TOTAL, ABDOMINAL, AND HIP FAT MASS AND MARKERS FOR CORONARY HEART DISEASE IN PRE AND POSTMENOPAUSAL OBESE WOMEN

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To the Dean of the Graduate School:

I am submitting herewith a thesis written by Melinda Villarreal entitled "Total, Abdominal, and Hip Fat Mass and Markers for Coronary Heart Disease in Pre and Postmenopausal Obese Women." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Masters with a major in Exercise & Sports Nutrition.

David Nichols, Ph.D., Major Professor

We have read this thesis and recommend its acceptance:

Department Chair

Accepted:

Dean of the Graduate School

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ABSTRACT

MELINDA VILLARREAL

TOTAL, ABDOMINAL, AND HIP FAT MASS AND MARKERS FOR CORONARY HEART DISEASE IN PRE AND POSTMENOPAUSAL OBESE WOMEN

AUGUST 2009

The purpose of this investigation is to examine the relationship between total, abdominal, and hip fat mass and serum markers for CHD in pre and postmenopausal obese women. Participants included non-smoking, sedentary and obese premenopausal women (n = 19) and postmenopausal women (n = 13). Resting blood samples were previously collected and analyzed for lipid and lipoprotein cholesterol concentrations. Multiple regression analysis was used to determine relationships among regional fat mass and lipid and lipoproteins and MANOVA was used to assess differences in these variables between pre and postmenopausal women. Menopausal status had an effect on concentrations of lipid and lipoprotein-cholesterol and on total and regional fat mass. Total and regional fat mass were also found to have associations with lipid and lipoprotein-cholesterol concentrations.

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CHAPTER I

INTRODUCTION

An estimated 16 million people have coronary heart disease (CHD) and it is considered to be the leading cause of death in the United States. It is also known that more women than men die each year from cardiovascular disease (CVD). Roughly every 26 seconds an American suffers a coronary event, and it is expected that there will be 450,000 recurrences every year. About every minute, someone will die from a coronary event. The estimated direct and indirect cost of CHD for 2008 is $156.4 billion. Prevention remains the key strategy for reducing CHD mortality and Medicare costs (Rosamond et al., 2008).

Blockage of the blood vessels of the heart results in development of CHD. In addition, blocked vessels will cause low blood flow to the heart and in turn cause chest pains or "angina." If left untreated, sections of the heart muscles may die causing heart attacks or myocardial infarctions. Therefore, the major developments of CHD events are nonfatal myocardial infarction and coronary death of the heart (Fowler-Brown et al., 2004). There are other multiple factors that are involved with the development of CHD which include: obesity, diabetes, high blood pressure, abnormal blood lipid concentration, dietary habits, sedentary life style, and poor aerobic fitness. These are modifiable risk factors for CHD (Leon, 1987; Villareal et al., 2006). Biological factors that cannot be

modified include: age, sex, and family history. A person's risk for CHD can be estimated based on the presence of all these factors.

Similar risk factors also underlie the metabolic syndrome. The syndrome indentifies individuals at an elevated risk for atherosclerotic cardiovascular disease no matter the cause (Grundy et al., 2005). Individuals without metabolic syndrome have the lowest risk for CVD events, those with metabolic syndrome have an intermediate level of risk, and those with diabetes have the highest level of risk (Malik et al., 2004). Furthermore, at least 65% of people with diabetes die of some form of heart disease or stroke (Rosamond et al., 2008). Overall the more risk factors one has, the greater the incidence of CHD and CHD mortality. Features of the metabolic syndrome resulting from the increasing prevalence of obesity have been associated with risks for CHD (Despres, 2006). It has been shown that the metabolic syndrome increases risk of CHD by approximately 1.5 to 2.0 fold (Despres, Arsenault, Cote, Carter, & Lemieux, 2008). There is a new notion of a "global cardiometabolic risk" of CVD that has recently been introduced (Despres et al., 2008). It has been described as the classical risk factors (age, sex, smoking, hypertension, LDL and HDL cholesterol, diabetes, and family history of CHD) plus the further risk of the metabolic syndrome (Despres et al., 2008).

Obesity, insulin resistance, and dyslipidemia are associated with increased risk for metabolic and cardiovascular diseases. The prevalence of obesity has been increasing and remains a major public health concern (Hedley

et al., 2004). The most recent results from the 2005-2006 National Health and Nutrition Examination Survey (NHANES), using measured heights and weights, indicate that an estimated 67% of U.S. adults are either overweight or obese and 32% of adults are obese (Ogden et al., 2006). World wide, more than 1 billion adults are considered overweight (BMI > 25 kg/m^2) and 300 million are obese (BMI > 30 kg/m^2; World Health Organization, 2003).

In addition, excess abdominal fat is a well known independent coronary heart disease risk factor (Hubert, Feinleib, McNamara, & Castelli, 1983). An increase in central adiposity, as indicated by an enlarged waist circumference (WC), is associated with the presence of CHD, Type 2 diabetes mellitus, hypertension (HTN), and an increased mortality risk (Bigaard et al., 2005; Haffner, 2006).

Today, CHD remains the leading cause of death among women older than 55 years of age in comparison to women of reproductive age (Saltiki & Alevizaki, 2008). Younger women tend to have a lower incidence of CVD than men of similar age due to the greater accumulation of abdominal fat in men, whereas women accumulate more hip fat (Astrup, 1999). It is known that larger hip circumferences are associated with a protective effect against CVD (Lissner, Björkelund, Heitmann, Seidell, & Bengtsson, 2001). Kannel et al. (1991) showed that estrogen promotes the accumulation of gluteo-femoral fat.

Furthermore, transitioning from pre to postmenopause is associated with an increase in total and abdominal fat due to its direct relation to estrogen

deficiency accompanied by aging (Kimura et al., 2006). This may help to explain why an increase in abdominal fat may accelerate the incidence of CVD in women after menopause (Carr, 2003). Therefore, the development of practical techniques to quantify total, abdominal, and hip adiposity may be important in predicting CHD. Dual energy x-ray absorptiometry may be useful in determining regions of interest (ROI) such as abdominal and hip fat mass (Paradisi et al., 1999).

Purpose of the Study

The purpose of this investigation is to examine the relationship between total, abdominal, and hip fat mass, quantified by dual energy x-ray absorptiometry (DXA), and serum markers for CHD in pre and postmenopausal obese women. Also, to determine which of the body fat distribution variables may have independent relations with CHD risk factors in all women. Improvements in technology have made it possible to precisely measure total and regional body fat distribution. It remains unclear, however, if abdominal and hip fat mass quantified by DXA is associated with risk factors for CHD in pre and postmenopausal women who are at risk for developing metabolic and cardiovascular disease. The development of practical techniques to quantify total, abdominal and hip adiposity may be useful as they are important predictors of CHD.

Null Hypothesis

Postmenopausal women will not have (a) greater abdominal fat compared to premenopausal women; (b) increased concentrations of blood total cholesterol, LDL-C, triglycerides or lower HDL-C levels compared to premenopausal women; and (c) abdominal fat mass related to fasting insulin, glucose, triglyceride, LDL-C, HDL-C and total cholesterol concentrations.

Definitions

Body mass index (BMI): is a ratio calculated from a person's weight and height. Calculations are used for assessments of overweight and obesity populations. Can be calculated with the following formulas: weight (kg) / height $(m)^2$ or weight (lb) / height $(in)^2$ x 703.

Cardiovascular Disease (CVD): Disease affecting the heart or blood vessels.

Cholesterol: A fat-like substance that is made by the human body and eaten in animal products.

Coronary Heart Disease (CHD): Narrowing of the small blood vessels that supply blood and oxygen to the heart. It may also be referred to as coronary artery disease (CAD).

Diabetes Mellitus (DM): The World Health Organization (WHO) defines (2006) diabetes mellitus as a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. According to the WHO (2006), recommendations for the

diagnostic criteria for diabetes are: a fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or 2 hr plasma glucose ≥ 11.1mmol/l (200mg/dl).

Dual Energy X-Ray Absorptiometry (DXA): A test in which two X-ray beams are used to measure bone mineral density and soft tissue composition. In this study it will be used solely to measure total, abdominal, and hip fat tissue composition.

Dyslipidemia: According to the National Cholesterol Education Program Adult Treatment Panel III (2001), it is defined as a condition marked by abnormal concentrations of lipids (cholesterol and or triglycerides) or lipoprotein cholesterol concentrations (high and or low density lipoprotein) in the blood.

Estrogen: A female steroid hormone produced by the ovaries.

Estradiol: An esterified natural estrogen.

Glucose: A monosaccharide sugar occurring widely in most plant and animal tissue. It is the principal circulating sugar in the blood and the major energy source of the body. The body makes glucose from proteins, fats and, in largest part, carbohydrates.

High Density Lipoprotein (HDL): a class of plasma lipoproteins that promote transport of cholesterol from extrahepatic tissue to the liver for excretion in the bile. Serum levels have been negatively correlated with premature coronary heart disease.

High Density Lipoprotein-Cholesterol (HDL-C): The serum cholesterol carried by HDL.

Insulin: A protein pancreatic hormone secreted by the beta cells of the islets of Langerhans. It is essential for the metabolism of carbohydrates and the regulation of glucose levels in the blood and that when insufficiently produced results in diabetes mellitus.

Lipoprotein: A complex of lipids and apolipoproteins that are the principal means by which lipids are transported in the blood.

Lipoprotein lipase (LPL): A specific lipase that hydrolyzes triglyceride (TG) carried by lipoproteins in the periphery into free fatty acids and glycerol (Goldberg & Merkel, 2001).

Low Density Lipoprotein (LDL): a class of plasma lipoproteins that transport cholesterol to extrahepatic tissues. High serum levels have been correlated with premature coronary heart disease.

Low Density Lipoprotein-Cholesterol (LDL-C): The serum cholesterol carried in LDL.

Menopause: The permanent cessation of menses.

Metabolic Syndrome: Refers to a cluster of risk factors for CVD and type 2 DM (Rosamond et al., 2008). According to the National Cholesterol Education Program Adult Treatment Panel III, it is diagnosed as having at least three of the following five risk factors present (Grundy et al., 2005):

1. Fasting plasma glucose > 100 mg/dL
2. HDL cholesterol < 40 mg/dL in men or > 50 mg/dL in women
3. Triglycerides > 150 mg/dL

4. Waist circumference > 102 cm in men or > 88 cm in women

   BP > 130 mm Hg systolic or 85 mm Hg diastolic or drug treatment for

5. Hypertension

Normal weight: Defined as a BMI between 18.5 – 24.9 kg/m^2

Obese: According to the National Cholesterol Education Program Adult Treatment Panel III, obesity is defined as a body mass index (BMI) ≥ 30 kg/m^2 and/or a waist circumference > 88 cm (NCEP ATPIII, 2001).

Postmenopause: The status of no menses for more than 1 year prior to the study.

Premenopause: Individuals who were not experiencing menopause. Therefore, the pre-menopausal group consisted of women who declared having regular menstrual cycles in the year prior to the study.

Risk Factors: Behaviors or conditions that increase the chance of developing a disease.

Sedentary: No participation or minimal participation of 2 days or less per week for 20 min per session in aerobic exercise for 6 months prior to entry into the study.

Triglyceride (TG): A compound consisting of three molecules of fatty acid esterified to glycerol. It is a lipid that serves as a storage form of energy, primarily in adipose tissue.

Total Cholesterol (TC): A combination of the LDL, HDL, and VLDL concentrations in the bloodstream. This is the cholesterol measurement that is given by the standard home cholesterol test kit. Less than 200 mg/dL is desirable, 200 mg/dL to 239 mg/dL is considered borderline high, over 240 mg/dL is considered high.

Assumptions/Limitations

It is assumed that using anthropometric measurements such as waist circumference are useful indices for determining central fat obesity. It is also assumed that the existing data from the two previous researchers used for this investigation are valid and accurate.

Since the study is investigating only women, results may not be generalized to the entire population. In addition, the study design has no randomization. Also, there is a wide age range of women between the ages of 18-71 years.

Significance of the Study

Since women have a higher risk for CHD after menopause compared to premenopausal women, this study is of importance (Carr, 2003). An important area of inquiry is to determine methods to identify which women, with regard to menopause status, are at risk for CHD in relation to their body fat distribution. Some studies have shown mixed results by demonstrating an increased risk of CVD after menopause (Gohlke-Barwolf, 2000). More research is needed to clarify the independent effects of fat distribution on cardiovascular risk factors in

pre and postmenopausal women, in relationship to menopausal status, to help in determining appropriate interventions.

CHAPTER II

REVIEW OF LITERATURE

Lipid and Lipoprotein Metabolism

Lipids, more commonly known as fats and oil, are a concentrated source of energy and stored mainly in adipose tissue and the liver (Brooks, Fahey, & Baldwin, 2005). Lipids are insoluble in water but soluble in organic solvents. There are many different substances with different structures and functions that are included in the lipid category, such as triglycerides (TG), phospholipids, glycolipids, unesterified fatty acids, and cholesterol (Matthews, Freedland, & Miesfeld, 1997). The two main forms of lipids are cholesterol and triglycerides. The body produces varying amounts of cholesterol and can also be obtained in the diet. Fat in the form of triglycerides exists in food as well as in the body. Both travel in the blood stream attached to proteins called lipoproteins (Tirosh et al., 2007). Lipoproteins are macromolecular complexes composed of a lipid monolayer surrounding a hydrophobic core that contains cholesterol esters and TG (Ginsberg, 1998). The monolayer is composed of amphipathic molecules of phospholipids, apoproteins, and free cholesterol (Figure 1).

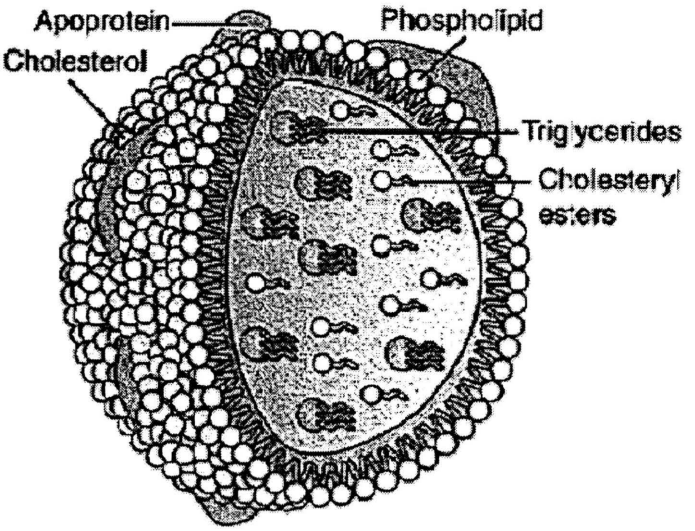

Figure 1. Lipoprotein structure

Note. The structure of a serum lipoprotein. A lipoprotein typically compromises a hydrophilic shell composed of phospholipids, apoproteins, and free cholesterol surrounding a hydrophobic core that contains triglycerides and cholesterol esters. From "Atherogenic Lipoprotein Subprofiling," by A. Ellington & I. Kullo, 2008, *Advances in Clinical Chemistry, 46, p.* 297. Copyright 2008 by the Academic Press. Adapted with permission of the author.

Lipoprotein particles are classified by associated apolipoproteins, content of cholesterol, TG and phospholipid that each particle carries. Apolipoproteins are critical regulators of lipid transport and provide structural stability (Ginsberg, 1998). The ten major apolipoproteins (apo) are: AI, AII, AIV, B48, B100, CI, CII, CIII, E, and (a). In addition, the protein and lipid content defines the particles buoyant gravitational density, in which low density particles have a higher lipid/protein ratio than do more dense particles (Ellington & Kullo, 2008). Lipoprotein subclasses, large and small lipoprotein particle sizes, are also a

function of the nuclear lipid content, with larger particles containing increased lipid mass. The major lipoprotein classes include intestinally derived chylomicrons, hepatic-derived very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), and hepatic and intestinally derived high density lipoprotein (HDL). The characteristics of lipids and lipoproteins are listed in Table 1.

Table 1

Characteristics of Plasma Lipids and Lipoproteins

			Major Constituents of lipoproteins (%)				
Lipoprotein	Size (nm)	Density (g/ml)	TG	Phos	Chol	Protein	Apolipoproteins
CM	75 - 1200	<0.94	85	7	7	1	B48, AI, AII, AIV, CI-II, E
VLDL	30 - 80	0.940 - 1.006	50	18	23	9	B100, CI-III, E
IDL	25 - 35	1.006 - 1.019	29	34	26	11	B100, CI-III, E
LDL	18 - 25	1.019 - 1.063	10	22	47	21	B100
HDL	5 - 12	1.063 - 1.125	8	29	30	33	AI, AII, AIV, CI-III, E

Note. CM= Chylomicron; VLDL= very low density lipoproteins; IDL= intermediate density lipoprotein; LDL= low density lipoproteins; HDL= high density lipoproteins; TG= triglyceride; Phos= phospholipid; Chol=cholesterol. Adapted from (Brooks, Fahey, & Baldwin, 2005; Ellington & Kullo, 2008; Ginsberg, 1998).

Very Low and Low-Density Lipoprotein Metabolism

Lipoproteins VLDL, intermediate density lipoprotein (IDL), and LDL are linked in a continuous metabolic cascade in which lipid (mainly TG) is lost in a series of small lipolytic steps (Packard & Sheperd, 1997). As plasma TG rises, there is an increase in hepatic synthesis of VLDL particles. Interaction with lipoprotein lipase (LPL) on endothelial cell surfaces initiates VLDL catabolism (Figure 2). When delipidated, large VLDL ($VLDL_1$) give rise to remnants in the smaller $VLDL_2$ and IDL density intervals, and are inefficiently converted to a class of LDL that is cleared slowly from the plasma (Tan et al., 1995). Esterified cholesterol is delivered to cells by way of LDL receptor mediated endocytosis (Brown & Goldstein, 1986). The predominant function of LDL and VLDL is to transport TG and cholesterol from the liver to peripheral tissues where TG are either used for energy or storage and cholesterol is used for biosynthesis of cell membranes, bile acids and steroid hormones (Brooks, Fahey, & Baldwin, 2005; Brown & Goldstein, 1986).

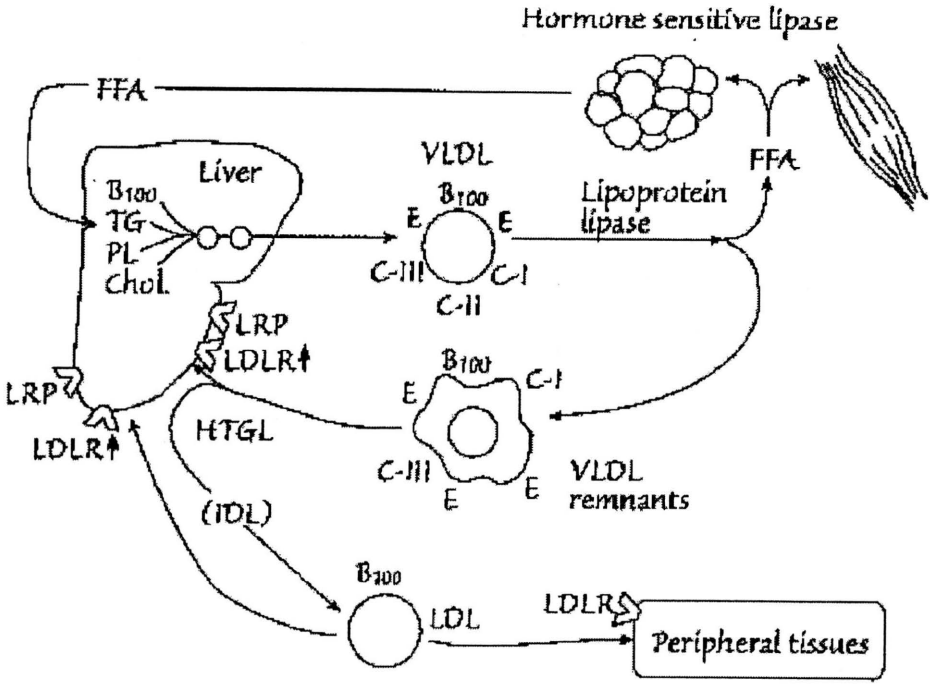

Figure 2. Very Low Density Lipoprotein Catabolism

Note. Transport of VLDL, IDL, and LDL. The availability of core lipids, TG, and cholesterol in the liver stimulates the assembly and secretion of VLDL, which then interacts with LPL in adipose and muscle tissue. The VLDL remnant (IDL) can then be removed via receptor mediated pathways in the liver or be converted to LDL. LDL is removed primarily by the liver and peripheral tissues via the LDL receptor pathway. VLDL= Very low density lipoprotein; IDL= intermediate density lipoprotein; LDL= low density lipoprotein; TG= triglyceride; LPL= lipoprotein lipase. Adapted from (Ginsberg, 1998). From "Lipoprotein Physiology," by H.N. Ginsberg, 1998, *Endocrinology and Metabolism Clinics of North America, 27, p.* 513. Adapted with permission of the author.

High Density Lipoprotein Metabolism

High density lipoprotein particles contain mostly apo AI which is synthesized mainly in the liver and is secreted into the plasma in a lipid-free or lipid-poor form (Tabet & Rye, 2009). As apo AI enters the circulation it gathers phospholipids and unesterified cholesterol from cell membranes and other

lipoproteins, is converted into discoidal HDL, then is rapidly converted into large spherical HDL by lecithin cholesterol transferase (LCAT; Hamilton, Williams, Fielding, & Havel, 1976). The enzyme LCAT generates almost all of the cholesteryl esters, which are hydrophobic, which then move into the center of the HDL particle, converting them into large spherical HDL particles (Hamilton et al.; Tabet & Rye). In the peripheral tissues, HDL removes excess cholesterol for elimination by the liver and secretion into bile through the process of reverse cholesterol transport (RCT; Klerkx et al., 2006). Therefore, RCT is generally thought to provide the rationale for the antiatherogenic properties of HDL (Klerkx et al.). Briefly, the RCT depends mainly on the presence of cholesterol transporters in the cell membrane and the presence of extracellular cholesterol acceptor particles, mostly thought of as HDL (Yancey et al., 2003).

Atherosclerosis and CHD Risk Factors

Plasma lipoproteins are known to play a causative role in atherosclerosis and its clinical manifestation, CHD. A risk factor for CHD is the aggregation of blood lipid abnormalities. Among these metabolic risk factors of CHD, LDL-C represents the lipoprotein cholesterol fraction which is the primary target of therapy (NCEP ATP III, 2001). The major cholesterol carrying lipoprotein in plasma, LDL, is the fraction most strongly implicated in atherogenesis. Austin, King, Vranizan, and Krauss (1990) have demonstrated an association between a particular LDL phenotype and CHD risk. The atherogenic lipoprotein phenotype, defined by the predominance of small, dense LDL and moderately elevated

plasma TG and low HDL-C levels, is associated with a three fold increase in CHD risk (Austin et al.). Several modified forms of LDL could contribute to atherogenesis such as oxidized LDL, aggregated LDL or LDL containing immune complexes (Steinberg, 1997). Two hallmark cell types in atherosclerotic lesions that give rise to cholesterol laden foam cells are the monocyte/macrophage and the smooth muscle cells (Steinberg). According to Watson et al. (1995) HDL inhibits these modifications by preventing oxidized LDL formation. There is also evidence that HDL has antithrombotic properties that involve the promotion of blood flow and the attenuation of thrombin generation and endothelial and platelet activation, thus causing a prolongation in the time of plaque rupture (Mineo, Deguchi, Griffin & Shaul, 2006).

The relative CHD risk associated with elevated lipid levels is different in men than in women. Postmenopausal women have a greater risk for developing CHD as a result of elevated triglyceride and low HDL-C compared to men (Castelli, 1988; Castelli & Anderson, 1986). Premenopausal women have a lower overall incidence of atherosclerosis and CHD compared with men of similar age (Astrop, 1999; Carr, 2003). By the age of 70 years, the incidence of cardiovascular risk is equal in both sexes. This may imply that a decrease in ovarian and especially, estrogen function, as occurs in the transition from pre to postmenopausal status, plays a significant role in the increased risk (Carr). Other risk factors include increased concentrations of serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), decreased high-density

lipoprotein cholesterol (HDL-C), increased abdominal fat distribution, and decreased physical activity.

Menopause and CHD Risk Factors

Premenopause is defined as women who are experiencing regular menstrual cycles. The menstrual cycle is a recurring cycle of physiologic changes that occurs in reproductive age females. Prior to reaching menopause, women undergo a perimenopausal phase that can extend up to 4 years in duration and consist of irregular patterns of the menstrual cycle (Carr, 2003). Carr best defines menopause as the absence of menses for 12 consecutive months.

The acceleration of atherosclerosis and CVD risk increases as women transition from premenopausal to postmenopausal status due to estrogen deprivation. Postmenopausal status is characterized as a deficiency of estrogen formation and may emerge with features such as dyslipidemia, insulin resistance and abdominal obesity, which increase cardiovascular risk. Dyslipidemia is distinguished by a decrease in HDL-C, increase in LDL-C and TG along with features of the metabolic syndrome. Women who have developed menopause at an earlier age have a higher risk for CHD independent of those features previously mentioned (Carr, 2003; Matthews et al., 1989). Premenopausal women, in comparison to men of similar age, are largely protected from CHD (Sullivan & Fowlkes, 1996). However this disparity tends to disappear after

menopause (Barrett-Connor, 2003). Estrogen deprivation may play an important role in the appearance of early CHD in women (Mathews et al., 1989)

Menopause and Estrogen

The main source of estrogen production in premenopausal women is the ovaries but it is the adipose tissue during menopause. Naturally occurring estrogens such as 17β-estradiol (E2), estriol (E3), and estrone (E1), are C18 steroids derived from cholesterol through a series of enzymatic reactions in steroidogenic cells (Figure 3). During steroidogenesis, androgens are aromatized to estrogens with the enzyme aromatase in the granulose cells of the ovaries. Estrogens bind to protein transporters in the circulation, such as sex hormone binding globulin (SHBG) produced in the liver and only 2-3% of estrogens remain unbound. Changes in SHBG may also influence free androgens, since the latter are also bound to it. Estrogens increase SHBG levels while androgens and high insulin levels have the opposite effect (Carr, 2003; Gouva & Tsatsoulis, 2004; Selby, 1990).

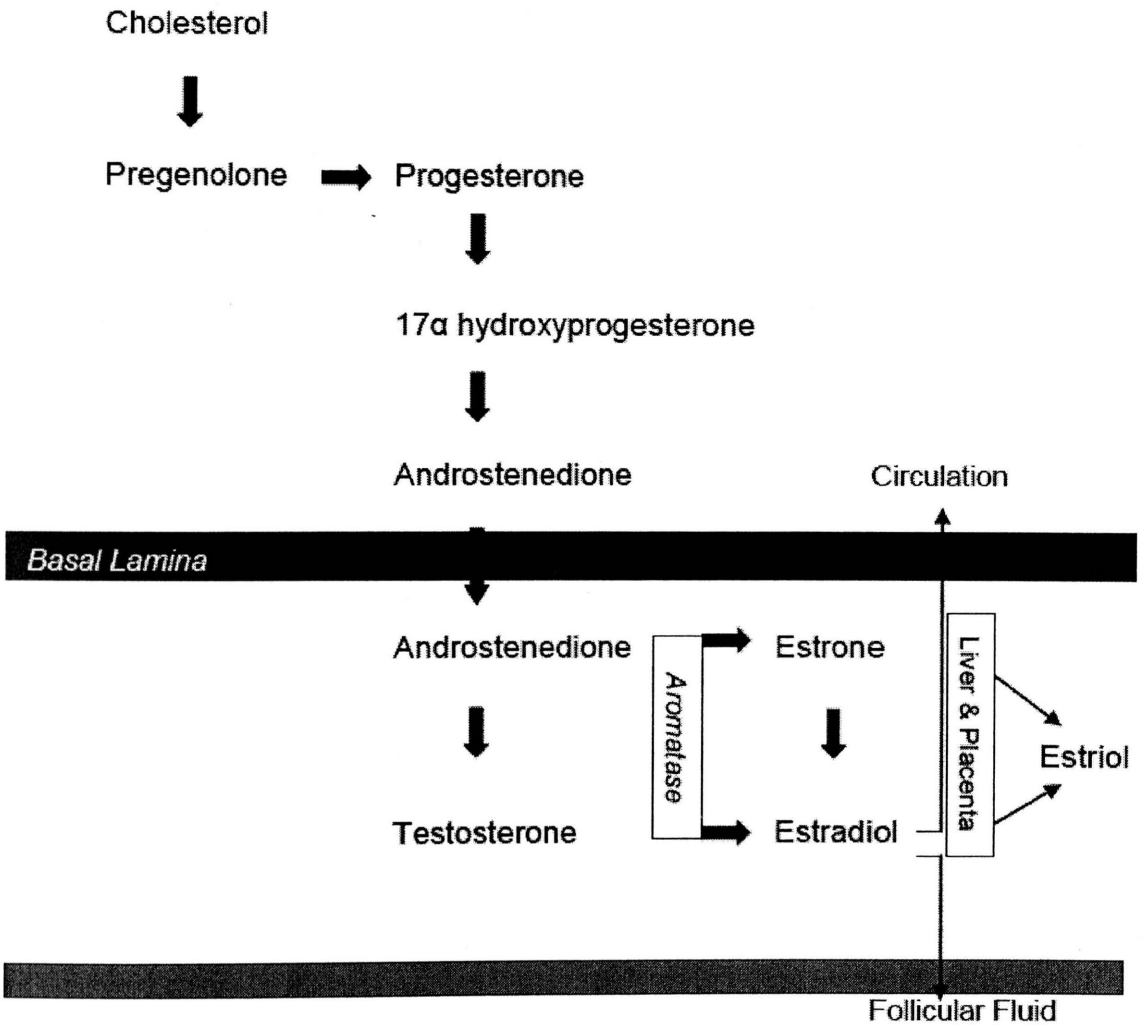

Figure 3. Steroidogenesis

Note. Theca and granulosa cells coordinate the production of estrogen. The secretion of estradiol by the dominant follicle requires cooperation between theca cells, which synthesize cholesterol to androstenedione and testosterone. Granulosa cells of mature follicles convert androgens to estradiol and estrone. Estradiol is the predominate form in reproductive females, estrone is produced during menopause, and estriol is the primary estrogen of pregnancy. From "Endocrine Physiology" by P. Molina, 2006. McGraw-Hill, *p.* 212. Copyright 2006 by the McGraw-Hill Medical Publishing Division. Adapted with permission of the author.

With menopause, there is a natural lack of endogenous estrogen production in the form of 17β-estradiol that is associated with reduced SHBG leading to decreased binding and thus an increase in the level of free androgens. Prior to menopause, average concentrations of 17β-estradiol range from 10-80 pg/ml during the follicular phase and reach levels of 600 pg/ml at midcycle. After menopause, estrogen concentration is between 5-30 pg/ml. In postmenopausal women, the main estrogen is E1, which is 50-70% less active than 17β-estradiol. The production of estrogens via extragonadal tissue, during menopause, is dependent on age and weight. In addition, estrogens are mainly produced by the adipose tissue (Saltiki & Alevizaki, 2007). Moreover, the alteration in the balance between androgens and estrogens may contribute to changes in body composition and other symptoms associated with menopause (Carr, 2003; Gouva & Tsatsoulis, 2004; Saltiki & Alevizaki). Furthermore, obesity, in particular visceral adiposity, is associated with hyperinsulinemia, a condition leading to a reduction in the synthesis of measured SHBG that, in turn, increases the bioavailability of estradiol in adipose tissues (Calle & Kaaks, 2004).

Estrogen and Cardiovascular Effects

Estrogens act on cellular function through genomic and nongenomic mechanisms. Estrogens act in target tissues by binding to estrogen receptors (ERs) that are members of the superfamily of nuclear receptors to which androgen, progesterone and glucocorticoid receptors also belong (Weigel, 1996). There are two different genes encoding ERs known as estrogen receptor-α (ER-

α) and estrogen receptor-β (ER-β). Once the receptors are bound by estrogen, the estrogen receptor can undergo genomic actions on where an alteration of the expression of various genes occurs. A conformational change occurs allowing the receptor to bind with high affinity to specific DNA sequences and modulate transcription of target genes (Murdoch & Gorski, 1991). Estrogens, through genomic actions, can effect changes in the metabolic profile, the immune process and the response to vascular injury (Gruber, Tscuhgguel, Schneeberger, & Huber, 2002; Mendelsohn & Karas, 1999). Both ERs are present in various human tissues as well as in cells of the cardiovascular system. Estrogens act on the cardiovascular system through their receptors which are expressed in vascular endothelial cells and smooth muscle cells of vessels (Kim-Schultze et al., 1996; Mendelsohn & Karas; Venkov, Rankin, & Vaughn, 1996).

Estrogens also show nongenomic actions that are critical for the proper function of the cardiovascular system. The nongenomic mechanism is not dependent on changes in gene expression and occurs after estrogens bind with receptors and are situated on the cellular membrane. Estrogens are believed to play a role in vascular homeostasis. Nongenomic actions regulate rapid vasodilation of coronary and other vessels by opening Ca^{++} channels and activation of K^{+} channels, as well as through the secretion of vaso-relaxing factor molecules such as nitric oxide (NO) produced by endothelium cells. The release of NO activates smooth muscle cells and leads to vasodilation (Rubanyi, 1993). It also has an anti-inflammatory and anti-atherogenic effect because it prevents

platelet aggregation on the wall of affected vessels and decreases proliferation of vascular muscle cells which ultimately prevents endothelial dysfunction (Post et al., 2003). Similarly through a nongenomic action, insulin secreted by pancreatic β cells, is possibly regulated by estrogen (Nadal et al., 1998). Endothelial dysfunction is regarded as an early manifestation of atherosclerosis (Quyyumi, 1998).

If estrogen deficiency occurs in postmenopausal women, more calcified atherosclerotic plaque occurs in the coronaries than premenopausal women or postmenopausal women receiving hormone replacement therapy (Saltiki & Alevizaki, 2007). Therefore, estrogen deficiency may play a role in the importance of vascular health (Janowitz, Agatston, Kaplan & Viamonte, 1993). Women have less calcified plaque in the arteries compared to men until the age of 60, and thereafter, gender differences are attenuated.

Estrogen and Lipid Metabolism

Estrogens play a role in both lipogenesis and lipolysis and are mediated by ERα. The effects on these processes depend on the type of estrogen being used. Studies have shown that 17β estradiol decreases TC and LDL-C concentrations by about 5-15%, due to an augmentation of the break down and clearance of LDL as well as an increase in hepatic expression of the LDL receptors (Gouva & Tsatsoulis, 2004). An increase in hepatic expression of apoprotein genes and decrease in the transcription of the LPL gene also occurs. LPL is synthesized and secreted by the adipocyte and transported to the capillary

endothelium where it hydrolyzes triglyceride into free fatty acids (FFA) and glycerol (Goldberg & Merkel, 2001). Estrogens also reduce apolipoprotein (a) and increase HDL-C by 10%, due to a decrease in hepatic expression of the HDL-receptors. Also, estrogens increase plasma triglycerides by about 20-25% (Gouva & Tsatsoulis).

Studies have shown regional difference in lipolysis or uptake of triglycerides via LPL to account for differences in body fat distribution in obese women (Raison, Basdevant, Sitt, & Guy-Grand, 1988). Shadid, Koutsari, and Jensen (2007) showed that there are regional and sex differences in adipose tissue FFA uptake in obesity. It resulted that the efficiency of FFA uptake by lower body adipose tissue was more efficient (11 ± 3% vs 3 ± 1%, $p = .02$, respectively) in women than men (Shadid et al.). This implies that the efficiency of FFA uptake is related to body fat distribution in regards to sex.

On the other hand, after menopause, estrogen levels decrease and an increase of the LPL activity is observed which can contribute to the accumulation of abdominal fat (Mayes & Watson, 2004). Furthermore, the period of menstrual irregularity prior to menopause tends to shift lipid metabolism to a more atherogenic lipid profile. This shift is similar to the metabolic syndrome which includes increased LDL and TG concentrations, reduced HDL_2 concentration, and smaller, denser LDL particles. Dyslipidemia and abdominal obesity are known to be associated and are likely to contribute to future CVD risk during menopausal transition (Carr, 2003). Furthermore, an increase of HDL-C by 1

mg/dL will reduce CHD risk by 2-3%, independent of LDL and triglycerides (Toth, 2005). Increasing HDL-C and decreasing LDL-C are important in reducing the risk for CHD. Also, prevention of obesity may help reduce the aggregation of lipid abnormalities which is important in reducing CHD risk.

Estrogen and Obesity

As mentioned earlier, weight is an important predictor of SHBG in postmenopausal women (Saltiki & Alevizaki, 2007). It has been suggested that there is an inverse relationship between obesity and SHBG in postmenopausal women, particularly related to abdominal obesity rather than to general obesity (Haffner, Katz & Dunn, 1991). Furthermore, body mass index (BMI) and waist circumference have been directly correlated with the total and bioavailability of estrogens in adipose tissue of postmenopausal women (Lukanova et al., 2004; McTiernan et al., 2003).

Baglietto et al. (2008) attempted to identify which anthropometric measures among height, fat mass, fat free mass and waist circumference play predominant roles in determining SHBG and steroid hormone concentrations in postmenopausal women. Fat and fat free mass were quantified by bioelectrical impedance. Concentration of free estradiol was positively associated with BMI (per 5 kg/m^2), fat mass (per 10 kg) and waist circumference (per 10 cm) at any time since menopause, in which the strongest association was observed for the period of 6-9 years after menopause. Time since menopause was represented in quartiles: 0-5 years, 6-9 years, 10-14 yrs and 15 or more years. In addition,

BMI, fat mass, fat free mass, and waist circumference were positively associated with free estradiol concentration, for which up to 7% of its total variability was explained by fat mass and waist circumference. BMI was found to be positively associated with estrogen and androgens and negatively associated with SHBG. Overall, this data suggests that the association between circulating concentrations of estrogens and body size varies with time since menopause, with the strongest association being observed at least 6 years after menopause. This may reflect a balance between the lower levels of SHBG due to visceral fat and the increased production of SHBG secondary to higher estradiol levels in heavier women (Baglietto et al.).

Regions of Interest and Lipid Metabolism

Many of the same factors that influence the amount of body fat, such as addition of adipose tissue LPL and lipolytic activity, also influence regional abdominal fat distribution (Bouchard, Bray & Hubbard, 1990). Generally, regional fat distribution changes during growth, sex maturation, and with aging. As mentioned earlier, a shift of fat distribution to the abdominal region is thought to occur after menopause (Carr, 2003). In addition, some research has also shown the existence of racial differences (Kanaley, Giannopoulou, Tillapaugh-Fay, Nappi & Ploutz-Snyder, 2003). Furthermore, it is important to know how regional fat distribution expressed as abdominal fat and hip fat play a role in lipid metabolism in regards to menopausal status. Currently, regional fat exhibits different influences on lipid metabolism, with central fat mass promoting and

peripheral fat mass counteracting cardiovascular risk in postmenopausal women (Tanko, Bagger, Alexandersen, Larsen & Christiansen, 2003). There has been conflicting data on the effects of abdominal fat distribution on blood lipid changes associated with menopause. Also, studies are not uniform in the methods of measuring regional body distribution, particularly by the lumbar vertebrae in abdominal fat mass measured by DXA. Some studies identify abdominal fat mass measured between L1 - L4, while others measure between L2 - L4. Trunk fat mass measured between regions L2-L4 in postmenopausal women has been shown to be associated with insulin resistance and dyslipidemia, whereas leg fat mass characterizes protective effects against metabolic dysfunction (Van Pelt, Evans, Schechtman, Ehsani & Kohrt, 2002).

Rissanen, Hamalainen, Vanninen, Tenhunen-Eskelinen and Uusitupa (1997) observed that measuring total abdominal fat mass with DXA, specifically between the L1 – L4 regions, correlated significantly with fasting insulin, glucose, triglyceride and HDL-C concentrations ($r = .54$; $r = .32$; $r = .30$; $r = -.49$, respectively). Participants were obese middle-aged women ($n = 43$) ages 29 – 64 years. Furthermore, the lower lumbar fat (L4 – L5) to hip fat (gauged downward the trochanter and same height as lower lumbar fat area) ratio was significantly correlated with triglyceride and HDL-C concentrations as opposed to the hip fat mass area alone ($r = .35$; $r = -.57$, respectively). A limitation to this study was that they did not categorize women according to their menopausal status.

Paradisi et al. (1999) compared abdominal fat defined as the DXA L1 – L4 region of interest, to BMI, waist-to-hip ratio and waist circumference. Healthy men (n = 87) were divided into two groups: lean men (n = 63) and obese men (n = 24). In addition, the DXA L1-L4 region of interest technique resulted as the best predictive value for cardiovascular risk factors such as insulin sensitivity, triglyceride and cholesterol concentrations in men (r = -.267, $p < .05$; r = .316, $p < .005$; r = -.319, $p < .005$, respectively). A limitation of this study was that only men served as participants. More research in women is needed.

Feng et al. (2008) observed the effects of menopause on metabolic risk factors for CVD. Participants included lean women (N = 9,097) ages 25-64 years. Women were further sub grouped as premenopausal (N = 2391) and postmenopausal (N = 1429) categories. Women, whose menopause was reported < 35 years of age and because of undetermined menopause status, were excluded. Surprisingly, postmenopausal women had significantly lower BMI but had a higher percentage of abdominal fat quantified by DXA between regions L2 - L4 compared to premenopausal women ($p < .05$). Also, significant increases in total cholesterol, LDL-C, HDL-C and triglyceride concentrations were observed in postmenopausal women, even after adjustment for age, compared to premenopausal women ($p < .05$). Interestingly, the age at menarche was taken into account and was seen to have an inverse relationship with body fatness, which was assessed by BMI, WC, total body fat percent and abdominal fat percent quantified by DXA. The menarche age range for the terms: early

menarche (8-13 yrs), intermediate age at menarche (14-16 yrs), and late menarche (17-19 yrs) were defined. Early menarche was significantly associated with an increase in TG and TC, and decreased HDL-C. In general, an earlier onset of menarche may be associated with unfavorable changes in risk factors for metabolic and cardiovascular diseases.

Williams, Hunter, Kekes-Szabo, Snyder and Treuth (1997) determined that trunk fat quantified by DXA was positively correlated with risk factors for CVD, whereas leg fat was negatively correlated in women independent of menopausal status and age. This indicates that trunk fat may put women at risk of developing CVD whereas leg fat does not. A limitation to this study was that trunk and leg fat were not defined, nor was the study done in men.

Faloia, Tirabassi, Canibus and Boscaro (2008) analyzed abdominal and leg fat, quantified by DXA, in 80 premenopausal women subdivided as severely obese (BMI > 40 kg/m^2) or obese (BMI < 40 kg/m^2). Abdominal fat (L2-L4) was positively correlated with triglycerides ($r = .33$, $p = .041$) in obese women whereas it was not significant with severely obese women. In addition, even though severely obese women had greater abdominal fat than obese women, there were no associations with any cardiovascular risk factors. However, leg fat was negatively correlated with TG ($r = -.30$, $p = .03$) and TC ($r = -.20$, $p = .045$) in severely obese women whereas it was not negatively significant in obese women. This shows that leg fat may play a protective role against cardiovascular

risk factors particularly in severely obese premenopausal women. However, this study does not state if leg fat itself is the protector.

Anthropometrics

Waist circumference has been widely used as an indicator of abdominal obesity and has been used in equations for predicting body fatness in the past several decades. Waist and hip circumferences do not necessarily represent the amount of fat at that region (Snijder et al., 2004). In addition, larger waist circumferences reflect increased higher trunk fat whereas the hip fat reflected fat and lean mass from the legs in men and women. Previous studies have shown that waist circumference is associated with an increased risk for CHD whereas measuring height, weight and hip circumference do not (Yang, Kuper & Weiderpass, 2008).

Increased waist circumference and small hip circumference have independent and often opposite effects on CVD risk factors (Seidell, Perusse, Despres, & Bouchard, 2001). With menopause, there is an increase in waist measurements which is suspected to cause a shift of fat deposition (Kanaley et al., 2003). Furthermore, Snijder et al. (2003) determined that larger waist circumference is associated with a higher risk of type 2 diabetes in both men and women, whereas a larger hip or thigh circumference is associated with a lower risk, independently of BMI, age and waist circumference. Particularly in women, large thigh and hip circumferences could reflect increased femoral and gluteal subcutaneous fat masses. These depots have relatively low rates of basal and

stimulated lipolysis which may protect the liver and muscle from the accumulation of FFA through uptake and storage (Snijder et al., 2003). A limitation to this study is that menopausal status was not considered. The study included men and women aged 50-75 years.

Another common assessment is BMI. The BMI method is an indirect assessment of fat and cannot distinguish fat mass from fat free mass, nor determine fat distribution (Neovius, Udden & Hemmingsson, 2007). There has previously been evidence to believe that body composition rather than BMI is related to cardiovascular disease risk (Segal et al., 1987). Therefore more emphasis on the assessment of body composition should be stressed.

Body Composition Techniques

Accurate assessments of body composition are important in individuals, as body fat is related to health. Factors affecting body composition include age, gender, diet and level of physical activity. Newer technology has been used for more detailed measures of body composition such as: DXA, computed tomography (CT) and magnetic resonance imaging (MRI). The application of CT and MRI for body composition assessments are limited because of expense. In addition, DXA exposes participants to minimal amounts of ionizing radiation in comparison to CT. Originally, DXA has been used to measure bone density and total body composition. Now, studies have used DXA to separate the body into regions of interest (ROI). Studies have referred to measuring abdominal body fat

as the fat mass located from the first to the fourth lumbar intervertebral disk (Paradisi et al., 1999; Rissanen et al., 1997; Svendsen & Hassager, 1998).

Svendsen, Haarbo, Hassager & Christiansen (1993) have reported that DXA is an accurate and precise method for measuring body composition. Seven pigs were measured by DXA in vivo and after postmortem homogenization, chemical analysis was determined. There were no significant mean differences using DXA and chemical fat extraction in assessing the pigs' body fat percent, fat mass and lean mass (-2.2 ± 1.0%, -1.7 ± 0.8 kg, 0.4 ± 1.2 kg, $p > .05$, respectively).

Glickman et al. (2004) reported that the DXA L1 – L4 region of interest technique (ROI) was correlated to CT for quantifying abdominal fat mass ($r = .967$, $p < .0001$). To test the reliability of the DXA technique, two separate experiments were conducted to measure changes in body composition at the L1-L4 area. Scans were performed before and after fat packets of porcine lard (~2.5 cm thick) were placed over the L1-L4 area. An increase of approximately 10% (0.5-1.0 kg) of fat was placed over the L1-L4 area. As expected, DXA estimates were significantly different for total tissue mass with and without added fat from porcine lard (7.38 ± 3.92 g vs 6.58 ± 3.69 g, $p < .0001$). To test for reliability, scans between regions L1-L4 with and without the added fat were compared but scans with the added fat were corrected by subtracting the known fat packet mass. There was no significant difference between the two estimates for fat mass in the L1-L4 region (2.81 ± 0.24 g vs 2.26 ± 3.69 g, $p < .0001$). Overall, the

DXA L1-L4 ROI proved to be both reliable and accurate in comparison with the CT method to determine abdominal obesity.

CHAPTER III

METHODS

Participants

Participant data was derived from two separate studies performed at Texas Woman's University; one study included premenopausal women and the other including postmenopausal women. Both data sets were combined to make this present study possible. Premenopausal data was derived from a study by Wooten (unpublished data, 2008) and was titled: *Acute Response Of Lipid And Lipoprotein Metabolism Following Aerobic Exercise In Women Who Are Obese.* Postmenopausal data was derived from Koh (unpublished data, 2008) and the study was titled: *The Effects Of Niacin And A Single Bout Of Exercise On Blood Lipid And Lipoprotein Profiles In Postmenopausal Women.*

Participants for this study included 32 women between the ages of 18-71 years. Women were further categorized according to their menstrual status and were sub-grouped as premenopausal or postmenopausal. The premenopausal group (n = 19) included women between the ages of 18-44 years and the postmenopausal group (n = 13) included women between the ages of 46-71 years. The study by Koh (2008) originally had 18 postmenopausal women, although 5 women were excluded for this study because they did not meet the obesity requirement. So to unify this study population to only obese women, 13

postmenopausal women were included in this study as opposed to 18 postmenopausal women. Recruitment of participants was conducted through advertisement in the Denton newspaper and flyers posted around the Texas Woman's University campus. All participants were non-smokers, sedentary, obese and were not taking any medications for the regulation of dyslipidemia, diabetes, hypertension, birth control, and/or weight-loss for six months prior to entry into the study. Sedentary was defined as no participation or minimal participation of two days or less per week for 20 min per session in aerobic exercise for six months prior to entry into the study. According to the National Cholesterol Education Program Adult Treatment Panel III, obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2 and or a waist circumference > 88 cm (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults & NCEP, 2001). The methods and procedures for this study were submitted to and approved by the Institutional Review Board of Texas Woman's University (Appendix A).

Anthropometrics

Body mass data was measured to the nearest 0.1 kg with a calibrated digital scale (Tanita Corp., Arlington Heights, IL) and height to the nearest 1 mm with a stadiometer (Perspective Enterprises, Kalamazoo, MI). Body mass index was calculated by dividing body weight in kg by height in meters squared. Waist circumference was measured to the nearest 1 mm with a tape measure placed immediately above the iliac crest in premenopausal women, as recommended by

the National Institutes of Health guidelines (National Institutes of Health & National Heart Lung and Blood Institute, 2000). Waist circumference was measured at the narrowest part of the abdominal region in postmenopausal women.

Dual Energy X-ray Absorptiometry

Dual energy X-ray absorptiometry is widely used for scanning the total body to determine total body fat percent, total tissue, total lean tissue, total fat tissue, and bone mineral content (Svendsen, Haarbo, Hassager & Christiansen, 1993). Total fat tissue is considered to be the sum of the fatty elements of all soft tissue and not just adipose tissue. Total lean tissue mass represents the sum of all fat-free soft tissue elements. Regions of interest can be determined for legs, arms, and trunk fat separately.

Only total body fat mass was established from the studies mentioned above and was measured in grams using a whole-body Lunar DPX-IQ DXA scanner (Lunar Radiation Corp., Madison, WI). Scans were conducted at the Institute for Women's Health at Texas Woman's University by a registered technician and the data was safely kept stored in a database. The DXA was calibrated prior to scans with a known calibration marker to ensure quality assurance. Participants were fully dressed and laid on a DXA table in the supine position with their arms separated from the trunk. Participants were instructed to remain still throughout the duration of the scanning procedure.

Additional DXA data was further established from the participants who participated in the above mentioned studies, which were retrieved from a saved database. Regions of interest (ROI) were additionally evaluated using the Lunar 4.7V smart scan, which allowed the operator to manually draw a quadrilateral box on a specific body region. Abdominal and hip fat mass were identified as specific regions of interest within the analysis program (Figure 4). The "abdominal fat" area was measured at the superior of the iliac crest and between T12-L1 (Heiss et al., 1995). The "hip fat" area was measured downwards from the greater trochanter so that the height of the area was equal to the height of the "abdominal fat" area.

Total and regional fat mass was estimated for four participants in the premenopausal group that did not fit within the DXA scanning area. This was done by positioning a central line in the scanning area through the midpoint of the body, splitting it into two equal halves. The side of the body that was completely included in the scanning area was used for calculation of total and regional body fat mass. The side of the body that was not completely included in the scan area was not used. Total and regional fat mass was calculated by multiplying the measured tissue mass by 2. Tataranni and Ravussin (1995) demonstrated that measuring half of the body can significantly predict whole body composition with minimal differences when using either side of the body.

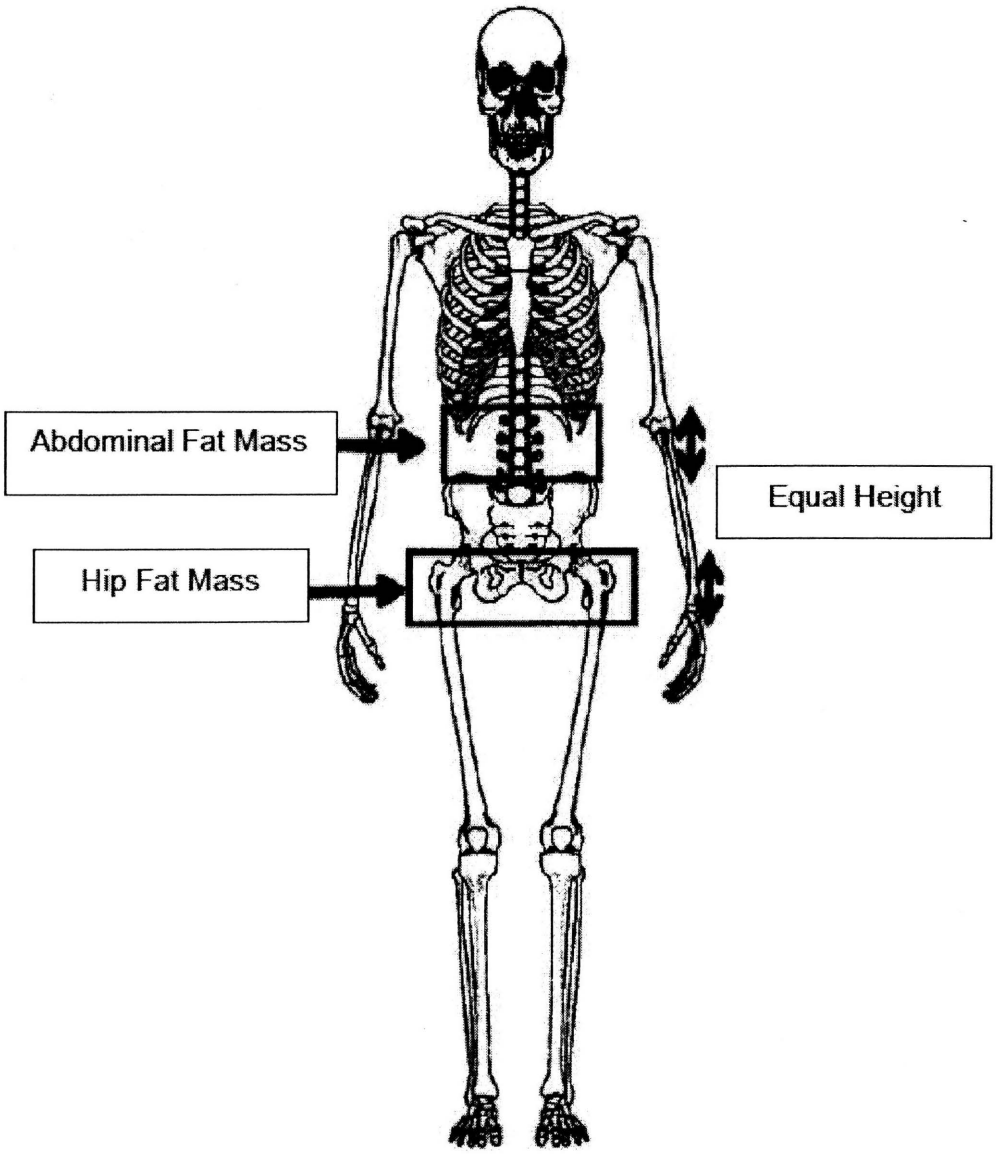

Figure 4. Regions of Interest Determined by DXA

Note. DXA= Dual energy x-ray absorptiometry. From " Abdominal and Gynoid Fat Mass are Associated with Cardiovascular Risk Factors in Men and Women," by P. Wiklund, 2008, 93, p. 4361. Copyright 2008 by The Endocrine Society. Adapted with permission of the author.

Blood Collection

Blood was collected from participants in a fasted (10 hr) state between the hours of 0600 and 0800. Participants were in a seated position for 20 min prior to venous blood collection. The amount of blood collected into vacutainer tubes was approximately 25 ml [1 X 7.0 ml and 2 X 9 ml] and 20 ml [1 X 5.0 ml and 2 X 7.5 ml] in premenopausal and postmenopausal women, respectively. Only baseline blood samples from both studies were used. All blood collection was performed by a trained phlebotomist. Aliquots of serum were stored at -70 °C until analyses.

Monitoring Menstrual Cycle

Prior to the experimental investigation, participants in the premenopausal subgroup kept a menstrual flow record and documented the beginning and ending dates of their menstrual cycle for two consecutive menses to provide accurate estimates of when the follicular phase of the menstrual cycle began. Based on these records, blood was collected 3-5 days after menses or during the early to mid-follicular phase of the menstrual cycle in each participant of this subgroup.

Bioanalyses

Established data was collected from the studies mentioned above, which both included concentrations of blood: TC, HDL-C, LDL-C, TG, insulin, and glucose. Blood serum was assayed in triplicates and duplicates in premenopausal and postmenopausal women, respectively. Blood serum was

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assayed for total cholesterol (TR13421, Thermo Fischer, Pittsburgh, PA; Kit# 85430, Raichem, Columbia, MD), triglyceride (TR22321, Thermo Fischer, Louisville, CO; Kit# 85460, Raichem, Columbia, MD), HDL-C (Kit#82051, Raichem, San Diego, CA; Kit# 82051, Raichem, Columbia, MD) and glucose (#80038, Raichem, San Diego, CA) concentrations using standard enzymatic techniques in premenopausal and postmenopausal women, respectively. All enzymatic measurements were performed using the PowerWave™ XS microplate spectrophotometer (BioTek instruments, Winooski, VT) and Infinite 200 microplate reader (Tecan, Austria).

The concentration of HDL-C was determined by the precipitation of VLDL and LDL from serum or plasma by dextran sulfate and magnesium and the remaining supernatant contained HDL that was used for the determination of HDL-C subfractions (#82051, Raichem, San Diego, CA; Kit# KP481, Wako, Richmond, VA) in premenopausal and postmenopausal women, respectively. The Friedewald's equation ([LDL-C]=[TC]-[HDL-C]-[Tg]/5) was used to approximate LDL-C concentrations, rather than measured directly (Friedewald, Levy & Fredrickson, 1972). The influence of VLDL associated cholesterol was adjusted with a correction term of Tg/5. Prior to statistical analysis, all lipid and lipoprotein concentrations were adjusted for changes in plasma volume. The insulin ELISA kit (#DSL-10-1600, Diagnostic Systems Laboratories, Webster, TX), the Columbus Pro™ Microplate washer (Tecan, Grödig, Austria) and the

Infinite M200 microplate reader (Tecan, Grödig, Austria) were used in the analysis of insulin concentrations.

Design/Analysis

All data were entered and analyzed with Statistical Package for the Social Sciences (SPSS, Chicago, IL, version 15.0) and were presented as means ± SD. Descriptive statistics were computed for the following variables: age, height, weight, menopause status, BMI, waist circumference, body fat percent, total body fat mass, abdominal fat mass, hip fat mass, abdominal to hip fat mass ratio, triglyceride, total cholesterol, LDL-C, HDL-C, HDL_2-C, HDL_3-C, insulin and glucose. A multiple regression analysis was used to determine correlations with abdominal fat, hip fat, and waist circumference (independent variables) and LDL-C, HDL-C, and insulin concentrations (dependent variables). A MANOVA was used to look for differences in pre and postmenopausal women (independent variable) and abdominal fat, hip fat, waist circumference, HDL-C, and LDL-C concentrations (dependent variables). Criterion reference for statistical significance was set at $p < .05$.

CHAPTER IV

RESULTS

Participant Characteristics

As presented in Table 2, the mean age of premenopausal and postmenopausal women was 29.5 ± 7.8 and 58.8 ± 8.0 years, respectively. Pre and postmenopausal women had a mean height of (165.3 ± 7.3 and 160.1 ± 6.9 cm) and mean weight of (95.4 ± 17.0 and 82.1 ± 13.5 kg), respectively. Pre and postmenopausal women had a mean BMI of (35.0 ± 5.9 and 31.9 ± 3.6 kg/m^2) and a mean waist circumference of (110.9 ± 12.5 and 91.8 ± 8.6 cm), respectively. Of the 32 participants who completed the study, 30 participants had a waist circumference greater than 88 cm, whereas 7 of the 32 participants had a BMI between 25 and 30 kg/m^2. Overall, all 32 women were considered obese as defined by the NCEP ATPIII as a BMI ≥ 30 kg/m^2 and or a waist circumference > 88 cm (NCEP ATP III, 2001).

In pre and postmenopausal women, the mean insulin concentration was (21.2 ± 17.3 and 20.9 ± 17.3 μIU/mL) and the mean glucose concentration was (104.6 ± 14.2 and 97.2 ± 12.7 mg/dL), respectively. Pre and postmenopausal women had average fasting insulin concentrations between 2.5 – 41 μIU/mL, which is considered a normal range. The definition of a normal fasting plasma glucose level has recently been revised by the Expert Committee on the

Diagnosis and Classification of Diabetes Mellitus, 2003, of the American Diabetes Association. Normal fasting glucose levels are < 100 mg/dL; IFG (impaired fasting glucose) levels range between 100 - 125 mg/dL; and diabetes is ≥ 126 mg/dL. Premenopausal women were considered to have IFG plasma glucose levels whereas postmenopausal women were within normal levels. A multivariate analysis of variance (MANOVA) was used to look for differences in glucose and insulin between participant groups; no statistical difference was found ($F = 1.1$; $p = .34$).

A MANOVA was also used to look for differences in waist circumference (WC) and weight, using the independent variable of, menopausal status. A significant difference in weight and WC was observed between groups ($F = 11.3$; $p < .001$). Although premenopausal women were heavier ($p < .05$) and had larger waist circumferences ($p < .001$) compared to postmenopausal women, there were no significant differences in BMI between groups.

Table 2

Characteristics of Premenopausal (n=19) and Postmenopausal (n=13) Women

Characteristic	Premenopause Mean ± S.D	Premenopause Min - Max	Postmenopause Mean ± S.D	Postmenopause Min - Max
Age (yr)	29.5 ± 7.8†	18.0 - 44.0	58.8 ± 8.0	46.0 - 71.0
Height (cm)	165.3 ± 7.3	151.4 - 178.6	160.1 ± 6.9	147.0 - 171.4
Weight (kg)	95.4 ± 17.0*	76.4 - 139.4	82.1 ± 13.5	66.8 - 115.4
BMI (kg/m^2)	35.0 ± 5.9	25.5 - 46.6	31.9 ± 3.6	26.2 - 39.3
WC (cm)	110.9 ± 12.5†	90.5 - 134.8	91.8 ± 8.6	74.0 - 106.9
Insulin (µIU/mL)	21.2 ± 17.3	1.4 - 72.0	20.9 ± 17.3	6.7 - 68.4
Glucose (mg/dL)	104.6 ± 14.2	76.0 - 141.2	97.2 ± 12.7	72.3 - 118.9

Note. BMI= body mass index; WC= waist circumference

* $p<.05$; † $p<.001$

Lipid and Lipoprotein-Cholesterol Characteristics

A MANOVA was used to look for differences in lipid and lipoprotein-cholesterol concentrations, using the independent variable of, menopausal status. As the multivariate F was significant ($F = 712.5$; $p < .001$) follow-up univariate ANOVAs were done; those results are presented in Table 3. As presented in Figure 4, postmenopausal women had significantly higher TC ($p < .001$) and LDL-C ($p < .001$) compared to premenopausal women. In pre and postmenopausal women, the mean TC concentration was (177.0 ± 31.4 and 224.8 ± 26.7 mg/dL) and the mean LDL-C concentration was (111.7 ± 26.8 and 163.6 ± 24.8 mg/dL), respectively. There were no significant differences in TG concentrations between groups. The mean TG concentration in pre and postmenopausal women was (100.0 ± 41.0 and 99.1 ± 40.6 mg/dL), respectively.

As presented in Figure 5, premenopausal women had significantly higher HDL_3-C ($p = .011$) than postmenopausal women. The mean HDL_3-C in pre and postmenopausal women was (41.4 ± 7.7 and 31.3 ± 4.8 mg/dL), respectively. There were no significant differences in HDL-C and HDL_2-C concentrations between groups. In pre and postmenopausal, the mean HDL-C concentration was (45.3 ± 4.6 and 35.7 ± 4.2 mg/dL) and HDL_2-C concentration was (9.6 ± 4.2 and 10.0 ± 4.9 mg/dL), respectively.

Table 3

Lipid and Lipoprotein-Cholesterol in Premenopausal (n=19) and Postmenopausal (n=13) Women

Metabolic Variables	Premenopause Mean ± S.D	Range	Postmenopause Mean ± S.D	Range
Cholesterol (mg/dL)	177.0 ± 31.4	121.5 - 235.8	224.8 ± 26.7*	174.9 - 263.2
Triglyceride (mg/dL)	100.0 ± 41.0	39.2 - 187.1	99.1 ± 40.6	61.1 - 204.5
LDL-C (mg/dL)	111.7 ± 26.8	73.6 - 161.7	163.6 ± 24.8*	116.5 - 191.2
HDL-C (mg/dL)	45.3 ± 4.6	36.2 - 53.2	41.4 ± 7.7†	29.1 - 54.7
HDL_2-C (mg/dL)	9.6 ± 4.2	3.3 - 18.4	10.0 ± 4.9	3.4 - 18.9
HDL_3-C (mg/dL)	35.7 ± 4.2	27.0 - 44.0	31.3 ± 4.8†	25.5 - 42.3

Note. LDL-C= low density lipoprotein cholesterol, HDL-C= high density lipoprotein cholesterol, HDL_2-C= high density lipoprotein subfraction 2, HDL_3-C = high density lipoprotein subfraction 3.
* = p<.001 ; † = p<.01

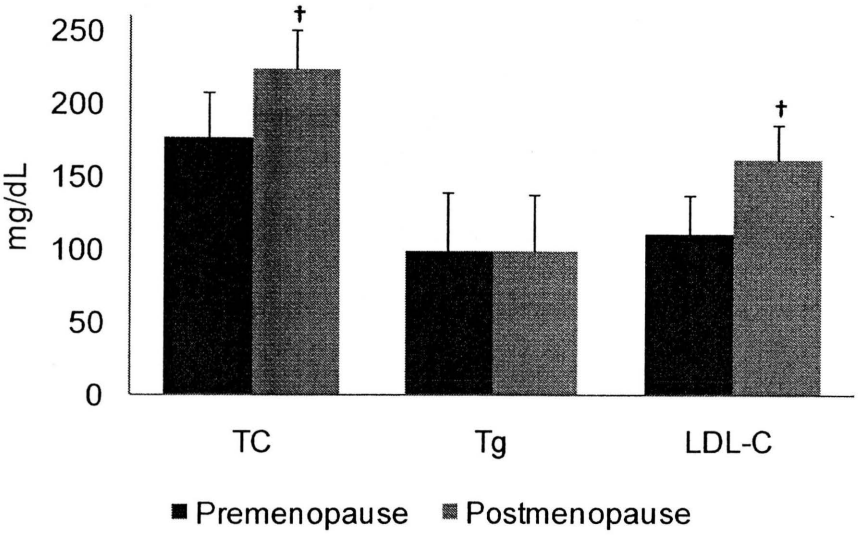

Figure 5. Lipid and Lipoprotein-Cholesterol in Premenopausal (n=19) and Postmenopausal (n=13) Women

Note. TC= Total cholesterol, Tg= triglyceride, LDL-C= low density lipoprotein cholesterol. Data are expressed as mean ± standard deviation. † $p < .001$ – values are significantly different than premenopausal women.

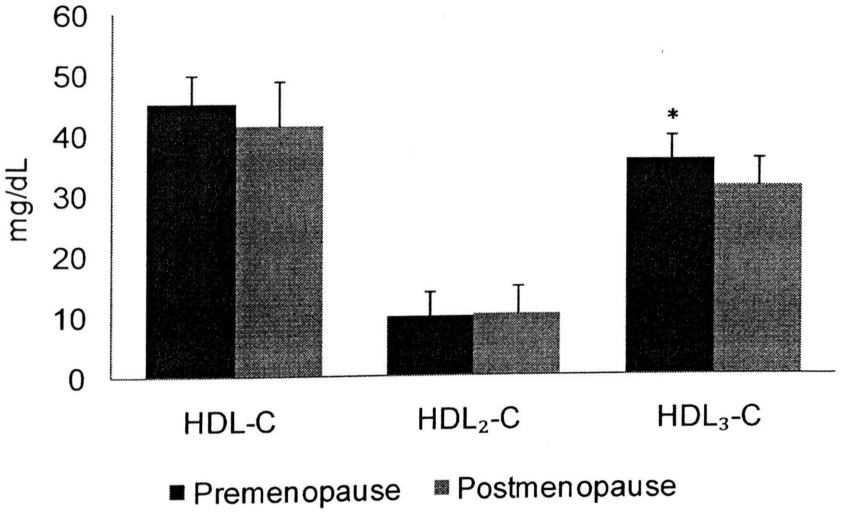

Figure 6. High Density Lipoprotein-Cholesterol in Premenopausal (n=19) and Postmenopausal (n=13) Women

Note. HDL-C= high density lipoprotein cholesterol, HDL2-C= high density lipoprotein subfraction 2, HDL3-C = high density lipoprotein subfraction 3. Data are expressed as mean ± standard deviation. * $p < .05$ – values are significantly different than postmenopausal women.

Body Fat Distribution Characteristics

A MANOVA was used to look for differences in body fat percent (BF%), total fat mass (TF), regions of interest of abdominal fat (AF) and hip fat (HF) mass, using the independent variable of, menopausal status. As the multivariate F was significant ($F = 3.6$; $p = .02$) follow-up univariate ANOVAs were done; those results are presented in Table 4.

The mean percent body fat for premenopausal and postmenopausal women was 49.9 ± 5.8 and 47.4 ± 6.2%, respectively. There was no significant difference ($p = .251$) in mean BF% between groups. There was a significant difference ($p = .006$) in mean TF (45,240.0 ± 10,922.1 and 34,697.0 ± 8,051.7g) for premenopausal and postmenopausal women, respectively.

As presented in Figure 6, premenopausal women had significantly higher AF ($p = .003$) and HF ($p = .011$) mass compared to postmenopausal women. The mean AF mass in pre and postmenopausal women was (4,370.4 ± 1,315.5 and 3,071.7 ± 726.2 g), respectively. The mean HF mass was (4,777.8 ± 1,159.6; 3,748.5 ± 887.7 g) in pre and postmenopausal women, respectively.

Table 4

Regions of Interest of Premenopausal (n=19) and Postmenopausal (n=13) Women

Characteristic	Premenopause Mean ± S.D	Range	Postmenopause Mean ± S.D	Range
Body Fat (%)	49.9 ± 5.8	39.2 - 58.4	47.4 ± 6.2	34.6 - 55.6
Abdominal Fat Mass (g)	4,370.4 ± 1,315.5*	2,091.0 - 7,722.0	3,071.7 ± 726.2	1,596.0 - 3,845.0
Hip Fat Mass (g)	4,777.8 ± 1,159.6*	3,155.0 - 7,624.0	3,748.5 ± 887.7	1,987.0 - 4,733.0

Note. Body Fat %, Total Fat Mass, Abdominal Fat Mass and Hip Fat Mass are measured by DXA
* = $p<.01$

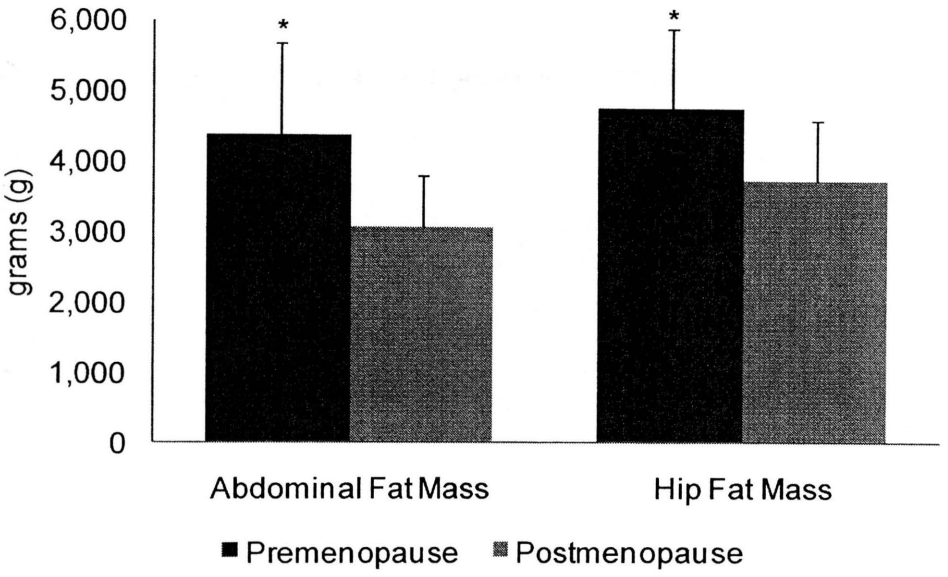

Figure 7. Regional Fat Mass of Premenopausal (n=19) and Postmenopausal (n=13) Women

Note. Abdominal Fat Mass and Hip Fat Mass were measured by DXA. Data are expressed as mean ± standard deviation. * $p < .05$ – values are significantly different than postmenopausal women.

Body Fat Distribution Associations with Lipid and Lipoprotein-Cholesterol

A multiple regression analysis was used to determine correlations with WC, regions of interest (total fat, TF; abdominal fat, AF and hip fat, HF) and lipid and lipoprotein-cholesterol, and insulin concentrations. There were no associations between body fat distributions and insulin, glucose, and HDL-C concentrations in obese women. There was a significant association of TF (β = 1.3; p =.02) and HF (β = -1.2; p =.01) with TG concentrations in obese women (see Table 5). As presented in Tables 6 and 7, WC is significantly associated with TC (β = -.765; p =.04) and LDL-C (β = -.817; p =.03) concentrations.

Table 5

Regression Analysis of Body Fat Distributions Associations with Tg Model in Obese Women (n = 32)

	Unstandardized Coefficients		Standardized Coefficients		
Tg Model	B	Std. Error	Beta	t	Sig.
WC (cm)	- .163	.925	- .059	- .177	.861
TF (g)	.005	.002	1.293	2.493	.019*
AF (g)	.009	.017	.285	.536	.596
HF (g)	- .043	.015	- 1.229	- 2.782	.010

Note. (R = .601) Regions of interest (total fat, TF; abdominal fat, AF; hip fat, HF) and waist circumference (WC) were put into test models to examine the interrelationship of varabilities of serum concentrations of (Tg).
* $p < .05$

Table 6

Regression Analysis of Body Fat Distributions Associations with TC Model in Obese Women (n = 32)

	Unstandardized Coefficients		Standardized Coefficients		
TC Model	B	Std. Error	Beta	t	Sig.
WC (cm)	- 1.989	.942	- .765	- 2.111	.044*
TF (g)	.000	.002	.515	.912	.370
AF (g)	.012	.017	.390	.675	.505
HF (g)	- .042	.016	- .606	- 1.260	.218

Note. (R = .494) Regions of interest (total fat, TF; abdominal fat, AF; hip fat, HF) and waist circumference (WC) were put into test models to examine the interrelationship of varabilities of serum concentrations of (TC)
* $p < .05$

Table 7

Regression Analysis of Body Fat Distributions Associations with LDL-C Model in Obese Women (n = 32)

LDL-C Model	Undstandardized Coefficients		Standardized Coefficients		Sig.
	B	Std. Error	Beta	t	
WC (cm)	- 2.054	.868	- .817	- 2.367	.025*
TF (g)	.001	.002	.419	.781	.442
AF (g)	.008	.016	.282	.513	.612
HF (g)	- .013	.014	- .427	- .932	.360

Note. (R = .561) Regions of interest (total fat, TF; abdominal fat, AF; hip fat, HF) and waist circumference (WC) were put into test models to examine the interrelationship of varabilities of serum concentrations of (LDL-C).

* $p < .05$

CHAPTER V

DISCUSSION

Statement of the Problem

The purpose of this study was to examine the relationship between total, abdominal, and hip fat mass and serum markers for CHD in premenopausal and postmenopausal obese women. Menopause has been referred to as a period when unfavorable lipoprotein changes occur as a consequence, therefore increasing the risk for CHD (Matthews et al., 1989). Studies have shown mixed results in determining an increased risk for CHD in relation to their body fat distribution after menopause (Gohlke-Barwolf, 2000; Rissanen et al., 1997; Van Pelt et al., 2002).

Responses of Total and Low Density Lipoprotein-Cholesterol Concentrations

The mean total cholesterol concentrations in pre and postmenopausal women were 177.0 ± 31.4 and 224.8 ± 26.7 mg/dL, respectively. Based on NCEP ATP III guidelines, postmenopausal women were borderline high (200 - 239 mg/dL), whereas premenopausal women had desirable total cholesterol concentrations (< 200 mg/dL). The mean LDL-C concentrations in pre and postmenopausal women were 111.7 ± 26.8 and 163.6 ± 24.8 mg/dL, respectively. Based on NCEP ATP III (2001) guidelines, postmenopausal

women had high (160 - 189 mg/dL), whereas premenopausal women had near optimal/above optimal (100 - 129 mg/dL) LDL-C concentrations.

In agreement with literature, postmenopausal women had significantly higher TC and LDL-C concentrations compared to premenopausal women (Brown et al., 1993). It is interesting to note that although premenopausal women were significantly heavier, TC was considered normal compared to postmenopausal women who were borderline high. It may be that menopausal status is an important factor for the changes seen in these lipoproteins. Elevated TC is known as hypercholesterolemia and is undoubtedly associated with an increased risk of CHD when there is an accumulation of cholesterol within an artery wall leading to the development of atherosclerotic plaque (Brown & Goldstein, 1986). It has been documented that TC and LDL-C tend to increase after menopause, and LDL-C accounts for most of the increase in TC (Mauriege et al., 2000). Although not measured in this study, this may be explained by higher hepatic lipase (HL) activity in postmenopausal women (Berg et al., 2001). A higher HL activity promotes more hydrolyzed Tg and phospholipid in LDL, leading to formation of smaller more atherogenic LDL particles. Smaller LDL particles have shown to increase in postmenopausal women compared to premenopausal women (Carr et al., 2000; Carr, 2003). Smaller and more dense LDL is associated with a 3 fold increased risk for CVD compared to women with large LDL (Austin et al., 1988). Other factors such as aging may also be contributing factors in the diminution of the removal of LDL-C from blood plasma,

resulting in increased LDL-C levels (Giribela, Melo, Latrilha, Baracat, & Maranhao, 2009).

Responses of High Density Lipoprotein-Cholesterol and Triglyceride Concentrations

The mean HDL-C concentrations in pre and postmenopausal women were 45.3 ± 4.6 and 41.4 ± 7.7 mg/dL, respectively. According to NCEP ATP III (2001), women in this study, on average, had normal HDL-C concentrations (40 - 59 mg/dL). The mean TG concentrations in pre and postmenopausal women were 100.0 ± 41.0 and 99.1 ± 40.6 mg/dL, respectively and were considered to have normal (< 150 mg/dL) levels (NCEP ATP III). In postmenopausal women, low plasma levels of HDL-C (< 40 mg/dL) and elevated triglycerides are important secondary targets to decrease CHD risk (Mosca et al., 2007). Low levels of HDL-C concentrations are normally observed in patients with elevated triglycerides, and reciprocally (Bainton et al., 1992; Grundy et al., 2005). The average participants in this study were normotriglyceridemic and may explain them having normal levels of HDL-C concentrations. Premenopausal women had significantly greater HDL_3-C concentrations compared to postmenopausal women. Greater HDL_3-C concentrations may be explained by the hydrolysis of Tg by LPL which promotes an increase in HDL and HDL_3- cholesterol concentrations, although LPL was not measured in this study.

Generally, decreases in plasma levels of HDL-C (≤ 10%) and elevated levels of TG are key discriminators of an elevated coronary risk in

postmenopausal women (Matthews et al., 1989; Shai et al., 2004). It is known that there is an inverse and independent association between HDL-C levels and CHD risk (Tabet & Rye, 2009). The effect of menopausal status on HDL metabolism is still controversial. Discrepancies among studies may be explained by other factors such as age, physical activity, and weight gain (Brown et al., 1993; Johnson, 1993). In women, HDL-C levels tend to increase progressively to the 6th decade, and then decrease, which tells us that age, may be a major factor (Brown; Johnson). It has also been considered that changes in distribution of HDL subclasses of smaller or larger particles are more closely related with atherosclerosis than low plasma levels of HDL-C (Jia et al., 2006). Although mean HDL particle size was not measured in this study, some studies have reported that small size HDL particles compared to large size HDL particles are associated with an increased risk for CHD (Jia et al.). Further investigation should be implemented to better understand the role of menopausal status on HDL metabolism.

In the present study no significant differences in baseline TG, and insulin concentrations were found between groups. Premenopausal women were considered to have impaired fasting glucose, whereas postmenopausal women were within normal levels. Some studies have shown increases in TG levels early in the postmenopausal period in women transitioning to menopause, while others have seen no changes (Franklin, Ploutz-Snyder, & Kanaley, 2009; Matthews, Kuller, Sutton-Tyrrell, & Chang, 2001). This may be related to the fact

that TG levels during menopause are correlated with an increase in abdominal fat (Carr, 2003). An accumulation of abdominal fat may result from an increase in LPL resulting from a deficiency in estrogen and leading to an increase in plasma free fatty acids (Saltiki & Alevizaki, 2007).

Body Fat Distribution

Several methods are used to assess adiposity in humans. Body fat distribution was measured by waist circumference (WC) and DXA. In this present study, DXA was used for 2 purposes: to determine the differences in total and regional fat mass between pre and postmenopausal obese women, and to investigate the relationship between total, abdominal, and hip fat mass and CHD risk factors in obese women. Although DXA does not differentiate between subcutaneous and visceral adipose tissue, studies have shown significant effects of menopause on body fat distribution using DXA (Feng et al., 2008; Paradisi et al., 1999; Rissanen et al., 1997).

Postmenopausal women tend to gain weight the first year of menopause and experience changes in body fat distribution, shifting from the hip to the abdomen (Feng et al., 2008; Rosano et al., 2007). Reuffe-Scrive et al. (1986) and Lindberg, Crona, Silverstolpe, Bjorntorp and Rebuffe-Scrive (1990) best explain this as postmenopausal women having lower LPL activity in the femoral adipose tissue compared to premenopausal women, whereas there is no differentiation in abdominal tissue LPL activity in premenopausal women.

Abdominal obesity is known to be associated with metabolic and cardiovascular complications (Bigaard et al., 2005; Hubert et al., 1983). As a result, the assessment of body fat accumulation in postmenopausal women is an important screening tool for the prevention of health complications. In the present study, premenopausal women had significantly greater WC, total, abdominal, and hip fat mass in comparison to postmenopausal women, although no significant differences were seen in BF percent between groups. Although all women in this study were considered obese, premenopausal women were significantly heavier than postmenopausal women (95.4 ± 17.0 and 82.1 ± 13.5 kg respectively). Therefore, the greater degree of obesity in premenopausal women may explain why there were no differences seen in body fat distribution between pre and postmenopausal women. Postmenopausal women have a shift in body fat distribution from the hip to the abdominal region after 12, 24, and 36 months of observation (Gambacciani et al., 2001). Taking into account the time since menopause may lead to a progressive increase in the central distribution of body fat following menopause. Another explanation could be due to the small population (n = 32) in this study. Lastly, the exploration of other indices for measuring body fat distribution, such as computed tomography (CT), should also be considered. Using CT to measure body fat distribution is not necessarily more precise than DXA but may be helpful in providing further examination of quantities of subcutaneous and visceral adipose tissue, which DXA does not measure.

Body Fat Distribution Associations with Lipid and Lipoprotein-Cholesterol Concentrations

Regional fat exhibits different influences on lipid metabolism, whereas menopause related central body fat accumulation potentially contributes to the increased incidence of CVD disease in postmenopausal women compared with premenopausal women (Gambacciani et al., 2001; Tanko et al., 2003). In this present study, total and regional fat mass were found to have associations with lipid and lipoprotein-cholesterol concentrations. Total and hip fat mass had the strongest associations with TG concentrations. Similar to other research, Rissanen et al. (1997) showed that lower lumbar fat (L4 - L5) and hip fat mass quantified by DXA significantly correlated with TG as opposed to hip fat mass alone in obese women. Other studies have demonstrated that abdominal fat (L2-L4) quantified by DXA, has significant correlations with TG in obese women (Faloia et al., 2008). Surprisingly, the results from this study indicate that there are no associations between abdominal fat mass and cardiovascular risk factors in this study of obese women. It could be that a bigger sample is needed to see such relationships. The present study also indicates that no body fat distribution variable had an effect on HDL-C concentration in obese women. In general, the concentration of HDL-C tends to be adversely altered in obesity and associated with abdominal body fat distribution (Despres & Lemieux, 2006). The null hypothesis was not rejected: abdominal fat mass, as measured by DXA, is not related to HLD-C or any other lipid and lipoprotein concentrations.

Our findings indicate that WC was strongly associated with TC and LDL-C concentrations. This means that using WC may be a good indicator of abdominal fat distribution as a marker of evaluating metabolic risk factors in obese women. These results are in agreement with previous research that observed that anthropometric measures such as WC are not inferior to DXA measurements when examining the relationship between abdominal fat distribution and metabolic risk factors (Rissanen et al., 1997). Other studies show WC to be closely linked to abdominal visceral adipose tissue deposition measured by CT (Despres, Prud'homme, Pouliot, Tremblay, & Bouchard, 1991). Discrepancies among studies may result from technical error as well as differences in participants and overall adiposity. A limitation to using DXA is that it may be difficult to measure regional fat in obese individuals due to the possibility of not being able to fit in the scan area and cause an overlap of limbs and the trunk (Park, Heymsfield, & Gallagher, 2002). In this study, 4 participants did not fit in the DXA scan area and total and regional body fat mass was estimated by the method of Tataranni and Ravussin (1995). For simple routine clinical practice, WC may be more useful as it is easier and less expensive method compared with DXA or CT for detecting metabolic risk factors.

Summary

In summary, menopausal status had an effect on concentrations of lipid and lipoprotein-cholesterol. Abnormal plasma lipids are an important modifiable risk factor for CVD in menopausal women (Rosano et al., 2007).

Postmenopausal women had significantly higher TC, LDL-C, and lower HDL_3-C concentrations compared to premenopausal women. Menopause itself, or unknown age related factors may contribute to these potentially adverse changes in lipids and lipoproteins and the emergence of the metabolic syndrome, which together increase CHD risk. Although estrogen was not measured in this study, it is possible that changes in lipid and lipoproteins seen in this study may be due to the detrimental effects of estrogen withdrawal. Obesity may also be another underlying factor for the altered lipid profile seen in this study. Total and regional fat mass were found to have associations with lipid and lipoprotein-cholesterol concentrations in obese women (n = 32). Knowing the effects of total and regional fat distribution on blood lipid changes associated with menopause may help in the recognition of women at risk for future cardiovascular disease.

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APPENDIX A

Institutional Review Board Approval Letter

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378 Fax 940-898-3416
e-mail: IRB@twu.edu

June 17, 2008

Ms. Melinda Villarreal
2500 N. I-35 Apt. 141
Denton, TX 76201

Dear Ms. Villarreal:

Re: Total, Abdominal, and Hip Fat Mass with Markers for CHD in Pre Menopausal and Post Menopausal Women

The above referenced study has been reviewed by the TWU Institutional Review Board (IRB) and was determined to be exempt from further review.

If applicable, agency approval letters must be submitted to the IRB upon receipt PRIOR to any data collection at that agency. Because you do not use a signed consent form in your study, the filing of signatures of participants with the TWU IRB is not required.

Another review by the IRB is required if your project changes in any way, and the IRB must be notified immediately regarding any adverse events. If you have any questions, feel free to call the TWU Institutional Review Board.

Sincerely,

Kim Grover-Haskin

Dr. Kim Grover-Haskin, Co-Chair
Institutional Review Board - Denton

cc. Dr. Charlotte Sanborn, Department of Kinesiology
Dr. David Nichols, Department of Kinesiology
Graduate School

APPENDIX B

Participant Characteristics: Raw Data

Descriptive Characteristics of the Participants

ID	Menopause Status	Age (y)	Ht (cm)	Wt (kg)	BMI (kg/m^2)	WC (cm)
1	PM	34	167.0	78.2	28.0	90.5
7	PM	18	173.2	101.7	33.9	109.4
8	PM	36	154.2	97.3	40.9	110.3
13	PM	32	165.3	76.4	28.0	97.1
15	PM	21	172.0	100.2	33.9	117.5
16	PM	20	178.6	81.2	25.5	102.1
17	PM	22	155.5	93.4	38.6	122.2
19	PM	44	161.7	83.5	31.9	105.5
20	PM	21	160.1	85.2	33.2	105.8
21	PM	28	151.4	85.6	37.3	115.1
22	PM	24	171.1	105.6	36.1	109.6
24	PM	31	167.7	107.7	38.3	114.5
25	PM	25	159.5	87.9	34.6	99.1
26	PM	32	164.7	91.3	33.7	112.6
27	PM	31	172.3	118.8	40.0	133.8
29	PM	29	160.9	77.7	30.0	104.7
31	PM	28	173.0	139.4	46.6	134.8
32	PM	43	170.0	81.1	28.1	94.0
33	PM	41	162.6	121.1	45.8	128.3
1	M	60	159.6	66.8	26.2	88.2
2	M	46	151.0	70.4	30.9	96.1
4	M	71	166.0	90.9	33.0	106.8
5	M	58	163.8	89.8	33.5	74.0
7	M	67	160.0	75.9	29.7	91.2
9	M	54	162.0	80.7	30.8	96.1
11	M	63	171.4	115.4	39.3	89.5
13	M	56	161.0	81.2	31.3	90.1
15	M	54	147.0	78.0	36.1	78.6
16	M	60	159.6	66.8	26.2	91.0
17	M	46	151.0	70.4	30.9	100.0
19	M	71	166.0	90.9	33.0	93.2
20	M	58	163.8	89.8	33.5	98.5

Note. ID = identification number, PM = premenopause, M = menopause, Ht = height, Wt = weight, BMI = body mass index, WC = waist circumference

APPENDIX C

Participant Blood Variables: Raw Data

Lipid and Lipoprotein Cholesterol Concentrations of the Participants

ID	Menopause Status	TG (mg/dL)	TC (mg/dL)	LDL-C (mg/dL)
1	PM	104.60	235.80	161.70
7	PM	122.50	216.40	140.10
8	PM	105.50	206.00	136.50
13	PM	114.70	179.70	112.10
15	PM	53.60	173.60	120.70
16	PM	58.30	121.50	73.60
17	PM	86.60	151.10	88.50
19	PM	89.70	220.90	151.30
20	PM	95.80	134.90	73.70
21	PM	79.40	195.80	131.90
22	PM	108.10	166.90	105.00
24	PM	54.60	177.20	118.20
25	PM	39.20	138.10	83.30
26	PM	146.70	212.10	140.10
27	PM	187.10	175.30	96.50
29	PM	77.30	185.30	121.10
31	PM	135.50	155.70	86.50
32	PM	63.20	153.70	92.80
33	PM	178.70	163.70	88.60
1	M	95.36	241.45	185.91
2	M	87.22	239.43	174.64
4	M	150.59	238.22	178.91
5	M	71.36	183.37	132.18
7	M	204.55	263.23	191.20
9	M	88.93	197.08	127.05
11	M	101.35	232.58	171.01
13	M	78.67	213.62	159.17
15	M	61.10	249.92	183.01
16	M	84.66	212.01	149.47
17	M	65.81	245.89	187.23
19	M	69.24	174.90	116.53
20	M	130.04	231.37	170.37

Note. ID = identification number, PM = premenopause, M = menopause, TG = triglyceride, TC = total cholesterol, LDL-C = low density lipoprotein cholesterol

High Density Lipoprotein Cholesterol Concentrations of the Participants

ID	Menopause Status	HDL-C (mg/dL)	HDL_2-C (mg/dL)	HDL_3-C (mg/dL)
1	PM	53.20	18.40	34.80
7	PM	51.80	18.20	33.60
8	PM	48.40	5.80	42.60
13	PM	44.70	7.80	36.90
15	PM	42.20	5.30	36.90
16	PM	36.20	5.70	30.50
17	PM	45.30	8.30	37.00
19	PM	51.70	7.70	44.00
20	PM	42.00	4.50	37.50
21	PM	48.00	8.80	39.20
22	PM	40.30	3.30	37.00
24	PM	48.10	10.70	37.40
25	PM	47.00	10.20	36.80
26	PM	42.70	13.30	29.40
27	PM	41.40	8.60	32.80
29	PM	48.70	14.90	33.80
31	PM	42.10	9.20	32.90
32	PM	48.30	9.50	38.80
33	PM	39.40	12.40	27.00
1	M	36.47	5.27	31.20
2	M	47.35	8.76	38.59
4	M	29.19	3.70	25.49
5	M	36.92	6.95	29.97
7	M	31.12	3.42	27.70
9	M	52.24	18.97	33.27
11	M	41.30	10.04	31.26
13	M	38.72	8.76	29.96
15	M	54.69	12.40	42.29
16	M	45.61	11.43	34.18
17	M	45.50	15.60	29.90
19	M	44.52	17.35	27.17
20	M	34.99	8.27	26.72

Note. ID = identification number, PM = premenopause, M = menopause, HDL-C = high density lipoprotein cholesterol, HDL_2-C= high density lipoprotein subfraction 2, HDL_3-C = high density lipoprotein subfraction 3

Glucose and Insulin Concentrations of the Participants

ID	Menopause Status	Glucose (mg/dL)	Insulin (μIU/mL)
1	PM	109.90	25.60
7	PM	115.40	26.81
8	PM	104.90	33.43
13	PM	99.93	5.88
15	PM	87.12	19.68
16	PM	76.00	1.42
17	PM	141.20	24.92
19	PM	108.90	5.42
20	PM	92.20	11.22
21	PM	103.40	26.64
22	PM	112.40	6.04
24	PM	119.80	43.20
25	PM	84.06	6.95
26	PM	104.80	15.00
27	PM	102.50	14.78
29	PM	99.27	20.28
31	PM	110.40	72.01
32	PM	104.10	4.58
33	PM	110.50	39.28
1	M	79.47	32.83
2	M	101.89	9.78
4	M	112.18	22.65
5	M	105.39	9.43
7	M	90.58	38.71
9	M	98.60	68.41
11	M	99.42	18.19
13	M	99.22	6.65
15	M	118.97	6.69
16	M	103.75	19.30
17	M	86.26	18.86
19	M	95.72	11.38
20	M	72.26	8.86

Note. ID = identification number, PM = premenopause, M = menopause

APPENDIX D

Participant Body Composition: Raw Data

Body Composition Characteristics of the Participants

ID	Menopause Status	BF (%)	TF (g)	AF (g)	HF (g)
1	PM	44.6	33424	3425	3838
7	PM	56.2	53873	4363	4442
8	PM	40.6	35632	3259	3646
13	PM	42.7	31322	2769	3155
15	PM	53.2	51113	4577	4963
16	PM	44.5	34128	3355	3996
17	PM	58.3	51193	4758	5515
19	PM	52.8	42165	4588	4671
20	PM	46.4	37584	3337	3727
21	PM	53.9	44103	4951	4804
22	PM	49.0	48988	4221	5048
24	PM	58.4	59214	5592	7624
25	PM	53.1	44538	4869	5400
26	PM	47.4	41204	4227	4471
27	PM	51.0	57960	6036	5440
29	PM	47.7	35399	3269	3788
31	PM	54.3	65370	7722	7032
32	PM	39.2	30491	2091	3667
33	PM	54.1	61878	5628	5552
1	M	50.1	31937	2578	3028
2	M	48.4	37304	3594	3878
4	M	46.4	40328	3390	3817
5	M	34.6	20174	1596	1987
7	M	51.5	43798	3616	4733
9	M	41.0	26609	2754	3012
11	M	43.4	31121	2904	3364
13	M	53.1	41942	3803	4669
15	M	40.1	21612	1804	2662
16	M	55.6	43238	3518	4663
17	M	46.2	32714	3048	3837
19	M	50.8	40190	3482	4424
20	M	54.4	40094	3845	4657

Note. ID = identification number, PM = premenopause, M = menopause, BF% = body fat percent, TF = total fat mass, AF = abdominal fat mass, HF = hip fat mass. Body composition was quantified by DXA.