestion of 75 grams of glucose load during an oral glucose tolerance test. In addition to this absolute requirement for insulin resistance, two additional criteria have to be met. These include obesity, dyslipidemia and hypertension. In 1999, the European Group for the study of Insulin Resistance (EGIR) proposed a modification to the WHO definition (Balkau and Charles, 1999). Like the WHO, the EGIR felt that insulin resistance is central to the pathophysiology of the metabolic syndrome, so it also requires it for the definition. In this case IR is defined by a fasting plasma insulin value that is greater than the 75^{th} percentile. The use of elevated fasting insulin alone as a reflection of insulin resistance simplifies the definition, but it also means that patients with T2D cannot be diagnosed as having metabolic syndrome, since fasting insulin may not be a useful measure of insulin resistance in such patients. Also, similar to the WHO definition, the EGIR definition requires two additional criteria which can be selected from obesity, hypertension and dyslipidemia. The obesity criteria were simplified to waist circumference whereas the WHO definition used a choice of body mass index. In 2001, the National Cholesterol Education Program (NCEP) Adult Treatment Panel 111(ATP 111) devised a definition for the metabolic syndrome (NCEP, 2002), which was updated by the American Heart Association and the National Heart Lung and Blood Institute in 2005 (Grundy *et al*, 2005). According to the NCEP ATP 111 definition, metabolic syndrome is present if three or more of the following five criteria are met, waist circumference over 40 inches (men) or 35 inches (women), blood pressure over 130/85mmHg, fasting triglycerides (TG) level over 150mg/dl, fasting high-density lipoprotein (HDL) cholesterol level less than 40mg/dl (men) or 50mg/dl (women) and fasting blood sugar over100mg/dl. The NCEP ATP 111definition is one of the most widely used criteria of metabolic syndrome. It incorporates the key features of hyperglycemia/insulin resistance, visceral obesity, atherogenic dyslipidemia and hypertension. It uses measurements and laboratory results that are readily available to physicians, facilitating its clinical and epidemiological application. In 2005, the International Diabetic Federation (IDF) published new criteria for metabolic syndrome (Zimmet *et al*, 2005). Although it includes the same

general criteria as the other definitions, it requires that obesity not insulin resistance, be present. The obesity requirement is met by population-specific cutoff points. This accounts for the fact that different populations, ethnicities and nationalities have different distributions of norms for body weight and waist circumference. It also recognizes that the relationship between these values and the risk for T2D or CVD differs in different populations. For example, south Asian populations have an increased risk for T2D and CVD at smaller waist circumference that would not be considered to meet the criteria in a western population.

CHAPTER THREE
MATERIALS AND METHOD

3.1 Area of study

The study was carried out in Enugu metropolis. Enugu is the capital of Enugu State. It spans across three local government areas that is Enugu North, Enugu East and Enugu South. The city had a population of 722,664 people according to 2006 Nigerian census. The people of Enugu metropolis are basically of Igbo tribe, though other tribes are well represented in the town. Two Hospitals were chosen for the collection of samples and analysis, Enugu State University Teaching Hospitals (ESUTH) and Mother of Christ Specialist Hospital Enugu. About twenty-five women were recruited from Holy Spirit Catholic Church Golf Estate G.R.A. Enugu, thirty from Methodist Church Ndiagu Enugu, ten from Urban Girls secondary schools and fifteen from Government secondary school Enugu. The other participants involved in this study were about seventy women from ESUTH and fifty from Mother of Christ Specialist Hospital, this include the female staff of these Hospitals and some women that visited the Hospital that met with the inclusion criteria.

3.2 Experimental design

The study was a cross sectional study. It essentially involved Igbo perimenopausal (45-55years $\bar{X}$=50 years) and premenopausal (30-40years $\bar{X}$= 35 years) women living in Enugu metropolis. The sample collection for the hormone analysis were taken during the early follicular phase (menstrual phase day 3- day 5) of menstrual cycle for the premenopausal women, while in the perimenopausal women because of irregularities of menstrual cycle the sample collection day was not specific. All sample collection was taken in the morning hours and in fasting state. Ten milliterss of venous blood was collected from each participant, 2mls was dispersed into a fluoride oxalate bottle for the determination of blood glucose while the rest of the blood was transferred into a plain tube, allowed to clot, centrifuged and the serum obtained was used for hormonal and biochemical analysis within one week of sample collection. The results obtained were analysed statistically using SPSS version 20 computer software at 95% confidence level.

3.3 Sample technique

The sample technique used in this study was simple random sampling technique. The participants were recruited from Enugu metropolis. Each participant was chosen entirely by convenience sampling and every woman has an equal chance of being included in the study provided all the inclusion criteria were met and informed consent obtained.

3.4 Subjects

3.4.1 Inclusion criteria

A total of two hundred apparently healthy women were recruited for the study. One hundred and twenty women between ages 45-55years who were in their perimenopausal stage in life were recruited while eighty women between ages 30-40years who were in their premenopausal years but not pregnant were also recruited. These participants who consented to the study were given a structured questionnaire to fill and their baseline clinical and demographic data were obtained. Women whose menstrual cycles were regular at younger age and started having menstrual irregularities between the ages of 45-55years and whose menstrual cycle have not ceased for one year were recruited as perimenopause Igbo women. Women whose menstrual cycle were regular every month and were not pregnant, within the ages of 30-40years were recruited as premenopause Igbo women. These women were apparently healthy and were not having any health challenges during the time of the study.

3.4.2 Exclusion criteria:

Women who were pregnant, those having irregular menses since puberty, HIV positive, known diabetes and hypertensive women including those that have not menstruated for one year or more and women who had hysterectomy, premature ovarian failure and hypothyroidism were all excluded.

3.4.3 Ethical clearance:

Ethical clearance was obtained from the Research Ethics Committee of ESUTH before commencement of the study. A written informed consent was also collected from each participant before enrolment in the study.

3.5 Sample size: The sample size was calculated using the formular of Naing et al (2006) which is $N= z^2 \times p (1- p) / d^2$

Where N = Minimum sample size

d = Desired level of significance (0.05)

z = Confidence interval (1.96)

p = Prevalence rate (12.1%) Adeoye et al (2015).

Using this formula, the minimum number of sample size was calculated to be 163

$N = 1.96^2 \times 0.121 (1- 0.121) / 0.0025$

$= 3.8416 \times 0.121 (0.879) / 0.0025$

$N = 163.4$

3.6 Sample collection:

Ten milliliters of venous blood samples was obtained in a fasting state from each participant for the determination of hormone profile (estradiol, luteinizing hormone, and follicle stimulating hormone)), lipid profile (total cholesterol, triglyceride, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol), calcium, alkaline phosphatase, uric-acid, inorganic phosphate and blood glucose level. Two milliliters of blood was dispersed into a fluoride oxalate bottle for blood glucose determination while the rest of the blood transferred into a plain tube was allowed to clot, then centrifuged to obtain the serum which was deep frozen at -20°c and analyzed for hormonal and biochemical parameters within one week of sample collection.

3.7 Methods:

3.7.1 Measurements of waist circumference and body mass index.

The height, weight and waist circumference of the respondents were recorded. Waist circumference was measured on standing subjects with a soft tape midway between the lowest rib and the iliac crest. Height was measured in meters on standing position without shoes and the body weight in kilograms using a standardized bathroom weighing scale (Camry BR 9011). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

3.7.2 Measurement of blood pressure

Blood pressure measurements were carried out using a standardized automatic blood pressure monitor (Reli-On HEM 8724). Two blood pressure recordings were obtained from the left arm of participants in a sitting position after 30 minutes of rest. Measurements were taken in 5 minutes intervals and the average of the two measurements was used in the analysis.

3.7.3 Estradiol (E_2) Hormone :(Tietz, 1995)

Principle:

A fixed amount of HRP (horse radish peroxidase) labeled E_2 competes with the endogenous E_2 in the standard, sample or quality control serum for a fixed number of binding sites of the specific E_2 antibody in the micro wells on incubation. The unbound E_2 peroxidase conjugate is removed by washing while the bound antibodies are reacted with TMB (tetra methyl benzidine) to produce a blue colour. The reaction is stopped by using 1N HCL. The colour produced is inversely related to the amount of E_2 in the sample.

Procedure of Assay: (Procedure was as described by the manufacturer of the kit from Diagnostic Automation, INC, Calabasas, California, U.S.A. (Cat No: 2046z)

The desired number of coated wells in the holder was secured and 25µl of standards, specimens or controls were dispensed into appropriately labeled wells. 100µl of estradiol HRP conjugate reagent was also dispensed into the wells, then 50µl of rabbit anti-Estradiol (E_2) reagent was added into each well and the wells were mixed for 30 seconds and incubated at room temperature for 90 minutes. After the incubation the micro wells were rinsed and flicked 5 times with distilled water. 100µl of TMB Reagent was dispensed into each well and mixed gently for 10 seconds then incubated for 20 minutes at room temperature. The reaction was stopped with the addition of 100µl of stop solution into each well. The wells were gently mixed for 30 seconds making sure that the entire blue colour changed to yellow then the absorbance were read at 450nm with a microtiter well reader within 15 minutes.

Calculation:

A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on a linear-graph paper with absorbance values on the vertical (y-axis) and concentration on the horizontal (x-axis). Then the mean absorbance value for each specimen was used to determine the corresponding concentration of estradiol in pg/ml from the standard curve as shown in the appendix.

3.7.4 Luteinizing, Hormone (LH) (Uotila et al, 1981):

Principle:

The LH in the test sample is sandwiched between a solid phase and enzyme-linked antibodies. The unbound antibodies are removed by washing while the bound antibodies are reacted with TMB to produce a blue colour on incubation. This reaction is stopped by using HCL which changes the colour to yellow. The intensity of the colour produced is directly proportional to the concentration of LH present in the sample.

Procedure: (Procedure was as described by the manufacturer of the kit from Diagnostic Automation, INC, Calabasas, California, U.S.A (Cat No: 4225-16).

The desired number of coated wells in the holder was secured. 50µl of standard, specimens and controls were dispensed into the appropriately labeled wells; 100µl of enzyme conjugate Reagent was dispensed into each well then the wells were mixed thoroughly for 30 seconds and incubated at room temperature for 60 minutes. The wells were flicked and rinsed for 5 times with washing buffer and the residual water droplets were removed by striking the wells on paper towels. 100µl of TMB solution was dispensed into each well and gently mixed for 5

seconds, then incubated at room temperature for 20 minutes. The reaction was stopped with the addition of 100µl of stop solution to each well and mixed gently for 30 seconds to make sure that the blue colour has changed to yellow completely. The absorbance was read at 450nm with a micro titer well reader within 15 minutes.

Calculation:

The mean absorbance value for each set of reference standards, specimens, controls and patient samples were calculated. A standard curve was constructed by plotting the mean absorbance obtained from each reference standard on the vertical (y-axis) against the concentration in mIu/ml on the horizontal (x-axis) on a graph paper. Then using the mean absorbance values for each specimen the corresponding concentration of LH in mIu/ml was determined from the standard curve as shown in the Appendix.

3.7.5 Follicle Stimulating Hormone (FSH) (Uotila et al, 1981):

Principle:

On incubation, FSH in the test sample is sandwiched between a solid phase and enzyme-linked antibodies. The unbound antibodies are removed by washing while the bound antibodies are reacted with TMB to produce a blue colour. This reaction is stopped by using HCL which also converts the colour to shades of yellow. The intensity of the colour produced is directly proportional to the concentration of FSH present in the test sample.

Procedure: (Procedure was as described by the manufacturer of the kit from Diagnostic Automation, INC, Calabasas, California, U.S.A. (Cat No: 4224-16).

The same procedure for the assay of luteinizing hormone above was used to assay FSH.

Calculation:

A standard curve was constructed by plotting the mean absorbance obtained from each reference standard on y-axis against its concentration on x-axis in mIu/ml on a graph paper. Then using the mean absorbance values for each specimen, the corresponding concentration of FSH in mIu/ml was determined from the standard curve as shown in the appendix.

3.7.6 Determination of Blood Glucose (tietz, 1995)

Principle:

The Glucose is oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of phenol and 4-

aminoantipyrine (4-AA) is oxidized by hydrogen peroxide, to form a red quinoneimine dye proportional to the concentration of glucose in the sample.

ß-D-Glucose + H_20 + O_2 $\xrightarrow{GOD}$ D-Gluconate + H_20_2

4 aminoantipyrine + phenol + H_20 $\xrightarrow{peroxidase}$ Quinoneimine + H_20

Procedure: (procedure was as described by the manufacturer of the kit from Ccromatest Montgat-Barcelonia (Spain)

Three test tubes were set up and labeled as blank, test sample and standard respectively. The reagents and test samples were brought to room temperature. 10µl of water, test sample and standard were added to the three tubes respectively. Then 1.0ml of monoreagent was added to all the three tubes. The contents of the tubes were mixed well and incubated at 37°c for 5minutes. They were read colourimetrically at 520nm against the reagent blank.

Calculation

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{100\text{mg/dl}}{1} = \text{mg/dl glucose}$$

3.7.7 Lipid profile determination:

Determination of serum Cholesterol (Allain et al, 1974):

Principle of the Method:

Cholesterol esters in the sample are hydrolyzed enzymatically to cholesterol and fatty acids. In the presence of oxygen, the cholesterol produced and the free cholesterol in the sample are oxidized by cholesterol oxidase to cholestenone and hydrogen peroxide. The hydrogen peroxide formed is detected by a chromogenic oxygen acceptor (phenol-ampyrone, (PAP) in the presence of peroxidase and phenol. The red quinoneimine formed is proportional to the amount of cholesterol present in the sample.

Cholesteryl esters +H_20 $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + fatty acids

Cholesterol +½ 0_2+H_20 $\xrightarrow{\text{cholesterol oxidase}}$ Cholestenone+ H_20_2

$2H_20_2$+4 Aminoantipyrine+ Phenol $\xrightarrow{\text{peroxidase}}$ Quinoneimine+$4H_20$

Procedure: (Procedure was as described by the manufacturer of the kit from Ccromatest Montgat- Barcelona(Spain).

Three test tubes were set up and labeled as sample, standard, and blank respectively. 0.02ml of serum sample, standard and water were added to the three tubes respectively. Then 2.0ml of the working reagent was added to all the three tubes. The contents of the tubes were mixed

well and incubated at room temperature for 10 minutes. They were read colourimetrically at 540nm against the blank.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{5.18\text{mmol/l}}{1} = \text{mmol/l of cholesterol}$$

3.7.8 Determination of serum Triglyceride (Fossati and Prencipe, 1982):

Principle of the Method:

Triglyceride is hydrolyzed by lipase to form fatty acids and glycerol. The glycerol concentration is then determined by enzymatic assay. Coupled with Trinder's reaction that terminates in the formation of a quinoneimine dye. The colour intensity is directly proportional to the concentration of triglycerides in the sample.

Triglycerides + H_20 $\xrightarrow{\text{lipase}}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ Glycerol-3-P + ADP

Glycerol-3-P + 0_2 $\xrightarrow{\text{G-3-P-Oxidase}}$ Dihydroxyacetone-p + H_20_2

$2H_20_2$ + 4-Aminoantipyrine + 4-Chlorophenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_20$

Procedure: (Procedure was as described by the manufacturer of the kit from Ccromatest Montgat- Barcelona (Spain).

0.02ml of serum sample, standard and water were added into the three test tubes labeled test, standard and blank. Then 2.0ml of the working reagent was added into all the three tubes. The contents of the tubes were well mixed and allow standing for 15 minutes at room temperature. The absorbance (Ab) was read spectrophotometrically at 520nm using blank to zero the instrument.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{2.26}{1} = \text{mmol/l of Triglyceride}$$

3.7.9 Determination of serum High Density Lipoprotein (HDL) Cholesterol (Burstein et al, 1980):

Principle of the Method:

Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the serum sample are precipitated with phosphotungstate and magnesium ions. The HDL-C present in the

supernatant is then enzymatically and spectrophotometrically measured by means of the coupled reaction described below.

Cholesteryl esters $+H_2O$ cholesterol esterase → Cholesterol + fatty acids

Cholesterol $+1/2O_2+H_20$ cholesterol oxidase → Cholestenone + H_2O_2

$2H_2O_2$+ 4-Aminoantipyrine+ Dichlorophenol Sulfonate peroxidase → Quinoneimine$+4H_2O$.

Procedure (Procedure was as described by the manufacturer of the kit from Ccromatest Montgat-Barcelona (Spain).

To 0.2ml of sample in a clean centrifuge tube was added 0.4ml of reagent A. The tube was mixed and allowed to stand for 10minutes at room temperature and then centrifuged for 10minutes at a minimum of 4000 r.p.m. The supernatant (0.05ml) was carefully transferred into another tube and cholesterol was then determined using the method described above.

Calculation

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{1.36\text{mmol/l}}{1} = \text{HDL-Cholesterol}$$

3.7.10 Determination of Low Density Lipoprotein (LDL) Cholesterol (Sachiko, 1997):

Principle of the Method:

The cholesterol in lipoproteins other than LDL in the test sample is decomposed by the simultaneous action of cholesterol esterase and cholesterol oxidase at pH 7.0, giving cholestenone and hydrogen peroxide. A surfactant which specifically acts on LDL is added to the reaction product above in which the aniline derivate N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, and 4-aminoantipyrine as a coupling reagent are condensed by the H_2O_2 in the presence of peroxidase to form a red quinoneimine dye which is directly proportional to the concentration of LDL-cholesterol present in the sample.

Procedure:

The samples and reagents were brought to room temperature. 0.04ml of water, serum sample and standard were pipetted into three test tubes labelled blank, sample and standard. Then 0.3ml of reagent A were added into the three test tubes mixed and incubated for 5 minutes at 37°c after which 0.1ml of reagent B were added into the three tubes, mixed thoroughly and incubated for further 5 minutes at 37°c. Absorbance of the sample and standard was read at 600nm against the reagent blank.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{concentration of standard}}{1} \times \frac{\text{dilution factor}}{1}$$

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{2.59\,\text{mmol/l}}{1} = \text{LDL- Cholesterol}$$

3.7.11 Estimation of Very Low Density Lipoprotein (VLDL) Cholesterol:

VLDL was estimated using Friedewald formula (Friedewald et al, 1972)

$$\text{VLDL in mmol/l} = \frac{\text{Triglyceride}}{2.2}$$

3.7.12 Determination of Uric Acid (Fossati et al, 1980):

Principle of the Method:

Uric acid in the serum samples in the presence of oxygen are hydrolyzed by uricase enzymes to Alantoina, carbondioxide and Hydrogen Peroxide.

The hydrogen peroxide formed reacts with 4-Aminoantipyrine and 3, 5-dichloro-2-hydroxy-sulphonate in the presence of peroxidase to produce a coloured quinonic derivative and four molecules of water. The colour intensity is proportional to the amount of Uric acid present in the sample.

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{Uricase} \text{Alantoina} + Co_2 + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{3,5-Dichloro-2-hydroxy-Sulphonate} \xrightarrow{POD} \text{coloured quinonic derivative} + 4H_2O.$$

Procedure: (Procedure was as described by the manufacturer of the kit from Qumica Clinica Aplicada S.A. Spain).

Three tubes were set up and labeled as sample, standard and blank respectively. 0.02ml of serum sample and 0.02ml of the standard were added to the first and second tubes, while the third tube was used as blank. Then 1.0ml of the working reagent was added to all the three tubes. The contents were well mixed and left to stand for 10 minutes at 37^{0c}. The Absorbance was read spectrophotometrically at 520nm against the blank.

Calculation:

Uric acid in mg/dl = $\frac{\text{Ab of Test}}{\text{Ab of standard}}$ x $\frac{5}{1}$

(mg/dl) x 0.0595 = mmol/l Uric acid

Uric acid in mmol/l = $\frac{\text{Ab of Test}}{\text{Ab of standard}}$ x $\frac{0.2975}{1}$

3.7.13 Determination of serum Calcium (Biggs et al, 1974):

Principle:

The principle is based on specific binding of o-cresolphthlein. At alkaline PH values, serum calcium forms a coloured complex with 0-cresolphthalein; the intensity of the chromophore formed is directly proportional to the concentration of the total calcium in the sample.

Procedure: (Procedure was as described by the manufacturer from Qumica Clinica Aplicada S.A. Spain).

Three tubes were set up and labeled as sample, standard and blank respectively. 0.01ml of serum sample and 0.01ml of the standard were added to the first and second tubes, while the third tube was used as blank. Then 1.0ml of the working reagent was added to all the three tubes. The contents were well mixed and left to stand for 5 minutes at room temperature (20-25^{0c}). Absorbance was read at 570nm colourimetrically.

Calculation:

$\frac{\text{Ab of Test}}{\text{Ab of Standard}}$ × 10 x 0.2495 = mmol/l calcium.

3.7.14 Determination of serum Inorganic Phosphorous (Fiske and Subbarow, 1925):

Principle:

The phosphate ion reacts with molybdate to produce phosphomolybdate, which is finally reduced to a molybdenum blue, which is photometrically measured.

Procedure: (Procedure was as described by the manufacturer from Qumica Clinica Aplicada S.A Spain).

Three test tubes were set up and labeled as sample standard and blank respectively. 0.05ml 0f serum sample and 0.05ml of standard were added to the first and second tubes. Then 1.0ml of the working reagent was added to the three tubes. The contents were well mixed and left to stand for 10 minutes at room temperature. Absorbance was read spectrophotometry at 650nm after zeroing with blank.

Calculation:

$$\frac{\text{Ab of Test}}{\text{Ab of Standard}} \times \frac{1.2916}{1} = \text{mmo/l Inorganic phosphorus}$$

3.7.15 Determination of Alkaline Phosphatase Activity (Babson 1965):

Principle:

Serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline PH values turns into a pink colour. The intensity of the colour is directly proportional to the concentration of alkaline phosphatase in the serum.

Procedure: (Procedure was as described by the manufacturer from Qumica Clinica Aplicada S.A. Spain).

Three test tubes were set up and labeled as sample standard and blank respectively. 1.0ml of alkaline phosphatase substrate was added into the three tubes. The tubes were incubated at 37^{0c} for 5minutes then 0.1ml of serum, standard and deionized water was added to the three tubes respectively. The contents of the tubes were mixed well and incubated at 37^0C for 10 minutes. Then 5.0ml of alkaline phosphatase colour developer was added into the three tubes. The contents were mixed well and read spectrophotometry at 550nm against blank.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{30}{1} = \text{u/l of Alkaline phosphatase}$$

3.8 Determination of Metabolic syndrome (MetS)

Metabolic syndrome was observed using three criteria NCEP-ATP 111, WHO and IDF

3.8.1 Metabolic syndrome according to NCEP-ATP 111 Criterion (NCEP 2001)

The national cholesterol education program-adult treatment panel 111 criterion state that the presence of three or more of the following components (FBG ≥ 110mg/dl, TG ≥ 1.7mmol/l, systolic BP ≥ 130mmHg, diastolic BP ≥ 85mmHg, WC ≥ 88cm and HDL-C < 1.29mmol/l) in an individual suggests the presence of metabolic syndrome.

3.8.2 Metabolic syndrome according to International Diabetes Federation Criterion (IDF, 2005)

The International Diabetes Federation defined metabolic syndrome as the presence of waist circumference (WC ≥ 80cm) plus any two or more of the following components (FBG ≥ 100mg/dl, TG ≥ 1.7mmol/l, systolic BP ≥ 130mmHg, diastolic BP≥ 85mmHg, and HDL-C < 1.29mmol/l) in an individual suggests the presence of metabolic syndrome.

3.8.3 Metabolic syndrome according to World Health Organization Criterion (WHO, 1998)

The World Health Organization defined metabolic syndrome as the presence of insulin resistance defined as fasting blood glucose (FBG > 100mg/dl) plus any two or more of the following components (TG ≥ 1.7mmol/l, systolic BP ≥ 140mmHg, diastolic BP ≥ 90mmHg, BMI ≥ 30kg/m² and HDL-C < 1.0mmol/l) in an individual suggests the presence of metabolic syndrome.

3.9: Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20 computer software at 95% confidence level. Results were expressed as mean ± standard deviation. Significant differences between means were determined using Independent Students T-test. The relationships between parameters were obtained using Correlations and Chi-square.

CHAPTER FOUR

RESULT

The results obtained from this study are as shown:

Table 4.1: Biophysical and anthropometric parameters of perimenopausal and premenopausal women. The result shows significantly higher mean values in SBP (121 ± 4), DBP (82 ± 11) and WC (88 ± 0.7) in perimenopausal compared with premenopausal SBP (117± 10), DBP (76 ± 9), WC (79 ± 0.4) ($p < 0.0001$). However, the BMI of perimenopausal women (30.1± 5) was not significantly different from that of (30.0 ± 4.7) premenopausal women ($p>0.05$)

Table 4.2: Hormonal profile (LH, FSH, & E_2) and fasting plasma glucose (FPG) of perimenopausal and premenopausal women. This table shows the mean ± SD of FPG (mmol/l), estradiol (pg/ml), luteinizing hormone (mIu/ml) and follicle stimulating hormone (mIu/ml). The result shows significantly higher FPG, LH and FSH in perimenopausal women compared to premenopausal women ($p< 0.05$). In contrast, significantly lower estradiol level was observed in the perimenopausal women compared to premenopausal women ($p < 0.05$).

Table 4.3 shows the mean ± SD of triglycerides (mmol/l), total cholesterol (mmol/l), high density lipoprotein- cholesterol (mmol/l), low density lipoprotein- cholesterol (mmol/l) and very low density lipoprotein- cholesterol (mmol/l) in the perimenopausal and premenopausal women. The result shows significantly higher level of serum TC (6.18 ± 1.2, 4.17 ± 0.8), and LDL-C (4.3 ± 1.1, 2.4 ± 0.7)) in perimenopause and premenopause respectively ($p < 0.0001$). However, HDL-C (1.3 ± 0.5, 1.2 ± 0.4), TG (1.3 ± 0.7, 1.3 ± 0.4) and VLDL-C (0.6 ± 0.3, 0.6 ± 0.2)) in perimenopausal and premenopausal women respectively was similar ($p> 0.05$).

Table 4.4 shows the mean ± SD of serum calcium (mmol/l), inorganic phosphate (mmol/l), alkaline phosphatase (u/l) and uric acid (mmol/l) level in the perimenopausal and premenopausal women. The result shows significantly higher level of serum calcium (2.64 ± 0.1, 2.45 ±0.3) and uric acid (0.28 ± 0.01, 0.26 ± 0.0) $p< 0.0001$ in perimenopausal and premenopausal women respectively while inorganic phosphate (1.43 ± 0.4, 1.33 ± 0.3) and alkaline phosphatase (24.58 ± 0.4, 23.78 ± 4.8) in perimenopausal and premenopausal women respectively were not significantly different ($p > 0.05$)

Table 4.1: Biophysical and Anthropometric parameters in perimenopausal and premenopausal women.

Groups	Systolic blood pressure(mmHg)	Diastolic blood pressure (mmHg)	Body mass index (kg/m^2)	Waist circumference (cm)
Perimenopausal Women N = 120	121 ± 4	82 ± 11	30.1 ± 5.1	88 ± 0.7
Premenopausal women N = 80	117 ± 10	76 ± 9	30.0 ± 4.7	79.1 ± 0.4
t-statistic	5.012	4.207	0.07	4.523
p-value	P< 0.0001	P< 0.0001	P = 0.944	P< 0.0001

Values are given as Mean ±SD

Table 4.2: The levels of LH, FSH, E_2 and Fasting plasma glucose in perimenopausal and premenopausal women.

Groups	Fasting blood glucose(mmol/l)	Estradiol(E_2) (pg/ml)	Luteinizing hormone (miu/ml)	Follicle stimulating hormone (miu/ml)
Perimenopausal Women N = 120	5.5 ± 12.2	17.0 ± 8.9	55.1 ± 28.0	122.7 ± 55.8
Premenopausal women N = 80	5.3. ± 7.7	90.2 ± 26.5	11.2 ± 4.6	17.6 ± 8.7
t-statistic	2.343	28.01	13.9	16.7
p- value	P < 0.0201	P <0.0001	P < 0.0001	P < 0.0001

Values are given as Mean ± SD

Table 4.3: The Lipid profile in perimenopausal and premenopausal women.

Groups	Triglycerides (mmol/l)	Total cholesterol (mmol/l)	High density lipoprotein-cholesterol (mmol/l)	Low density lipoprotein-cholesterol (mmol/l)	Very low density lipoprotein-cholesterol (mmol/l)
Perimenopausal women N = 120	1.3 ± 0.7	6.18 ± 1.2	1.3 ± 0.5	4.3 ± 1.1	0.6 ± 0.3
Premenopausal women N =80	1.3 ± 0.4	4.17 ± 0.8	1.2 ± 0.4	2.4 ± 0.7	0.6 ± 0.2
t-statistic	0.07	12.8	1.586	13.44	0.3543
p- value	P = 0.9442	p < 0.0001	P = 0.1142	P < 0.0001	P = 0.723

Values are given as Mean ± SD

Table 4.4: Levels of serum Calcium, Inorganic phosphate, Alkaline phosphatase and Uric-acid in perimenopausal and premenopausal women

Groups	Calcium (mmol/l)	Inorganic phosphate(mmol/l)	Alkaline phosphatase(u/l)	Uric acid(mmol/l)
Perimenopausal women N = 120	2.64 ± 0.1	1.43 ± 0.4	24.58 ± 0.4	0.28 ± 0.02
Premenopausal women N= 80	2.45 ± 0.3	1.33 ± 0.3	23.78 ± 4.8	0.26 ± 0.02
t-statistic	3.695	1.82	1.207	4.367
P- value	P < 0.003	P = 0.0703	P = 0.229	p < 0.0001

Values are given as Mean ± SD

Table 4.5 shows classification of metabolic syndrome in perimenopausal women according to NCEP-ATP 111 criterion (2001), where three or more of any of the following components (fasting plasma glucose ≥ 110mg/dl (6.1mmol/l), fasting triglyceride ≥ 1.7mmol/l, systolic blood pressure ≥ 130mmHg, diastolic blood pressure ≥ 85mmHg, waist circumference ≥ 88cm and high density lipoprotein cholesterol < 1.29mmol/l) suggest presence of metabolic syndrome. The result shows that 14 women had FPG ≥ 6.1mmol/l, 28 had TG ≥ 1.7mmol/l, 57 had SBP ≥ 130mmHg, 43 had DBP ≥ 85 mmHg, 55 had WC ≥ 88cm and 57 had HDL-C < 1.29mmol/l. Out of these women 30 had presence of three or more components, therefore were defined as having metabolic syndrome(MetS). Amongst the 30 women with MetS, the combination of components in them were as follows: SBP + DBP + TG + WC = 5, SBP + DBP + HDL-C + WC= 5 followed by SBP + DBP + FPG + WC= 4 and SBP + HDL-C + WC= 4 then SBP + DBP + HDL-C + TG + WC = 3, others were SBP + DBP + FPG + TG +WC =2, SBP + FPG + WC = 2, SBP +TG + WC =1, FPG + TG + WC =1, DBP + TG +WC = 1, SBP + DBP + FPG + HDL-C + WC =1 and SBP + HDL-C + TG + WC =1.

Table 4.6 shows classification of metabolic syndrome in premenopausal women according to NCEP-ATP 111 (2001) criterion, where three or more of any of the following components (fasting plasma glucose ≥ 6.1mmol/l, fasting triglyceride ≥ 1.7mmol/l, systolic blood pressure ≥ 130mmHg, diastolic blood pressure ≥ 85mmHg, waist circumference ≥ 88cm and high density lipoprotein cholesterol < 1.29mmol/l) suggest presence of metabolic syndrome. The result shows that out of 80 women 1 had FPG ≥ 6.1mmol/l, 11 had TG ≥ 1.7mmol/l, 14 had SBP ≥ 130mmHg, 11 had DBP ≥ 85mmHg, 7 had WC ≥ 88cm and 45 had HDL-C < 1.29mmol/l. Only 3 women had the presence of three or more of the above listed components and were tagged as MetS. The 3 women with MetS had this combination of components SBP, DBP TG, WC=1, SBP, DBP, HDL-C, WC=1 and SBP, DBP, FPG, WC= 1.

Table 4.5: NCEP-ATP 111 (2001) classification of metabolic syndrome in perimenopausal women.

Components	Criteria	Number of people affected	Number of people not affected	MetS	Non- MetS
FPG (mmol/l)	≥ 6.1	14	106	*30(25%)	90(75%)
Fasting TG (mmol/l)	≥ 1.7	28	92		
SBP (mmHg)	≥ 130	57	63		
DBP (mmHg)	≥ 85	43	77		
WC (cm)	≥ 88	55	65		
HDL-C (mmol/l)	< 1.29	57	63		

***** The components that constitute the 30 women with MetS were as follows

SBP + DBP +TG + WC =5, SBP + DBP +HDL-C + WC =5, SBP + HDL-C + WC =4

SBP + DBP +FBG + WC =4, SBP + DBP + HDL-C + TG + WC =3, SBP + DBP + FBG + TG +WC =2, SBP + FBG + WC = 2, SBP +TG + WC =1, FBG + TG + WC =1, DBP + TG +WC = 1, SBP + DBP + FBG + HDL-C + WC =1, SBP + HDL-C + TG + WC =1

Table 4.6: Metabolic syndrome classification of premenopausal women defined according to NCEP-ATP 111 criterion (2001)

Components	Criteria	Number of people affected	Number of people not affected	MetS	Non-MetS
FPG(mmol/l)	≥ 6.1	1	79	3(3.75)	77 (96.25)
Fasting TG (mmol/l)	≥ 1.7	11	69		
SBP (mmHg)	≥ 130	14	66		
DBP (mmHg)	≥ 85	11	69		
WC (cm)	≥ 88	7	73		
HDL-C (mmol/l)	< 1.29	45	35		

***Percentage in parenthesis**

* The combination of components in the 3 women with MetS were as follows:

SBP + DBP + TG + WC =1

SBP + DBP + HDL-C + WC =1

SBP + DBP + FBG + WC =1

Table 4.7 shows metabolic syndrome classification of perimenopausal women defined according to International Diabetes Federation (IDF, 2005) criterion, where central obesity defined as waist circumference (WC ≥80cm) is the central requirement plus any two of the following four components (fasting triglyceride ≥ 1.7mmol/l, systolic blood pressure ≥ 130mmHg or diastolic blood pressure ≥ 85mmHg, fasting plasma glucose ≥ 100mg/dl (5.6mmol/l) and high density lipoprotein cholesterol < 1.29mmol/l) suggest the presence of metabolic syndrome. Out of 120 women, 106 had WC ≥ 80cm, 28 had TG ≥ 1.7mmol/l, 57 had SBP ≥ 130mmHg, 43 had DBP ≥ 85mmHg, 56 had FPG ≥ 5.6mmol/l and 57 had HDL-C < 1.29mmol/l. From the 106 women with WC ≥ 80cm, 61 of them show the presence of two or more of the above listed components in them and were tagged as MetS. The 61 women with metabolic syndrome had this distribution of components in them. WC + hypertension (SBP or DBP) + dyslipidemia (HDL-C or TG) =21women, WC + FPG + dyslipidemia (HDL-C or TG) = 16 women, WC + hypertension (SBP or DBP) + FPG = 12 while WC + FPG + hypertension (SBP or DBP) + dyslipidemia (HDL-C or TG) = 12 women. Totally WC (61), SBP (43), FPG (40), DBP (30), HDL-C (33) and TG (21)

Table 4.8: shows IDF (2005) classification of metabolic syndrome in premenopausal women, where central obesity defined as waist circumference (WC) is the central criterion plus any two or more of the following four components (fasting plasma glucose ≥ 5.6mmol/l, high density lipoprotein cholesterol < 1.29mmol/l, fasting triglyceride ≥ 1.7mmol/l, systolic BP ≥ 130mmHg and diastolic BP ≥ 85mmHg) suggest metabolic syndrome. Out of the 80 premenopausal women, 38 had WC ≥ 80cm, 11 had TG ≥ 1.7mmol/l, 14 had SBP ≥ 130 mmHg, 11 had DBP ≥ 85mmHg, 30 had FPG ≥ 5.6mmol/l and 45 had HDL-C < 1.29mmol/l. From the 38 women with WC ≥ 80cm, only 14 of them had extra two or more of the above listed components in them and were defined as having MetS. The 14 women with MetS had this distribution of components in them. WC + hypertension (SBP or DBP) + dyslipidemia (TG or HDL-C) =2 women, WC + FPG + dyslipidemia (TG or HDL-C) = 6 women, WC + hypertension (SBP or DBP) + FPG =4 women and WC + hypertension (SBP or DBP) + FPG + dyslipidemia (TG or HDL-C) =2 women. All together were WC (14), FPG (12), SBP (8), DBP (4) and TG (4).

Table 4.7: IDF (2005) classification of metabolic syndrome in perimenopausal women

Components	Criteria	Number of people affected	Number of people not affected	MetS	Non-MetS
WC (cm)	≥ 80	106	14	61 (50.8)	59 (49.2)
Fasting TG (mmol/l)	≥ 1.7	28	92		
SBP (mmHg)	≥ 130	57	63		
DBP (mmHg)	≥ 85	43	77		
FPG (mmol/l)	≥ 5.6	56	64		
HDL-C (mmol/l)	< 1.29	57	63		

*** Percentage in parenthesis**

*The 61 women with MetS had the following distribution of components:

WC +FPG +HDL-C = 11, WC + SBP +DBP + HDL-C = 7, WC + FPG +SBP +DBP =6, WC + FPG +SBP =6, WC + FPG + TG =5, WC + FPG + SBP + DBP + TG =5, WC + SBP + HDL-C =5, WC + SBP + DBP + TG = 4, WC + SBP + DBP + FPG + HDL-C =3, WC + SBP + DBP + HDL-C +TG =2, WC + SBP + FPG + HDL-C =2, WC + DBP + TG = 1, WC + DBP + HDL-C + TG =1, WC + SBP + DBP + FPG + HDL-C + TG = 1, WC + SBP + FPG + HDL-C + TG =1, WC + SBP + TG =1

Table 4.8: IDF (2005) classification of metabolic syndrome in premenopausal women

Components	Criteria	Number of people affected	Number of people not affected	MetS	Non-MetS
WC (cm)	≥ 80	38	42	14 (17.5)	66 (82.5)
Fasting TG (mmol/l)	≥ 1.7	11	69		
SBP (mmHg)	≥ 130	14	66		
DBP (mmHg)	≥ 85	11	69		
FPG (mmol/l)	≥ 5.6	30	50		
HDL-C(mmol/l)	< 1.29	45	35		

*** Percentage in parenthesis**

* The components that constitute the 14 women with MetS were as follows:

WC + FPG + HDL-C = 3, WC + FPG + TG = 2, WC + FPG + SBP = 2, WC + SBP + DBP + FPG =2, WC + SBP + DBP + FPG + HDL-C = 1, WC + SBP + DBP + HDL-C =1, WC + FPG + HDL-C +TG = 1, WC + SBP + HDL-C + TG = 1, WC + SBP + FPG + HDL-C = 1

Table 4.9: Metabolic syndrome classification of perimenopausal women according to WHO definition and criterion (1998) was observed, where insulin resistance defined as fasting plasma glucose level above 100mg/dl (FPG > 5.6mmol/l) is a pivotal condition then, plus any other two or more of the following listed four components body mass index > 30kg/m², fasting triglyceride ≥ 1.7mmol/l, high density lipoprotein cholesterol < 1.0mmol/l, systolic BP ≥ 140mmHg and diastolic BP ≥ 90mmHg suggest metabolic syndrome. The result shows that 39 women had FPG > 5.6mmol/l, 28 had TG ≥ 1.7mmol/l, 29 had SBP ≥ 140mmHg, 40 had DBP ≥ 90 mmHg, 54 had BMI > 30kg/m² and 29 had HDL-C < 1.0mmol/l. Out of 39 women with FPG > 5.6mmol/l only 17 had extra two or more of the above listed components in them and were labeled as metabolic syndrome subjects. The 17 women with metabolic syndrome had this distribution of components in them, FPG + BMI + (HDL –C or TG) = 6, FPG + BMI + (SBP or DBP) = 5, FPG + (SBP or DBP) + (HDL-C or TG) =3, FPG + BMI + (SBP or DBP) + (HDL-C or TG) =3. Totally FPG (17), BMI (14), SBP (11), DBP (10), HDL-C (5) and TG (6)

Table 4.10: Shows premenopausal women classified with metabolic syndrome according to WHO criterion (1998), where insulin resistance defined as fasting glucose level above 100mg/dl (FPG > 5.6mmol/l) is a pivotal condition, plus any other two of the following listed four components body mass index > 30kg/m², fasting triglyceride ≥ 1.7mmol/l, high density lipoprotein cholesterol < 1.0mmol/l, systolic BP ≥ 140mmHg and diastolic BP ≥ 90 mmHg suggest metabolic syndrome. The result shows that 20 women had FPG > 5.6mmol/l, 11 had TG ≥ 1.7mmol/l, 2 had SBP ≥ 140mmHg, 9 had DBP ≥ 90mmHg, 37 had BMI > 30kg/m² and 23 had HDL-C < 1.0mmol/l. Out of 20 women with FPG > 5.6mmol/l only 6 had extra two or more of the above listed components in them and were labeled as metabolic syndrome subjects. The 6 women with metabolic syndrome had this distribution of components, FPG + BMI + SBP + DBP = 2, FPG + BMI + HDL-C = 2, FPG + BMI + HDL-C + TG = 1 and FPG + BMI + TG =1. Making a total of FPG (6), BMI (6), HDL-C (3) SBP (2), DBP (2) and TG (2). .

Table 4.9: Metabolic syndrome classification of perimenopausal women according to WHO (1998).

Components	Criteria	Number of people affected	Number of people not affected	MetS	Non- MetS
FPG (mmol/l)	> 5.6	39	81	17(14.2)	103(85.8)
Fasting TG (mmol/l)	≥ 1.7	28	92		
SBP (mmHg)	≥ 140	29	91		
DBP (mmHg)	≥ 90	40	80		
BMI (kg/m²)	> 30	54	66		
HDL-C (mmol/l)	< 1.0	29	91		

***Percentage in parenthesis**

* The 17 women with MetS have the following distribution of components:

FPG + BMI +SBP + DBP = 5, FPG + BMI + HDL-C = 4, FPG + SBP + DBP + TG = 3, FPG + BMI + TG = 1, FPG + SBP + BMI = 1, FPG + BMI + SBP + DBP + TG = 1, FPG + BMI + SBP + DBP + HDL-C =1 and FPG + BMI + HDL-C + TG = 1.

Table 4.10: Metabolic syndrome classification of premenopausal women according to WHO (1998)

Components	Criteria	Number of people affected	Number of people not affected	MetS	Non-MetS
FPG (mmol/l)	> 5.6	20	60	6(7.5)	74(92.5)
Fasting TG (mmol/l)	≥ 1.7	11	69		
SBP (mmHg)	≥ 140	2	78		
DBP (mmHg)	≥ 90	9	71		
BMI (kg/m²)	> 30	24	56		
HDL-C (mmol/l)	<1.0	23	57		

***Percentage in parenthesis**

* The components of the 6 women with metabolic syndrome were as follows:

FPG + BMI + SBP + DBP = 2, FPG + BMI + HDL-C = 2,

FPG + BMI + HDL-C + TG = 1, FPG + BMI + TG = 1

Table 4.11: The prevalence rate of metabolic syndrome of the perimenopausal and premenopausal women were compared using chi-square in the three different groups. The prevalence rate was higher in the perimenopausal women compared to the premenopausal women in all the three criteria assessed. NCEP ATP 111 criterion gave a prevalence of 25% and 3.75% (x =5.195; p =0.023), WHO criterion gave a prevalence of 14.2% and 7.5% (x =24.038; p=0.00) while IDF criterion has a prevalence rate of 50.8% and 17.5% (x=5.286; p=0.022) among perimenopausal and premenopausal women respectively.

Table 4.12: Shows the metabolic syndrome classification of perimenopausal women whose LH, E_2 and FSH were within the reference values (LH 5-20mIu/ml, FSH 2.5-36mIu/ml, E_2 50-150pg/ml) as those of the premenopausal women. In general fifteen (15) women were affected under this group. Applying the IDF and WHO criteria only one (1) that is 6.7% has metabolic syndrome respectively while in NCEP-ATP 111criterion none of the women was affected, thereby none had metabolic syndrome according to this criterion.

Table 4.13: shows the perimenopausal women with metabolic syndrome in the three groups that have serum uric-acid, inorganic phosphate, alkaline phosphatase and calcium levels above the cut-off points in the four parameters. Serum calcium level was predominant with prevalence rate of 47.1% (WHO), 32.8% (IDF) and 40% (NCEP- ATP 111) followed by serum inorganic phosphate 35.2% (WHO), 23% (IDF) and 26.7% (NCEP-ATP 111) then serum uric-acid 11.76% (WHO), 11.5% (IDF) and 16.7% (NCEP-ATP 111) while serum alkaline phosphatase level was the least with a prevalence rate of 5.8% (WHO), 3.3% (IDF) and 6.7% (NCEP-ATP 111).

Table 4.14: shows the premenopausal women with metabolic syndrome in the three groups that have serum uric-acid, inorganic phosphate, alkaline phosphatase and calcium levels above the cut-off points in the four parameters. Serum calcium level was also dominant having a prevalence rate of 50% (WHO), 35.7% (IDF) and 33.3% (NCEP-ATP 111) followed by serum inorganic phosphate 33.3% (WHO), 21.4% (IDF) and 0% (NCEP-ATP 111) then serum uric-acid 16.7% (WHO), 7.1% (IDF) and 0% (NCEP-ATP111) while serum alkaline phosphatase level was the least affected with a prevalence rate of 0% in all the three groups studied.

Table 4.11: The rate of prevalence of metabolic syndrome in perimenopausal and premenopausal women in the three groups compared using chi-square

Groups	Number of subjects	MetS	Non-MetS	Prevalence rate %	Chi-square X	P-value
NCEP-ATP 111	120 (PERIMENOPAUSAL) 80 (PREMENOPAUSAL)	30 3	90 77	25% 3.75%	5.195	0.023
WHO	120 (PERIMENOPAUSAL) 80 (PREMENOPAUSAL)	17 6	103 74	14.2% 7.5%	24.038	0.00
IDF	120 (PERIMENOPAUSAL) 80 (PREMENOPAUSAL)	61 14	59 66	50.8% 17.5%	5.286	0.022

Table 4.12: Metabolic syndrome classification of perimenopausal women with LH, E_2 and FSH within the reference range as those of the premenopausal women.

Components	Criteria	Number of women affected	Number of women not affected	Metabolic syndrome	Non- Metabolic syndrome
Waist Circumference (cm)	IDF ≥ 80	6	9	IDF = 1(6.7%)	IDF=14(93.3%)
	NCEP ≥ 88	4	11	WHO=1(6.7%)	WHO=14(93.3%)
				NCEP= 0(0%)	NCEP=15(100%)
BMI (kg/m²)	WHO > 30	3	12		
Fasting TG(mmol/l)	≥ 1.7 for all criteria	1	14		
Systolic Blood pressure (mmHg)	IDF ≥ 130	8	7		
	NCEP ≥130	8	7		
	WHO ≥ 140	4	11		
Diastolic Blood pressure (mmHg)	IDF ≥ 85	7	8		
	NCEP ≥ 85	7	8		
	WHO ≥ 90	6	9		
Fasting Plasma Glucose(mmol/l)	IDF ≥ 100	9	6		
	NCEP≥ 110	3	12		
	WHO > 100	7	8		
HDL-C(mmol/l)	IDF < 1.29	8	7		
	NCEP<1.29	8	7		
	WHO < 1.0	5	10		

Table 4.13: Perimenopausal women with MetS in the three groups that have serum uric-acid, inorganic phosphate, alkaline phosphatase and calcium levels above the cut-off values

Groups	Components	Cut-off values	Number of women affected	Number of women not affected	Prevalence %
WHO	Uric-acid (mmol/l)	> 0.3	2	15	11.76%
N =17	Inorganic Phosphate(mmol/l)	> 1.6	6	11	35.29%
	Alkaline Phosphatase(u/l)	> 35	1	16	5.8%
	Calcium (mmol/l)	< 2.1,>2.8	8	9	47.1%
IDF	Uric-acid (mmol/l)	> 0.3	7	54	11.5%
N = 61	Inorganic Phosphate(mmol/l)	> 1.6	14	47	23%
	Alkaline Phosphatase(u/l)	> 35	2	59	3.3%
	Calcium (mmol/l)	< 2.1,>2.8	20	41	32.8%
NCEP-	Uric-acid (mmol/l)	> 0.3	5	25	16.7%
ATP 111	Inorganic Phosphate(mmol/l)	> 1.6	8	22	26.7%
N = 30	Alkaline Phosphatase(u/l)	> 35	2	28	6.7%
	Calcium (mmol/l)	< 2.1,>2.8	12	18	40%

Table 4.14: Premenopausal women classified with MetS in the three groups that have serum uric-acid, inorganic phosphate, alkaline phosphatase and calcium levels above the cut-off values

Groups	Components	Cut-off values	Number of women affected	Number of women not affected	Prevalence %
WHO	Uric-acid (mmol/l)	> 0.3	1	5	16.7%
N =6	Inorganic Phosphate(mmol/l)	> 1.6	2	4	33.3%
	Alkaline Phosphatase(u/l)	> 35	0	6	0%
	Calcium (mmol/l)	< 2.1,>2.8	3	3	50%
IDF	Uric-acid (mmol/l)	> 0.3	1	13	7.1%
N = 14	Inorganic Phosphate(mmol/l)	> 1.6	3	11	21.4%
	Alkaline Phosphatase(u/l)	> 35	0	14	0%
	Calcium (mmol/l)	< 2.1,>2.8	5	9	35.7%
NCEP-	Uric-acid (mmol/l)	> 0.3	0	3	0%
ATP 111	Inorganic Phosphate(mmol/l)	> 1.6	0	3	0%
N = 3	Alkaline Phosphatase(u/l)	> 35	0	3	0%
	Calcium (mmol/l)	< 2.1,>2.8	1	2	33.3%

Table 4.15: shows the relationship between the indices of perimenopause (LH, FSH, and E_2) with TG, HDL-C, BP (systolic & diastolic), FPG, WC and BMI which are the indices of metabolic syndrome. The result shows a positive correlation between FPG and LH, FPG and FSH while a negative correlation exist between E_2 and TG as well as E_2 and FBG. Other parameters show no significant correlation.

Table 4.16: The relationship between LH, FSH and E_2 were correlated with the levels of serum calcium, inorganic phosphate, uric-acid and alkaline phosphatase in perimenopausal women. The result shows a positive significant relationship between luteinizing hormone and inorganic phosphate while a negative correlation exists between estradiol and alkaline phosphatase. Other parameters show no significant correlation.

Table 4.15: The relationship between parameters LH, FSH, & E_2) with metabolic syndrome indices (TG, HDL-C, BP (Sys & Dias) FPG, WC, BMI) in perimenopausal women

Parameters	(r) pearson	p-values
LH vs TG	0.002	0.9785
LH vs HDL-C	0.031	0.7347
LH vs SBP	-0.07	0.4478
LH vs DBP	-0.125	0.1749
LH vs FPG	0.185	0.0431 *
LH vs WC	0.030	0.7463
LH vs BMI	0.022	0.8109
FSH vs TG	0.126	0.1712
FSH vs HDL-C	0.064	0.4897
FSH vs SBP	-0.08	0.3867
FSH vs DBP	-0.062	0.5006
FSH vs FPG	0.211	0.0208 *
FSH vs WC	0.004	0.9675
FSH vs BMI	-0.099	0.2833
E_2 vs TG	-0.241	0.0080 *
E_2 vs HDL-C	0.041	0.6573
E_2 vs SBP	-0.118	0.2011
E_2 vs DBP	-0.149	0.1037
E_2 vs FPG	-0.204	0.0252 *
E_2 vs WC	-0.050	0.5849
E_2 vs BMI	-0.078	0.3994

*Significant correlation

T**able 4.16: The relationship between serum levels of calcium, inorganic phosphate, uric acid, alkaline phosphatase with luteinizing hormone, follicle stimulating hormone and estradiol in perimenopausal women**

Parameters	(r) Pearson	P-values
LH vs Calcium	0.024	0.7971
LH vs Inorganic Phosphate	0.181	0.0482 *
LH vs Uric acid	0.096	0.2945
LH vs Alkaline phosphatase	0.056	0.5431
FSH vs Calcium	-0.048	0.6028
FSH vs Inorganic Phosphate	0.045	0.6281
FSH vs Uric acid	0.116	0.2090
FSH vs Alkaline Phosphatase	0.044	0.6351
E_2 vs Calcium	0.111	0.2289
E_2 vs Inorganic Phosphate	-0.063	0.4976
E_2 vs Uric acid	-0.048	0.6057
E_2 vs Alkaline Phosphatase	-0.228	0.0124 *

* Significant correlation

CHAPTER FIVE

5.1 DISCUSSION

Perimenopause is associated with a lot of hormonal changes accounting for some biochemical changes which possibly give rise to metabolic syndrome in perimenopausal women. This study examined some biochemical changes and metabolic syndrome in cross sectional study of perimenopausal and premenopausal women. The result presented in this study shows that perimenopausal women showed significantly higher systolic and diastolic blood pressure compared to the premenopausal women. This rise in blood pressure could be as a result of endothelial dysfunction which deteriorates with aging leading to disruption in the release of nitric oxide within the vessel wall. This decreases the excretion of sodium and water by the renal distal tubules thereby promoting cardiac output (FareedKowNanse *et al*, 2013). Furthermore, Aging leads to arterial stiffness, increase pulse pressure, decrease in sex hormone (oestrogen) and increase in sensitivity to dietary sodium chloride (Manson *et al*, 2016). Oestrogen affects the expression of the renin-angiotensin system and the level of plasma catecholamines, which affect pathogenesis of hypertension in the sense that low oestrogen clearly elevates angiotensinogen thereby increasing angiotensin 1, which is converted to angiotensin 11 by the angiotensin-converting enzyme (ACE). Angiotensin 11 raises blood pressure in two ways; it induces vascular contraction stimulating aldosterone secretion by the adrenal gland which then increases tubular sodium and water reabsorption. Angiotensin 11 also binds to a receptor on the plasma membrane of smooth muscle cells within the arterioles, leading to contraction and reduction in the diameter of these vessels thus causing more resistance to blood flow (Catherine *et al*, 2012). This finding agrees with the works of Samat *et al,* (2008) and Tomiyana *et al,* (2009) who found significant increases in both the systolic and diastolic blood pressure of Caucasian menopausal transition women when compared to premenopausal women. It is also consistent with the work of FareedKowNanse *et al* (2013) who demonstrated significant increase in both systolic and diastolic blood pressure in Ghanaian women during their transition into menopause.

The BMI of both the premenopausal and perimenopausal women though high showed no significant difference. This finding is in line with Siminialayi *et al,* (2008)'s study among perimenopausal and premenopausal women of River State. It is also in agreement with a study in Caucasian menopausal women by Graziotan and Leiblum, (2005). Both studies demonstrated no significant difference in BMI between premenopausal and perimenopausal women. This result could also be attributed to lifestyle and dietary habit in the African

setting, and in this case the Igbo settings where a plump appearance is still desired in many tribes and fatness is seen as a measure of affluence, while usual African traditional diets, which were more of natural and non-processed food, is fast being replaced with processed and westernized (energy laden processed) food. All these result in obesity and increase in weight (Ahaneku *et al,* 2011). The lifestyle of our citizens have changed from the usual active and energy consuming life like going to the farm and gardening to more sedentary energy sparing ones with attendant increase in weight.

There was a significantly higher value in the waist circumference of the perimenopausal women when compared to the premenopausal women. This higher value in waist circumference may be attributed to oestrogen deficiency and changes in fat distribution, which is common in menopause transition. In menopause transition there are changes in how the body stores and metabolizes fat. The uterus and menstrual cycle no longer use the hormones produced by fat and the body does not store its fat in the hips and breast but in the waist (Achie *et al,* 2012). Thereby the body's metabolic rate reduces and abdominal circumferences increase This finding is consistent with the work by Achie *et al* (2012) on menopausal women in Zaria, Northern Nigeria and many other studies on menopausal Caucasians by Crawford et al (2000) and Donato et al (2006). Several studies indicated that even with a normal BMI, those with an elevated waist circumference can have a two to threefold increase in cardiovascular disease risk and premature death (Pischon *et al,* 2008; Dudeja *et al,* 2001). Inadvertently, the perimenopausal women in this study were at risk of developing diseases associated with increased waist circumference such as obesity, thrombosis and embolism.

The mean plasma glucose level of the perimenopausal women was significantly higher compared with the glucose value in premenopausal women. This could be as a result of insulin sensitivity which has been reported to decrease with age and decline in serum dehydroepiandrosterone sulfate (DHEAS) level (Oguoma *et al* 2017). Dehydroepiandrosterone sulfate is a hormone that can reduce visceral fat accumulation and improve insulin resistance. Moreover the declining oestrogen found in perimenopausal women could contribute to insulin insensitivity since oestrogen encourages muscle cells to absorb glucose, while declining oestrogen level during Perimenopause make the body more susceptible to insulin resistance (Carr, 2003). Oestrogen has a protective effect on pancreatic cells and prevents them from premature cell death (Janssen et al, 2008). It also acts on the cells of the pancreas to increase the production of insulin when required by certain conditions

such as hyperglycaemia. Therefore, the decline in oestrogen which is common in perimenopausal women seemed to cause the cells to become more insulin resistant, exacerbating raised blood glucose levels circulating in the body. Insulin resistance causes cells not to absorb glucose and metabolise it from the blood stream as readily so blood glucose levels get higher (Toth *et al,* 2000). Furthermore, some of the biochemical changes that characterize perimenopause can also raise blood glucose levels, such as weight gain, increase in waist circumference due to fat deposition on the waist line and no longer on thighs and breasts and general increase in body fat with less physical exercise (Fareedkownanse *et al,* 2013). Indeed, one of the challenges that menopausal women have during their transition is that some mistake menopause-related hot flashes or moodiness for symptoms of low blood glucose. Night sweats- hot flashes that occur at night can interrupt sleep leading to excessive daytime fatigue. This fatigue can also be mistaken for low blood glucose, thus leads to eating extra calories to raise a "low blood glucose level" thereby resulting to a high blood glucose level (Gutherie *et al,* 2001). This finding is in line with the works of many authors for example Fareedkownanse *et al* (2013) who demonstrated significantly high fasting plasma glucose in menopausal Ghanaian women while Onyesom *et al,* (2013) reported high plasma glucose level among menopausal women in delta state, Nigeria. Casiglia *et al* (2008) and Janssen *et al* (2008) who worked with Caucasians also reported similar significant high fasting plasma glucose levels in menopausal women compared to premenopausal ones.

The sex and pituitary hormones levels of these participants were also evaluated. The result presented in this study shows that perimenopausal women had significantly lower estradiol levels and higher follicle stimulating hormone and luteinizing hormone levels compared to premenopausal women. Bechilioulis *et al,* (2009) reported that major hormonal changes that occur in menopause transition are a decrease in estradiol levels with concomitant increases in follicular stimulating hormone and luteinizing hormone levels. The hormone levels in these participants were as expected since it has been established that during menopause transition there are very low levels of sex hormone (oestrogen) and high levels of pituitary hormones (FSH and LH) in the blood stream in response to depleted ovarian follicles (Isong *et al,* 2016). During this period the ovaries stop responding to FSH and LH due to ageing and without negative feedback the anterior pituitary keeps releasing FSH and LH thereby increasing their concentration in the blood stream while estradiol concentration is decreased (Saladin, 2012). Siminialayi *et al* (2008) also reported significantly higher FSH, LH levels and lower estradiol levels in River State perimenopausal women compared to premenopausal

women. Oestrogens have several cardio protective properties that change the vascular tone by increasing nitrous oxide production. They stabilize the endothelial cells, enhance antioxidant effects and alter fibrinolytic protein. All these are cardio protective effects which are lost with the onset of menopause (Sullivan, 2017).

The lipid profile in this study shows significantly higher levels of total cholesterol and LDL-C in perimenopause women while triglyceride, HDL-C, and VLDL-C show no significant differences ($P > 0.05$) between the two groups. This observation is in line with that of Berg *et al* (2001) who reported higher TC and LDL-C in menopausal transition in comparison with premenopausal women of South West Nigeria. Similar observations were earlier made by Berristein and Ross (1993), Matthew *et al,* (2001) and Carr (2003), in premenopausal and perimenopausal Caucasians.

High density lipoprotein-cholesterol reports have been inconsistent as it has been reported to remain unaffected (Jenson *et al,* 2010). An author reported elevated HDL-C among Caucasians (Mesech *et al,* 2006), while Igwe *et al* (2005) reported significantly lower level of HDL-C in perimenopausal women from South Eastern Nigeria. Igwe *et al* (2005) also observed no significant differences in total cholesterol and triglyceride, but reported significantly higher level of Low density lipoprotein-C as was also observed in this study. Zhu *et al* (2009) who studied serum lipid profile changes during menopausal transition among Chinese women observed higher level of TC as similar to the finding in this study but no significant difference in HDL-C and VLDL-C. Jensen *et al* (2010) in their study in Denmark premenopausal women undergoing menopause observed increase in TC, LDL-C as observed in this study but no difference in HDL-C. Mesech *et al* (2006) observed increases in all the lipid profile parameters (TC, TG, HDL-C, LDL-C and VLDL-C) during transition into menopause.

Lipid profile is influenced by geographical location, nationality, race, ethnicity and diet (Osakue, 2013), therefore these differences in these studies may be attributed to different races, ethnicity, geographical location, nationality and diet as well as different lifestyle of the participants in the different studies. The elevated total cholesterol and low density lipoprotein-cholesterol in perimenopausal women in this study could be attributed to hormonal changes and follicular atrophy due to aging and reduced level of estradiol in the women going into menopause. Low density lipoprotein has been implicated in the development of coronary heart diseases (CHD), which increases in menopause transition.

This increased risk may be associated with alteration in the lipid profile characterized by changes in LDL particle size and buoyancy (Carr *et al*, 2000). Oestrogen decreases the plasma LDL level by increasing its hepatic catabolism (Sullivan, 2017). Amount of LDL that enters the arterial wall decreases and foam cell formation rich in cholesterol esters were reduced. Oestrogen exerts its action on Hepatic lipase also known as Heparin Releasable Hepatic lipase (HRHL), to influence the metabolism of HDL. A study by Kalavathi *et al* (1991) showed that exogenous oestrogen administration caused cholesterol and LDL levels to be reduced and HDL levels to be increased. The elevated total cholesterol and low density lipoprotein-C in this study among the perimenopausal women could be an indication that they are both independent risk factors for developing cardiovascular disease in these women.

The mean Uric acid level of the perimenopausal women in this study was significantly higher compared to premenopausal women. The high level of uric acid observed in this study among the perimenopausal women may be attributed to stress which causes excess lactic acid build up, this hinders uric acid excretion by the kidneys thereby serum uric acid accumulates (Ekpenyong and Akpan, 2014). Again, the perimenopausal women in this study have high waist circumference or visceral fat and this is linked to insulin resistance, then insulin circulating throughout the body inhibits uric acid elimination by the kidneys (Achie et al, 2012). Overweight also increases the risk of developing hyperuricemia because there is more tissue available for turnover or breakdown, which leads to excess uric acid product (Ogbera et al, 2011).The perimenopausal women in this study may also be consuming high purine foods or foods rich in fructose. Increased fructose intake raises serum uric acid levels by increasing ATP degradation to AMP and activating the pathway of purine degradation to urate (Clarson, 2015). Environmental stress and socioeconomic confounding factors could also lead to high uric acid level in the perimenopausal women. This finding is in agreement with the work of Ogbera *et al* (2011) who observed hyperuricemia in menopausal women in Lagos, Nigeria. Ekpenyong and Akpan, (2014) stated that abnormal serum uric acid is an independent risk factor for metabolic and cardiovascular morbidity and mortality in women.

The serum inorganic phosphate level and alkaline phosphatase levels were not significantly different between perimenopausal women compared with premenopausal women in this study. This finding could be due to the fact that both the perimenopausal and premenopausal women in this study have a high mean value of BMI, which according to WHO indicate obesity. Sowers *et al,* (2010) stated that obese women appear to lose bone at lower rate than non-obese women across the menopause transition. Therefore, low BMI has been associated

with osteoporosis and women with obesity have been observed to be less at risk for osteoporosis and fracture. Moreover, Compston *et al,* (2011) reported serum inorganic phosphate to be higher in thin subjects than in obese or ideal weight subjects. Serum inorganic phosphate level and alkaline phosphatase level does not change in osteoporosis (Usoro et al, 2007). This finding is in line with the work of Usoro et al, (2007) who observed no significant differences in menopausal women in Calabar compared to premenopausal ones but not consistent with the work of Hafeez *et al,* (2011) who observed significant increase in serum inorganic phosphate and significant decrease in alkaline phosphatase level of perimenopausal women as compared to premenopausal women in Pakistan.

Furthermore, the serum calcium level was also determined in perimenopausal and premenopausal women. There exists significantly higher calcium level in the perimenopausal women when compared to the premenopausal women. This high calcium in the perimenopausal subjects may be attributed to oestrogen deficiency. It is well known that oestrogen deficiency induces synthesis of cytokines by the osteoblasts, monocytes and T-cells and thereby stimulating bone resorption by increasing osteoclastic activity (Usoro et al, 2007). Again the high calcium level could be as a result of increase in parathyroid hormone by the calcium sensing receptor in the parathyroid gland when there is low serum calcium level in the body. The increased parathyroid hormone levels induce 1α hydroxylase enzyme activity in the kidney, which converts inactive vitamin D to its active hormone form known as calcitriol. In turn, calcitriol stimulates the absorption of dietary calcium from the gastrointestinal tracts (GIT). It also increases renal tubular reabsorption of calcium, thus reducing the loss of calcium in urine (Matthew *et al,* 2016). Too much calcium in the blood can precipitate in the arteries, joints and ligaments killing muscle cells which only contract by deporting calcium outside the muscle cells and is harder if the blood contains more calcium thus leading to arteriosclerosis, bone-deformation, muscle cramps and fibromyalgia (Cakmak *et al,* 2015). This finding is in agreement with the work of Usoro et al, (2007) who reported higher serum calcium level in Calabar postmenopausal women compared to premenopausal women but not in line with the work of Hafeez *et al* (2011) who observed no significant different in calcium of both the pre and perimenopausal women of Pakistan.

The relationship between the indices of perimenopause (LH, FSH, and E_2) and the metabolic syndrome indices (TG, HDL-C, Systolic BP, Diastolic BP, FPG, WC and BMI) when compared shows that there exist significant negative correlation between E_2 and TG as well as E_2 and FPG. Positive correlations existed between fasting plasma glucose and luteinizing

hormone and also between fasting plasma glucose and follicle stimulating hormone. This is consistent with the work of Carr (2003) and Toth *et al,* (2000), which stated that decline in estradiol and increases in follicle stimulating hormone and luteinizing hormones seen in perimenopausal women caused cells to become more insulin resistant thereby increasing circulating blood glucose levels in the body. This association observed between perimenopausal indices (LH, FSH and E_2) and that of MetS (FPG) may be an indication of possible interaction between perimenopause and metabolic syndrome. This could also be indicative of the possible strategic role of fasting plasma glucose (FPG) in MetS amongst the perimenopausal Igbo women in Enugu metropolis. A positive correlation exist between LH and inorganic phosphate while a negative association exist between estradiol and alkaline phosphatase when the relationship between the indices of perimenopause (LH, FSH and E_2) and calcium, inorganic phosphate, uric-acid and alkaline phosphatase were compared. This finding is consistent with many reports on menopause transition and aging (Matthew *et al,* 2016 and Sullivan, 2017).

Metabolic syndrome is a constellation of interrelated risk factors of metabolic origin that appear to directly promote the development of thrombosis, embolism and atherosclerotic cardiovascular disease. Menopause and age are thought to predispose women to the development of metabolic syndrome. This study assessed the metabolic syndrome of both the premenopausal and perimenopausal women using three different criteria. Using the NCEP-ATP 111 diagnostic criterion metabolic syndrome classification among perimenopausal women was found to be higher 30 (25%) compared to their premenopausal counterparts 3 (3.75%). When WHO diagnostic criterion was used, metabolic syndrome classification among perimenopausal women was also found higher 17 (14.2%) compared to their premenopausal counterparts 6 (7.5%) and IDF diagnostic criterion gave the highest value of metabolic syndrome classification 61 (50.8%) in perimenopausal women as against 14 (17.5%) in the premenopausal women. This suggests that the perimenopausal women in this study are more predisposed to metabolic syndrome associated diseases than premenopausal women. It may also be inferred that metabolic syndrome classification can differ from population to population depending on the criterion used. The increase in metabolic syndrome among perimenopausal women could be attributed to decline in estradiol, which is linked to insulin resistant in the body, raising blood glucose levels. It may also cause increases in both the body mass index and waist circumference due to fat deposition on the

waist and also increases in the blood pressure due to endothelial dysfunction and arteriosclerosis arising from dyslipidemia.

In the NCEP-ATP 111 criteria for metabolic syndrome classification among the perimenopausal women (30) with MetS, this study revealed that central obesity which was defined as waist circumference in this study had the highest prevalence (100%) followed by hypertension SBP (93.3%) and DBP (70%) then dyslipidemia TG (46.6%), HDL-C (46.6%) and hyperglycaemia FPG (26.6%) while among the premenopausal women (3) with MetS, central obesity (WC) and hypertension (SBP and DBP) had the highest prevalence of 100% respectively and dyslipidemia (TG and HDL-C) and hyperglycaemia (FPG) had a prevalence of 33% respectively. This suggests that in the NCEP-ATP 111 criteria that waist circumference and hypertension were risk factors for the development of metabolic syndrome associated diseases in perimenopausal and premenopausal women with metabolic syndrome in Enugu metropolis.

In the IDF criteria for metabolic syndrome classification among the perimenopausal women (61) with MetS, the study shows that apart from abdominal obesity (WC) which was a central criterion that high SBP and high FPG demonstrated the highest prevalence of 70.5% and 65.6% respectively while among the premenopausal women (14) with MetS high FPG and high SBP also have the highest prevalence of 85.7% and 57.1% respectively. This suggests that in the IDF criteria FPG and SBP in combination with waist circumference are risk factors for the development of MetS associated diseases in both perimenopausal and premenopausal women with MetS in Enugu metropolis.

In the WHO criteria for metabolic syndrome classification among the perimenopausal women (17) with MetS, the study also observed that apart from insulin resistance/hyperglycaemia (FPG) which was a central criterion that obesity (BMI) had the highest prevalence of 82.4% followed by hypertension (SBP) with a prevalence of 64.7% while among the premenopausal women (6) with MetS obesity still dominant with a prevalence of 100%. This suggests that in the WHO criteria BMI in combination with FPG were risk factors for metabolic syndrome associated disorders such as risk of embolism, thrombosis and cardiovascular disorders.

Furthermore in this study central adiposity and obesity were the most prevalent components in the three (NCEP-ATP 111, IDF and WHO) criteria among perimenopausal and premenopausal women, suggesting that increase in central adiposity and general body fat predispose women to metabolic syndrome, therefore it is necessary to reduce this risk among

perimenopausal Igbo women in Enugu metropolis by changing the lifestyle leading to weight reduction by a healthy diet (low lipid diets with vegetables) and increase physical activity.

In addition, the rates of prevalence of MetS among the premenopausal and perimenopausal women were compared using chi-square tool in the three groups. It was observed that the prevalence of metabolic syndrome (MetS) was higher among the perimenopausal women than the premenopausal women in all the groups. International Diabetes Federation criterion (50.8% and 17.5% x=5.286; p= 0.022), WHO criterion (14.2% and 7.5% x=24.033; p=0.00) while NCEP-ATP111 criterion (25% and 3.75% x=5.195; p=0.023) respectively. These suggest that perimenopausal women in this study show higher prevalence of MetS than premenopausal women.

The metabolic syndrome classification was observed among perimenopausal women with gonadotropin hormones (LH, FSH) and E_2 within the reference ranges as those of the premenopausal women. The number of women affected in this group was fifteen and only one woman shows features of metabolic syndrome according to IDF and WHO criteria respectively. While applying the NCEP ATP 111 criterion none of the women show feature of metabolic syndrome. The prevalence rate in these women with MetS was 6.7% in both IDF and WHO criteria and 0% in NCEP ATP 111. This may suggest that increases in the gonadotrophin hormones (LH, FSH) and decrease sex hormone (E_2) levels which are perimenopausal indices affect the metabolic syndrome of women.

This study also compared the uric-acid, inorganic phosphate, alkaline phosphatase and calcium levels of the perimenopausal and premenopausal women with metabolic syndrome in all the three criteria using the different cut-off points in the four parameters. The result shows that calcium was the predominant components in both perimenopausal and premenopausal subjects WHO criterion (47.1% and 50%) IDF criterion (32.8% and 35.7%) and NCEP-ATP 111 criterion (40% and 33.3%) followed by inorganic phosphate WHO criterion (35.29% and 33.3%), IDF criterion (23% and 21.4%) and NCEP-ATP 111 criterion (26.7% and 0%) then uric-acid WHO criterion (11.76% and 16.7%), IDF criterion (11.5% and 7.1%) and NCEP-ATP 111 criterion (16.7% and 0%). This may indicate that metabolic syndrome affect these components mostly calcium and there is need to determine these parameters especially calcium, while assessing metabolic syndrome in women.

CONCLUSION

This study shows that there are changes in blood pressure, fasting plasma glucose, total cholesterol, LDL-C, waist circumference, uric acid, calcium levels as well as in the gonadotrophin (luteinizing hormone, follicle stimulating hormone) and sex hormone (estradiol) levels in perimenopausal Igbo women in Enugu metropolis. This biochemical changes .were related to metabolic syndrome.

Additionally the study demonstrated that there are differences in the prevalence of metabolic syndrome amongst Igbo women according to the three criteria used- National Cholesterol Education Program-Adult Treatment Panel 111(NCEP-ATP111), World Health Organization (WHO) and International Diabetes Federation (IDF).

The researcher state the need to include calcium as panel of components while assessing for MetS in Igbo women in Enugu metropolis since incidence of calcium was predominant in both perimenopausal and premenopausal women with MetS in this study.

It could also be concluded from the observed data that incidence of metabolic syndrome was higher in perimenopausal women than in premenopausal women of Igbo origin in Enugu metropolis and that surprisingly perimenopause is associated with an increased risk of metabolic syndrome.

RECOMMENDATIONS

It is recommended that components of MetS be determined frequently using different criteria in perimenopausal women. This is to prevent metabolic syndrome associated diseases like thrombosis, embolism and cardiovascular diseases in menopause since the incidence of MetS was higher in perimenopausal women compared to premenopausal women.

Furthermore, the researcher recommends frequent physical activities/exercising and cutting down on carbohydrates intake as a point of action to prevent the development of MetS amongst Igbo women in Enugu metropolis since WC and BMI were the most prevalent component among perimenopausal and premenopausal women with MetS in all the criteria studied. The researcher also encourages more research works on perimenopause. Perimenopause phase has been understudied, yet this is the period that many biochemical changes as well as many derangements in health start developing leading to cardiovascular events that manifest in menopause and post menopause.

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APPENDIX 1

INFORMED CONSENT FOR STUDY ON BIOCHEMICAL CHANGES AND METABOLIC SYNDROME IN PERIMENOPAUSAL IGBO WOMEN IN ENUGU METROPOLIS

INTRODUCTION: You are invited to participate in a research study on biochemical changes and metabolic syndrome in perimenopausal Igbo women in Enugu metropolis. Metabolic syndrome is a major public health issue which deserves more attention especially in Nigeria where there is paucity of information on it. Metabolic syndrome is the name for a group of risk factors that raise the risk for heart disease and other health problems, such as diabetes and stroke. Metabolic risk factors are the biochemical conditions, traits or habits in the body that increases the chance of developing a disease for example a large waist line (abdominal obesity), a high triglycerides (TG) level, a low high density lipoprotein cholesterol level, high blood pressure and high fasting blood glucose. Metabolic syndrome is diagnosed when there is the presence of three or more of the above listed risk factors (Adeoye et al, 2015). Perimenopause is known as menopause transition and is characterized with biochemical changes. These biochemical changes tend to increase the risk of metabolic syndrome leading to cardiovascular diseases. This study is geared towards understanding the biochemical changes and metabolic syndrome in perimenopausal Igbo women in Enugu metropolis and thereby proffering preventive approaches.

VOLUNTARY NATURE OF PARTICIPATION:

Participation in this study is voluntary. It does not affect your privileges.

STUDY: The participants will be asked about the events that took place before coming to the hospital. The drugs they have taken if any. They will be given a structured questionnaire to fill. There will be collection of samples (fasting blood) by vein-puncture by the pathologists in the clinic, into a clean-labeled plain tube.

RISKS: There will be no infection transferred since a new syringe will be used for each subject and the sample will be gently collected without undue pressure on the patients' arm.

DISPOSAL: Used syringes will be disposed by incineration to avoid contamination. Also after the test the labeled plain tubes will be disposed in the same way to avoid contamination.

CONFIDENTIALITY: All information gathered in this study will be confidential and participants will not be identified at publication or presentation of our findings.

COST: All the costs to be incurred in the laboratory tests will be borne by the researcher.

PROBLEMS OR QUESTIONS:

If you have any problem or question about the study, you should contact ***Mrs. Ifeoma Ikegwuonu (***Tel: 08035091156) of the medical laboratory department Unizik Nnewi Campus.

CONSENT

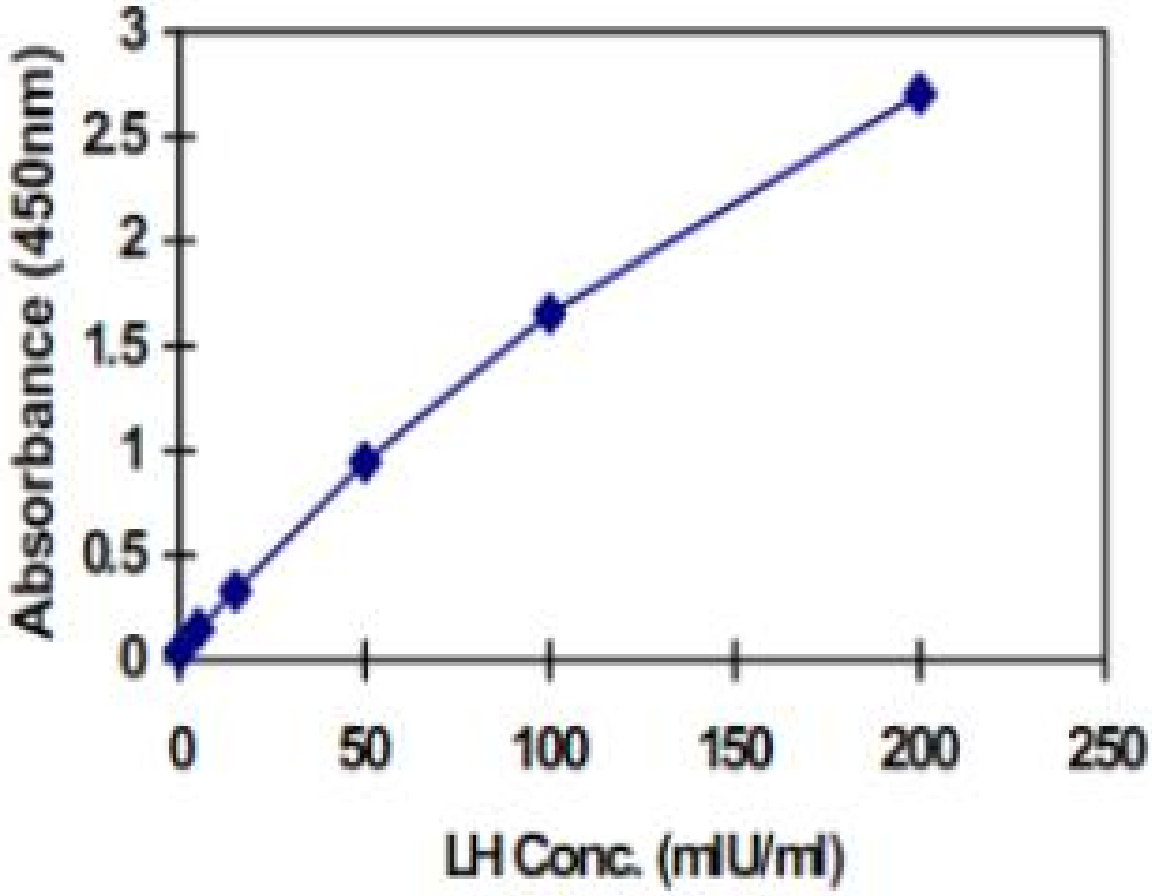
I,……..have read or had someone read to me the entire details of the study and have been given the opportunity to discuss any questions. I understand the nature, risks and benefits of this study. I hereby consent to be part of this study.

…… ……
Signature/Thumbprint of participant Name & signature of Researcher

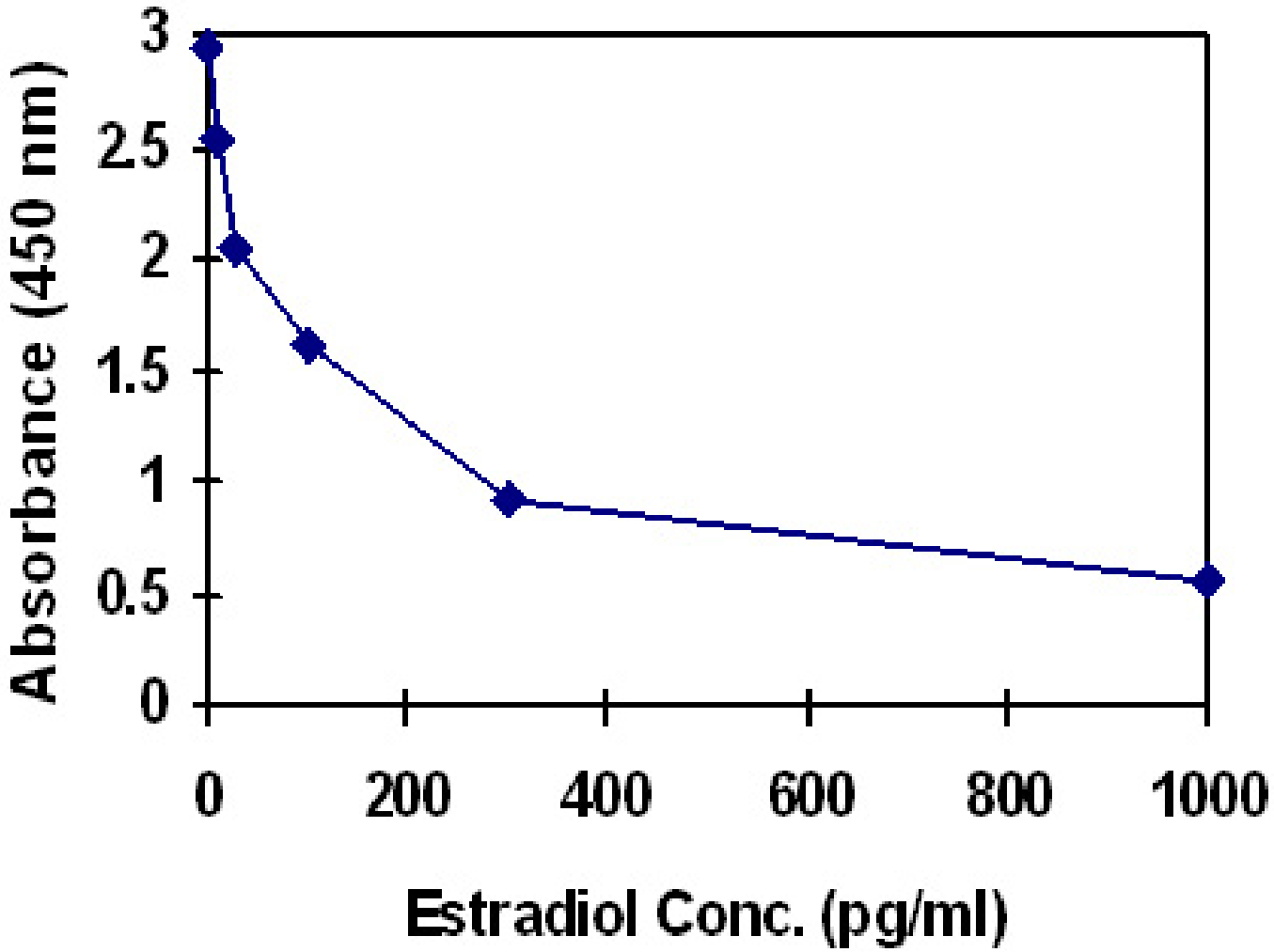
…….. …….
Date Date

……. …….
Name and signature of witness Date

STANDARD CURVE FOR LUTEINIZING HORMONE (LH)



STANDARD CURVE FOR ESTRADIOL



STANDARD CURVE FOR FOLLICLE STIMULATING HORMONE