Relationship of centrally-located body fat to appetitive hormones in healthy postmenopausal women

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Relationship of centrally-located body fat to appetitive hormones in healthy postmenopausal women

by

Laura Marie Ritland

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Nutritional Sciences

Program of Study Committee:
D. Lee Alekel, Major Professor
Manju Reddy
Kenneth Koehler

Iowa State University

Ames, Iowa

2008

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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control &amp; Prevention</td>
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<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<tr>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>17β-estradiol</td>
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<tr>
<td>ER</td>
<td>Estrogen receptor</td>
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<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
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<tr>
<td>GLUT-4</td>
<td>Glucose transporter</td>
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<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>Health ABC Study</td>
<td>Health, Aging, and Body Composition Study</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
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<td>HT</td>
<td>Hormone therapy</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>SERM</td>
<td>Selective estrogen receptor modulators</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-hip ratio</td>
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<td>WHO</td>
<td>World Health Organization</td>
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I would like to specially thank Dr. Manju Reddy and Dr. Kenneth Koehler for their advice and participation on my committee. Their expertise has been invaluable for the success of my thesis.

This thesis would not have been possible without my major professor, Dr. D. Lee Alekel. Her patience, guidance, support, and advice were endless and always seemed to come when most needed. I cannot thank her enough for the hours upon hours spent reviewing and discussing my project, manuscript, and thesis. Thank you for giving me the opportunity to work with SIRBL both as an undergraduate and graduate student. This experience has made me grow not only as a researcher but also as an individual.

I would also like to give a special thank you to Laura Hanson for her assistance, support, guidance, and friendship while working together the past four years. I would not be here if it wasn’t for her unending encouragement and advice about SIRBL, graduate school, and life. She made my experience with SIRBL very memorable.

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Finally, I would especially like to thank my mother for always being my biggest fan. Her everlasting support and advice have always pushed me through the toughest obstacles in my life. My mother’s love and guidance never stopped during my undergraduate and graduate careers and has always kept me focused. The love, support, and encouragement from the rest of my family and friends has been constant and endless. They have pushed me along my way and kept me smiling during my greatest obstacles.
Objective: Body composition and energy homeostasis are thought to affect the appetitive hormones: adiponectin, leptin, insulin, and ghrelin. This study examined whether centrally-located fat and/or overall adiposity were related to these appetitive hormones in healthy postmenopausal women.

Design: Overall and regional body composition was assessed by dual-energy X-ray absorptiometry in relation to plasma adiponectin, serum leptin, serum insulin, and plasma ghrelin in 242 postmenopausal women.

Results: Regression analyses revealed that the androidal-to-gynoidal fat mass ratio (18.0%), age (3.2%), and white blood cell count (1.8%) accounted for 28% of the variability in adiponectin ($F=22.2; P<0.0001$); androidal (waist + hip) fat mass (66.0%), $(\text{androidal fat mass})^2$ (6.2%), whole body lean mass (2.2%), and age (0.8%) accounted for 69% of the variability in leptin ($F=102.5; P<0.0001$). Regression analyses revealed that sagittal abdominal diameter (8.4%), glucose (5.4%), white blood cell count (2.6%), and dietary omega-3 fatty acids (2.0%) accounted for 32% of the variability in insulin ($F=20.8; P<0.0001$); waist circumference (12.7%), hip lean mass (2.0%), and white blood cell count (1.9%) accounted for 26% of the variability in ghrelin ($F=20.7; P<0.0001$). Our results indicated that centralized fat mass was the primary contributor to these appetitive hormones in healthy postmenopausal women.

Conclusion: Since central adiposity in postmenopausal women was related to appetitive hormones, minimizing weight gain during the menopausal transition may optimize appetitive hormones, thereby facilitating appetite control and weight maintenance.
GENERAL INTRODUCTION

Thesis Organization

This thesis begins with a general introduction describing the objectives, hypotheses, specific aims, limitations, and significance of this research project.

Objectives

The first objective is to identify at baseline key biologic factors (particularly central adiposity and thigh lean mass) and nutritional status indices related to adiponectin, leptin, insulin, and ghrelin (Figure 1). The second objective is to determine whether adiponectin, leptin, insulin, and ghrelin change during the course of one year. The third objective is to determine whether soy isoflavones affect central adiposity or thigh lean mass, as mediated by adiponectin, leptin, insulin, and ghrelin.

Hypotheses

1. At baseline, central adiposity (androidal fat mass) will be positively related to serum leptin and insulin, but inversely related to plasma adiponectin and ghrelin, whereas lean mass in the thigh region will be positively related to adiponectin, but inversely related to leptin, insulin, and ghrelin in 242 healthy postmenopausal women.

2. During the course of one year, we expect to document an increase in serum leptin and insulin, a decrease in plasma adiponectin, whereas no change in plasma ghrelin. Accordingly, we expect to document a gain in central adiposity, with a loss in thigh lean mass, among postmenopausal women who are not on active treatment.

3. Long-term intake of soy isoflavone extract will ameliorate the menopause-related increase in central adiposity and loss of thigh lean mass in healthy postmenopausal women.
   a. Adiponectin, leptin, and insulin will mediate the effect of soy isoflavones on central adiposity, with leptin and insulin stimulating the increase and adiponectin inhibiting the increase in fat deposition.
   b. Appetitive hormones will mediate effect of soy isoflavones to beneficially influence body composition.

Specific Aims

1. To determine androidal fat (waist + hip) and gynoidal lean (thigh) mass at baseline using dual-energy x-ray absorptiometry (DXA) and relate central adiposity to adiponectin, leptin, insulin, and ghrelin concentration in postmenopausal women.
2. To determine the change in central adiposity and lean mass from baseline to one year in postmenopausal women with respect to treatment and relate these changes to adiponectin, leptin, insulin, and ghrelin.

3. To examine the change, from baseline to one year, in central adiposity and thigh lean mass in postmenopausal women and determine whether change in body composition is influenced by soy isoflavone (80 or 120 mg/day) intake, as mediated by the hormones adiponectin, leptin, insulin, and/or ghrelin.

Limitations

The first limitation was that this ancillary cross-sectional study was hypothesis-generating and thus could not determine cause and effect. In addition, the participants in this study were healthy postmenopausal women, primarily of Caucasian descent. Thus, our results cannot be generalized to all women across ethnic groups. The third limitation was that UC-Davis enrolled eight women with a BMI > 29.9 and one woman with a BMI < 18.5, making the inclusion of protocol violators difficult to explain to reviewers. However, although these nine women did not meet our inclusion criteria, leading to greater variability in body weight, this may have allowed us to better examine the appetitive hormones in relation to central adiposity. The design of the overall Soy Isoflavones for Reducing Bone Loss (SIRBL) clinical trial was to determine the effect of two doses (80 and 120 μg) of soy isoflavones on bone, whereas this ancillary project examined the effect of isoflavones on central adiposity, taking adiponectin, leptin, insulin, and ghrelin into account. The fourth limitation of this study is that investigators will remain blinded until we have completed the intervention at both study sites in May 2008. Thus, the response-to-treatment statistical analyses will be completed this summer (2008) and this thesis does not include these analyses. Nevertheless, the manuscript in this thesis focuses on baseline data, irrespective of treatment. Thus, this thesis includes hypothesis and specific aim 1, but does not address the second and third hypotheses and specific aims, which will be the focus of a subsequent manuscript.

Significance

Obesity increases the risk of atherosclerotic cardiovascular disease (CVD), insulin resistance, type 2 diabetes, hypertension, stroke, and cancer, all of which increase the risk of premature death. The appetitive hormones adiponectin, leptin, insulin, and ghrelin have all been studied as circulating hormones related to obesity. The most intensively studied hormones released from adipose tissue, the adipocytokines
such as adiponectin and leptin, are involved in mediating the amount and frequency of food consumed. Evidence suggests that dysregulation of adipocytokine production promotes the development of the metabolic and vascular diseases related to obesity (Funahashi et al. 1999). Further, hormones produced by the stomach and pancreas, ghrelin and insulin, respectively, play a significant role in energy homeostasis.

As women transition through menopause they experience a rapid decline in ovarian function and a subsequent decline in circulating reproductive hormones, including estrogen. Menopause-induced hormonal changes result in an increase in overall adiposity (Tchernof et al. 2004), but especially visceral adiposity (Lovejoy et al. 2008). Postmenopausal women may experience an increase in adiposity in the central region that translates into a greater risk of obesity and subsequent chronic disease. Hormone therapy (HT) has been prescribed as a method to decrease women’s risk for chronic disease; however, the Women’s Health Initiative Trial demonstrated that HT may carry greater risks than benefits (Rossouw et al. 2002). Since this trial, HT use has declined (Ettinger et al. 2003) and women are searching for alternative therapies to relieve menopausal symptoms. Soy isoflavones, with their estrogen-like properties, are thought by some to be an ideal alternative to HT because of perceived health benefits (Messina 2002). However, we have sparse information on the effect of soy isoflavones on body composition in humans. Further, research on the effect of soy isoflavones on adiponectin, leptin, insulin, and ghrelin in postmenopausal women is limited and inconsistent. As this manuscript explains, central adiposity in postmenopausal women was related to appetitive hormones, despite the apparent health of the women enrolled in the study. Thus, minimizing weight gain during the menopausal transition may optimize appetitive hormones, thereby facilitating appetite control and weight maintenance.
Figure 1: Study design: Conceptual framework
REVIEW OF LITERATURE

OVERVIEW OF OBESITY

The prevalence of obesity in the United States (U.S.) has markedly increased in the past 25 years and needs urgent attention. Approximately 65% of U.S. adults are overweight or obese (Hedley et al. 2004). Obesity increases the risk of atherosclerotic CVD, insulin resistance, type 2 diabetes, hypertension, stroke, and cancer, all of which increase the risk of premature death. In 2000, approximately 365,000 deaths in the U.S. were associated with obesity (Mokdad et al. 2005). Further, the economic cost was more than $117 billion, with annual medical expenditures 36% higher for obese versus non-obese individuals (Sturm 2002). This disease is influenced by a variety of factors such as genetics, environment, socioeconomic status, and behavior (Rashid et al. 2003). One analogy for this chronic multifactorial disease is that genetics loads the gun but a permissive or toxic environment pulls the trigger (Bray & Champagne 2005). Obesity is attributed in part to the American lifestyle: physical inactivity, high consumption of energy-dense foods, large portion sizes, sleep deprivation, and continual high levels of stress related to fast-paced lifestyles. Currently, for most overweight individuals, there are no proven, effective approaches for meaningful and long-term weight loss (Hill & Peters 1998).

Definitions: Overall Adiposity, Androidal versus Gynoidal Adiposity

Obesity is a general term used to describe the overall accumulation of body fat. Overall adiposity is adipose tissue accumulated throughout the whole body (subcutaneously and internally), whereas regional fat distribution is the location of adipose tissue in specific regions, yet being very different across the life cycle, as well as between the sexes and among ethnic groups (Malina 1996). The location of body fat is critical in terms of risk of chronic disease, with central adiposity conferring greater health consequences. Typically, researchers classify individuals into two body types: those with either an androidal (“apple”) or a gynoidal (“pear”) shape. Androidal adiposity is characterized by adipose tissue deposited in the abdominal (trunkal or central) region, whereas gynoidal adiposity is characterized by adipose tissue deposited in the gluteofemoral (thigh) region (Malina 1996). The size of adipocytes varies, depending upon the weight and adiposity of the individual and upon the site of storage. For example, Garaulet et al. (2000) studied adipocytes of obese women undergoing abdominal surgery or laparoscopy and found those women with a gynoidal shape...
possessed significantly smaller and more numerous adipocytes than women with an androidal shape.

The terms fat and adipose tissue are used interchangeably; however, they have different meanings in the scientific community. Fat is a chemical component of the food system and is found in body compartments, such as adipose and muscle tissue, in the form of lipid or triacylglycerol (TAG). Adipose tissue is defined as specialized loose connective tissue composed of adipocytes (Shen et al. 2003). About 80% of adipose tissue is lipid, with the remaining 20% composed of water, protein, and minerals (Snyder et al. 1975). Adipose tissue is actively involved in energy storage of lipid, thermal insulation, provides a protective padding/cushioning around internal organs, and is an important endocrine organ that functions in the regulation of food intake.

**Implications for Disease Risk**

These two body shapes or types (androidal versus gynoidal) confer health implications for disease risk. Those individuals with androidal adiposity are at an increased risk of chronic diseases/conditions such as dyslipidemia, hypertension, atherosclerotic CVD, and diabetes. The reason for the difference between these two body types in disease risk is currently unknown, but it is thought to be related to the different types of adipose tissue. Further, visceral adipose tissue is more likely to release free fatty acids into the circulation because it is more sensitive to lipolytic stimuli (Snijder et al. 2006). However, other research suggests that these findings are inconsistent (Tchernof et al. 2006). This group studied healthy women and found that on an absolute basis, subcutaneous adipocytes located in the abdominal region are larger, have higher lipolysis rates per cell, and higher lipoprotein lipase activity compared with omental (deep visceral) adipose tissue. They suggested that larger subcutaneous adipocytes accumulate greater TAG and also release greater fatty acids than smaller adipocytes. Regardless, the released free fatty acids enter directly into the portal vein. Excess energy may be stored in the intra-abdominal adipose tissue, visceral organs (such as the liver), and/or skeletal muscle. However, excess fatty acids stored in the liver and skeletal muscle have metabolic consequences distinct from that of subcutaneous fat. Increased fatty acid storage in the liver causes dyslipidemia and hepatic insulin resistance (Seppala-Lindroos et al. 2002). Increased fatty acid storage in the skeletal muscle is associated with skeletal muscle insulin resistance (Sinha et al. 2002).
In contrast, gynoidal adiposity does not increase disease risk in the same manner as androidal obesity. In fact, some researchers consider gynoidal adiposity as protective against disease (Snijder et al. 2005), possibly due to the larger amount of muscle mass located in the thigh region or to the relatively large proportion of subcutaneous compared with internal fat located in the gluteofemoral (thigh) region. Also, it has been suggested that the thigh subcutaneous fat acts as a “metabolic sink” for circulating free fatty acids (Frayn 2002). Lipolytic activity in the thigh subcutaneous fat is different from that of abdominal subcutaneous fat due to high lipoprotein lipase activity in this region (Rebuffe-Scrive et al. 1985), especially in women. Thus, thigh subcutaneous fat is more likely to take up free fatty acids from and less likely to release free fatty acids to the circulation (Snijder et al. 2006). Further, Bos et al. (2005) found that for women, larger thigh fat mass was associated with higher lipoprotein lipase activity. Recent evidence supports this hypothesis by comparing lower and upper body subcutaneous adipose tissue in women. The women with greater adiposity, as assessed by DXA, stored greater free fatty acid in the lower body subcutaneous adipocytes versus the upper body subcutaneous or visceral adipocytes, as reflected by a free fatty acid tracer (Koutsari et al. 2008). Thus, the gynoidal body shape may protect other organs from ectopic fat exposure and accumulation, leading to a lower risk of insulin resistance.

**Adipose Tissue Compartments**

**Subcutaneous and intra-abdominal adipose tissue**

Adipose tissue is compartmentalized into specific regional depots, with each compartment serving as a storage location that carries specific disease risk. Adipose tissue storage is divided into two compartments, subcutaneous adipose tissue (fat accumulated under the skin) and intra-abdominal adipose tissue (visceral or fat accumulated around the organs). The literature is vague in describing these two compartments because they are much more complex than one might initially think. Shen et al. (2003) identified specific sub-compartments of subcutaneous and intra-abdominal (visceral) adipose tissue storage (Table 1). In the lower trunk and gluteal-thigh region, subcutaneous adipose tissue is divided by the fascial plane into superficial and deep subcutaneous adipose tissue (Figure 2). Superficial subcutaneous adipose tissue is the layer between the skin and the fascial plane in the gluteal-thigh area, whereas the deep subcutaneous adipose tissue layer is found between the muscle fascia and the fascial plane in the gluteal-thigh area. The deep subcutaneous adipose tissue is located in the
posterior half of the abdomen, whereas the superficial subcutaneous adipose tissue is evenly distributed around the abdomen. Shen et al. (2003) classify the intra-abdominal adipose tissue as internal adipose tissue, which is the total body adipose tissue minus the subcutaneous adipose tissue. Internal adipose tissue is further divided into visceral, or the adipose tissue within the chest, abdomen, and pelvis; and nonvisceral, the internal adipose tissue minus visceral adipose tissue. The visceral adipose tissue compartment is further divided into intra-thoracic and intra-abdominopelvic. Within these compartments are sub-compartments: intra-pericardial (e.g., omental and mesenteric) and extra-pericardial, as well as intra-peritoneal and extra-peritoneal adipose tissue.

<table>
<thead>
<tr>
<th>Adipose Tissue Compartment</th>
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<tbody>
<tr>
<td>Subcutaneous adipose tissue</td>
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<tr>
<td>Superficial subcutaneous adipose tissue</td>
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<td>Deep subcutaneous adipose tissue</td>
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<tr>
<td>Internal adipose tissue</td>
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<td>Visceral adipose tissue</td>
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<td>Intraperitoneal</td>
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<td>Extrapitoneal</td>
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**Table 1**: Proposed classification of adipose tissue (Adapted from Shen et al. 2003)
Figure 2: Abdominal axial computed tomography scan. White arrows indicate location of subcutaneous adipose tissue division into superficial and deep subcutaneous adipose tissue by a fascial plane.

Lean Tissue

The largest component of the body is lean tissue, comprised of skeletal muscle, smooth muscle, and bone. Lean tissue mass, according to Roche (1996), is the whole body minus the adipose tissue that is visible to the eye. Muscle, especially skeletal muscle, plays an important role in body weight homeostasis. Typically when we consider skeletal muscle, we think about its role in strength, but it also plays a major role in carbohydrate and lipid storage. Skeletal muscle is the major site for insulin-stimulated glucose disposal (DeFronzo et al. 1981). Lipid droplets, stored as TAG, accumulate within skeletal muscle and have been shown to be positively related to insulin resistance in European and South Asian men (Forouhi et al. 1999). Skeletal muscle, the largest component of lean tissue, is primarily located in the legs, with lesser amounts in the trunk, arms, and head (Lukaski 1996). Although lean tissue is typically not considered in obesity, it is important to assess because it is metabolically active, storing and releasing compounds related to metabolism. However, research relating lean tissue to obesity is
limited. It is difficult to measure lean tissue because it is located internally, yet surrounded by adipose tissue. Further, increased lean mass and muscle strength, particularly localized in the legs, has been associated with higher fat mass (Lebrun et al. 2006). This finding is most likely due to the greater overall mass that must be supported by the legs.

Lean tissue is commonly assessed by anthropometric measures, muscle metabolites, and/or radiologic measures. Although anthropometry is more commonly used to assess fat mass, lean mass may be assessed using circumference measurements of the arms or legs. Two of the most common muscle metabolites to assess lean mass are creatinine and 3-methylhistidine (Ballard & Tomas 1983). However, muscle metabolites are not commonly used because they require a 24-hour urine collection and 3-methylhistidine is technically difficult to accurately measure. Radiologic measures, such as computed tomography or DXA, are the most useful methods to assess lean tissue because they provide a direct visualization of body composition. However, radiation exposure may be a concern with these methods, particularly because computed tomography requires high doses of radiation. In addition, this method is most valuable to assess centrally-located fat, rather than whole body lean mass.

Methodology to Assess Regional Fat and Lean Tissue Distribution

Overall obesity may be assessed by calculating body mass index (BMI), but this is very crude. There are many anthropometric measures used to assess body fat distribution, particularly centrally-located fat, such as waist, abdominal, and hip circumferences; waist-to-hip ratio (WHR); sagittal abdominal diameter; and skinfold thickness (subscapular, waist, abdomen). Researchers debate about which method to use under what circumstances, since not all methods reflect internal fat, the metabolically active fat that is closely related to disease risk. Inconsistency among studies about which method is the most precise, reliable, and sensitive to change may be due to differences in sample characteristics and in measurement methods.

Anthropometric measurements

**Body mass index:** A commonly used but crude measure to assess relative weight is BMI (kg/m²), allowing a consistent classification system across study samples. The World Health Organization (WHO) (2004) classified individuals within the following BMI ranges: 18.5 to 24.9 kg/m² – normal or lean; 25.0 to 29.9 kg/m² – overweight; >29.9
kg/m\(^2\) – obese. Research has demonstrated a direct relation between BMI and risk of diabetes (Colditz et al. 1995). Individuals with a BMI of 25 or greater are at a higher risk for obesity-related adverse health outcomes than those with a BMI less than 25. Although BMI is widely used, there are many limitations to using BMI as a tool to quantify obesity or adiposity. Some individuals carry a disproportionate amount of lean mass, such as football players, and consequently may be considered overweight or obese if based on BMI when in fact their percent body fat may be quite low. Also, BMI may not be a good indicator of obesity across the races (Evans et al. 2006), since it has been shown to inaccurately indicate obesity in black but not white women. Although the black women had a higher (\(p < 0.05\)) BMI than the white women, percent body fat (based on DXA) was not different. These researchers also found that the BMI cut-off of 30 kg/m\(^2\) was too high to accurately classify obese versus nonobese black and white postmenopausal women. Further, the BMI cut-point for obesity that was equivalent to 38 percent body fat (recommended by Lohman et al. 1997 for middle-aged woman) was different in black versus white women. Lastly, BMI does not accurately estimate abdominal fat, which correlates most closely with chronic disease risk. Many studies indicate that it is more critical to assess abdominal fat than overall adiposity in assessing chronic disease risk.

**Body circumferences (waist, hip, thigh):** Waist circumference is an anthropometric girth measure around the smallest part of the waist, typically taken to accurately assess abdominal adiposity. Men and women are at an increased cardiometabolic risk with a waist circumference greater than 102 cm (40 inches) and 88 cm (35 inches), respectively. Recently, a consensus statement has been published from a number of health-related organizations (*Shaping America’s Health*: Association for Weight Management and Obesity Prevention; North American Association for the Study of Obesity, The Obesity Society; The American Society for Nutrition; The American Diabetes Association), suggesting that waist circumference be adopted as the best method of assessing adiposity in a clinical setting. This panel, organized by the National Heart, Lung, and Blood Institute, recommended using waist circumference because, when compared with BMI, it better predicts intra-abdominal adipose tissue (Klein et al. 2007). Since increased adiposity is associated with elevated CVD risk, the expert panel suggested using waist circumference as a clinical tool to assess the risk of developing elevated blood pressure, dyslipidemia, and hyperglycemia. Studies support the idea that
waist circumference is a key predictor of diabetes, CVD, and mortality, independent of blood pressure, glucose concentration, and lipoproteins (Yusuf et al. 2005; Wang et al. 2005). Although waist circumference cannot quantify the amount of abdominal adipose tissue, it is a useful method to assess abdominal adiposity and hence cardiometabolic risk. Interestingly, Wang et al. (2005) have also found that waist circumference was a better measure than BMI or WHR in predicting type 2 diabetes among 27,270 men. In fact, Moreira et al. (2007) found that waist circumference was a better measure than BMI or WHR for obese women to assess the prevalence of mood disorders and depressive symptoms. Although this assessment method is relatively easy, a definitive standard procedure for measurement is still needed because researchers define “waist” differently. Parikh et al. (2007) suggested that height be taken into consideration when measuring waist circumference to assess central adiposity. Their rationale is because individuals within the same group who have identical waist circumference measurements, but different heights, have dissimilar risk for chronic disease. These researchers define an index of central obesity as the ratio of waist circumference and height and suggest that in the U.S., the index of central obesity cutoff for men and women should be 0.58 and 0.54, respectively.

Another commonly used anthropometric index to assess body fat distribution is a combination of waist and hip circumferences expressed as WHR, with hip measured around the widest girth. The WHR is calculated using the waist circumference divided by the hip circumference. Both WHR and waist circumference are related to visceral adipose tissue; however, waist circumference is more strongly associated with visceral adipose tissue (Pouliot et al. 1994; Onat et al. 2004). Lemieux et al. (1996) suggested that a WHR of 0.94 for men and 0.88 for women relate to an accumulation of adipose tissue. However, recent research has determined that the 0.88 cut-off does not accurately predict CVD risk for pre- and postmenopausal women alike. Azizi et al. (2005) found that a WHR of ≥ 0.78 for premenopausal and ≥ 0.84 for postmenopausal women more accurately assesses the risk of CVD because of the increase in fat mass after menopause. However, more recent research, contrary to reports from the 1990s and earlier, has suggested that WHR is not an adequate measure to assess body fat distribution. In fact, the American Heart Association no longer recommends using this index to assess adiposity, stating that this method is not as suitable as BMI or waist circumference (americanheart.org, 2007). According to Gambacciani et al. (1997), the
WHR method may underestimate abdominal fat in obese individuals. Nevertheless, some researchers (Seidell et al. 2001; Okura et al. 2004; Heitmann et al. 2004) have found that WHR is inversely related to dyslipidemia, diabetes, hypertension, CVD, and death, suggesting that it relates more closely with central adiposity than overall. However, the WHR, because it includes the hip circumference, typically measured at the widest girth at the point of the greater trochanter, reflects muscle and fat mass but does not include the abdominal region. Hip circumference is a combination of fat and lean mass and thus is associated with subcutaneous fat, gluteal muscle, and total leg muscle mass (Seidell et al. 2001). Thus, the purported cardioprotective association of hip circumference may be related to the muscle mass that is included in this measurement. According to a meta-regression analysis of 15 prospective studies, a 0.01 increase in WHR was associated with a 5% increase in CVD risk (de Koning et al. 2007).

Thigh circumference is not as commonly assessed as waist or hip circumference. Thigh circumference is an indicator of both muscle and subcutaneous adipose tissue and is measured around one thigh, typically at the mid-thigh between the inguinal crease and the proximal border of the patella (Snijder et al. 2005). Snijder et al. (2003) found that both the waist-to-thigh circumference ratio and WHR were better predictors of type 2 diabetes risk than either waist circumference or BMI alone in a prospective study of 1357 (619 men and 738 women) French Canadian subjects. In fact, a larger thigh circumference was associated with a lower risk of diabetes, although this protective effect was only statistically significant in women. Another study by Snijder et al. (2005) called the Health, Aging, and Body Composition Study (Health ABC Study) found that high subcutaneous thigh fat was independently associated with more favorable glucose and lipid concentrations. Van Pelt et al. (2005) found that thigh fat mass was associated with a reduced risk of CVD, supporting the idea that a gynoidal body shape is considered protective against disease risk. This may be because the storage of fat is subcutaneous, away from the organs, instead of storage in the visceral region.

**Sagittal abdominal diameter:** Another method to assess abdominal adiposity is sagittal abdominal diameter that is taken in the supine position with knees bent using a portable, sliding-beam, abdominal caliper. It measures the anterior to posterior tissue thickness of the abdomen, midway between the iliac crests at the L4-L5 level (Kahn et al. 1996). Researchers (Riserus et al. 2004; Petersson et al. 2007) have shown that sagittal abdominal diameter better reflects obesity-related metabolic disturbances such
as CVD and inflammation than BMI, waist circumference, or WHR. Sagittal abdominal diameter is probably an excellent indicator of CVD risk because it is strongly associated with visceral fat (Zamboni et al. 1998), because abdominal subcutaneous fat shifts to the sides of the waist as the measurement is taken in the supine position (Mukuddem-Petersen et al. 2006). These researchers found that in an elderly sample, sagittal abdominal diameter was not more advantageous than BMI, waist circumference, or WHR measurements. Although this method of assessing abdominal adiposity needs further research to determine its validity across various subject samples, it holds promise because it has very high inter- and intra-observer precision (Zamboni et al. 1998) and does not require radiation or sophisticated equipment.

**Radiographic measurements**

Quantitative computed tomography; dual-energy x-ray absorptiometry: Currently, there are two main radiographic techniques for assessing regional fat and lean tissue distribution, computed tomography and DXA. The most accurate indirect method for assessing abdominal adipose tissue, particularly visceral adipose tissue, is computed tomography (Despres et al. 1996). This method is considered the reference “gold standard”, but use of this machine for research is limited because of expense, access to the scanner, and exposure of subjects to significant amounts of radiation (Plourde 1997). Thus, DXA is more commonly used in clinical practice because it provides a reliable estimate of body composition with minimal radiation exposure (Park et al. 2002). The DXA instrument is typically used to assess hard tissue (bone); however, DXA can also be used to assess soft tissue (lean and fat tissue). Although computed tomography is considered the gold standard, DXA has been shown to provide accurate and reliable estimates of overall body composition (Glickman et al. 2004). However, DXA cannot differentiate between subcutaneous and visceral adipose tissue because measurements are only two-dimensional. Although these fat depots cannot be determined directly using DXA, the soft tissue from the DXA scans are often used to estimate visceral adipose tissue by dividing the whole body scan into regions (Snijder et al. 2002). In addition, the investigator can partition DXA scans into particular regions of interest that relate significantly to visceral fat (Park et al. 2002; Glickman et al. 2004). Park et al. (2002) compared four centralized regions of interest from DXA scans: 1) the upper edge of the second lumbar vertebra to the lower edge of the fourth lumbar vertebra; 2) the upper edge of the second lumbar vertebra to above the iliac crest; 3) the lower costal margin to
above the iliac crest; and 4) the region of interest 3 excluding the spine. They found that
the fat tissue was related to total visceral adipose tissue and was similar to an actual
measure of visceral adipose tissue from magnetic resonance imaging. Glickman et al.
(2004) compared DXA and computed tomography scans, defining the abdominal region
as fat mass located between the first and fourth lumbar vertebrae (L1 and L4). According
to this research group, DXA provided a valid method to determine abdominal adiposity in
comparison with computed tomography and performed as well as computed tomography
with respect to reproducibility.

RELATIONSHIP OF OBESITY WITH KEY FACTORS
Physical Activity

Studies are underway to assess the current activity profiles and level of physical
activity among Americans. However, researchers debate about how to best assess
physical activity such that energy expenditure is accurately captured. It is difficult to
assess physical activity because it is a complex, multidimensional behavior (Wareham
2007). For example, individuals participate, consciously or unconsciously, in a variety of
physical activities throughout the day, including activities of daily living at home,
occupation-related activities, physical activity related to transport, and recreational
exercise. Thus, assessing physical activity in free-living situations is difficult and hence
researchers most often rely upon self-reported data. Physical activity assessment needs
to consider the type of activity, intensity, frequency, and duration to accurately estimate
total energy expenditure.

Regular physical activity, fitness, and exercise are critically important for the
health and well being of individuals across the life cycle. The Department of Health and
Human Services and United States Department of Agriculture jointly released in 2005
In addition to the dietary recommendations, these guidelines also recommended exercise
to encourage Americans to participate in an active lifestyle. These experts suggested
engaging in at least 30 minutes of moderate intensity physical activity on most days of
the week for weight maintenance and 60 minutes of moderate intensity activity on most
days to sustain weight loss. Incorporating physical activity into everyday life has well-
Activity and Health (1996), the benefits of physical activity include: lowered mortality,
decreased risk of CVD and some cancers, enhanced insulin sensitivity and subsequent reduced risk of developing type 2 diabetes, reduced risk of osteoporosis, greater muscle mass, and lesser fat mass, thus lowering the risk of associated comorbidities. However, the prevalence of obesity in the U.S. continues to rise, which simultaneously has coincided with decreased levels of physical activity. Research has also shown that increasing physical activity is a key element in treating obesity and in preventing weight regain in those who have successfully lost weight (Wareham 2007). Further, low levels of physical activity are associated with a high risk of CVD and mortality (Blair et al. 1989; Talbot et al. 2007). The prevalence of physical inactivity progressively increases with age (Talbot et al. 2007); approximately 30% of postmenopausal women report that they do not engage in physical activity (Centers for Disease Control 2005). In fact, during the postmenopausal years, fitness levels decline 1 to 2% per year (Fleg et al. 2005).

People who exercise typically have less abdominal fat than those who do not exercise (Lofgren et al. 2004). In fact, this study found that those individuals with a smaller waist circumference recorded more daily steps (as captured by pedometers) than those with a larger waist circumference. Similarly, Ross et al. (2003) found that subjects with higher levels of cardiorespiratory fitness had a lower risk of developing chronic diseases due to less abdominal fat. Further, exercise in women may affect various adipose tissue stores differently than in men. Women who exercise have markedly increased lipolysis in abdominal compared with femoral (thigh) subcutaneous adipose tissue (Horowitz et al. 2000). This suggests that exercise-induced weight loss would be associated with a preferential reduction in abdominal adiposity, although this was not tested directly. In a study of premenopausal obese women, substantial reductions in abdominal (10%) and visceral (18%) fat were noted in the women who exercised but who did not lose weight (Ross et al. 2004) compared with women who attempted only dietary-induced weight loss. Physical activity may also reduce appetite and fat intake (Saris 1999), thus reducing the amount of energy consumed. These studies combined illustrate the importance of exercise and cardiorespiratory fitness in weight loss and weight maintenance. However, substantial energy deficit through exercise alone is very difficult to achieve, demonstrating the importance of the combination of exercise and dietary change.
Dietary Intake

Although genetics play a role in weight gain, it cannot independently explain the dramatic increase in the development of obesity during the past several decades. In the U.S., diets high in fat and energy have paralleled the increase in the prevalence of obesity as an epidemic. The consumption of food involves many aspects of life, including social, cultural, and economic factors that determine food intake behavior. The prevailing thought is that the typical Western diet promotes excess energy intake and a positive energy balance, with an average of 37-42% of energy derived from fat (Uauy & Diaz 2005). Nutritional epidemiologic research typically assesses single nutrients to examine association with disease risk; however, this approach may not account for nutrient interactions in vivo. More recently, a dietary pattern approach has been studied, which uses cluster analysis to examine the effect of overall diet on disease risk, thereby allowing more conclusive results related to diet and disease (Newby & Tucker 2004; Okubo et al. 2007).

Energy

Obesity is often caused by excess energy intake over time (McCrory et al. 2000) relative to energy expenditure. Typical meals in the U.S. promote positive energy balance because of large portion sizes, consumption of energy dense convenience foods, lack of nutrient dense foods, and eating away from home, all of which contribute to our obesity epidemic. Per capita energy intake in the U.S. food supply has increased steadily in the past 40 years, as demonstrated by the figure below (Gerrior et al. 2004). According to the Nutrient Content of the U.S. Food Supply (1909-2000) report from the Home Economics Research Report No. 56 by the U.S. Department of Agriculture (USDA), ~3,100 kcal/day were consumed in 1965, whereas more recently (2000), energy intake has risen to ~3,900 kcal/day, based upon the available food supply. Further, data from the Centers for Disease Control & Prevention (CDC 2004) indicated that, between 1971 and 2000, men and women reported a combined increase in daily energy intake of 168 and 335 kcal, respectively, based upon actual estimated intake.

An increase in daily energy intake can translate into weight gain and increase the risk for disease. Findings from Howarth et al. (2006) suggest that an energy dense diet is a risk factor for elevated BMI in both men and women across ethnic groups. According to Howarth et al. (2007), total and snack and mealtime energy intake was positively associated with BMI in both younger (average = 38.5 years) and older (average = 71.0
years) adults. Although overweight/obese adults and their normal weight counterparts do not necessarily differ significantly in the amount of energy consumed, they do differ in the composition of their diet derived from fat and carbohydrate (Davis et al. 2006), suggesting that diet composition plays a vital role in promoting or preventing obesity. However, the role of diet composition in weight control and obesity remains controversial.

![Figure 3: Daily per capita food energy in the U.S. food supply (1909-2000)](image)

**Carbohydrate**

In 2004, the CDC reported that since 1970, Americans have increased their carbohydrate consumption by 60-70 g/day. Carbohydrate quality and quantity has been the subject of discussion among researchers for many years. Current recommendations emphasize the intake of complex, fiber-rich carbohydrate with a low glycemic index, whereas high-glycemic refined carbohydrate foods should be limited (USDA 2007). Gross et al. (2004) suggested that diets with a high glycemic index (glycemic load) increase the risk of obesity and its associated health problems. In a study of 63,307 postmenopausal women aged 50-74 years enrolled in the Cancer Prevention Study II Nutrition Cohort (Jonas et al. 2003), a higher intake of carbohydrate was associated with a lower risk of obesity and had an inverse relationship with BMI. Further, in a study of age- and height-matched normal and overweight/obese adults, the overweight/obese adults consumed a smaller proportion of their energy from carbohydrate, specifically complex carbohydrate and dietary fiber, than their normal weight counterparts (Davis et al. 2006). The overweight/obese adults consumed more simple sugars than the normal weight adults. Simple sugar intake is high among those living in the U.S., in part
because high-fructose corn syrup and other sweeteners are used in place of sucrose in many foods and most sweetened beverages. Coincidently, consumption of high-fructose corn syrup has increased in parallel with the rise in obesity. In fact, Bray & Champagne (2005) suggested that the high prevalence of obesity is due to the consumption of high-fructose corn syrup. However, others have suggested that the relationship between high-fructose corn syrup and elevated body weight is very weak (Forshee et al. 2007).

Fiber

The inverse relationship between carbohydrate consumption and BMI may partly be due to the fiber content of carbohydrate (Schulze et al. 2004). Dietary fiber may facilitate weight reduction because high fiber foods are typically nutrient dense, low in fat and/or energy content, and may displace energy dense foods, thus decreasing overall energy intake. Hence, a low fiber intake may, among other factors, contribute to excess energy intake. Dietary fiber slows gastric emptying, decreases the absorption of energy-yielding nutrients, and enhances satiety (Baer et al. 1997; Uauy & Diaz 2005), potentially decreasing energy intake. Dietary fiber may also decrease the risk of obesity because of its physical characteristics, such as its high viscosity, great water-holding capacity, and hence potential to increase the bulk/volume of stools. Women 20-59 years of age with a high dietary fiber intake, particularly in the context of a low fat intake, had a lower BMI than women with a low fiber intake (Howarth et al. 2005). Further, dietary fiber (assessed by a self-administered food frequency questionnaire) has been shown to be inversely related to diabetes risk in a prospective cohort study of 35,988 older women (Meyer et al. 2000) with insulin resistance (using the homeostasis model assessment of insulin resistance; HOMA-IR) in a cross-sectional study of 5,675 subjects aged 30-60 years (Lau et al. 2005). Many individuals in the U.S. do not consume the recommended 20-25 g/day of fiber, but research clearly suggests the benefits of dietary fiber intake.

Total fat

The total amount of fat is of concern in the U.S. diet because a diet high in fat, even in the absence of energy excess, has been shown to contribute to the obesity epidemic (Bray & Popkin 1998; Satia-Abouta et al. 2002). For examples, in a study of age- and height-matched overweight/obese versus normal weight adults, the overweight/obese individuals consumed a larger portion of their energy from fat (Davis et al. 2006). A longitudinal study of 14 years duration, with 782 Hispanic and non-Hispanic adults, found that dietary fat (independent of total energy intake) was positively
associated with weight gain and that high intake of dietary fat was strongly related to weight gain (Mosca et al. 2004). They also found that individuals with relative insulin resistance (measured by quantitative insulin sensitivity check index) at baseline, who also had a high dietary fat intake gained the greatest amount of weight. Dietary fat and its role in obesity is debatable because research supports that individuals who have attempted a low carbohydrate, high fat and/or high protein diet lost weight (Westman et al. 2002). Some health professionals suggest that it is the type of dietary fat that impacts health, although this remains controversial.

**Type of fat:** Fats are derived from animal and plant sources and are comprised of a variety of fatty acids. The degree of fatty acid saturation and the presence of double bonds have different implications for disease risk. Dietary saturated and trans fatty acids exert the most harmful effects because they tend to raise low density lipoprotein (LDL) cholesterol (Mensink & Katan 1992; Willet et al. 1993), thus increasing the risk of atherosclerosis (Hu et al. 1997). Interestingly, a low saturated fatty acid intake in men and women enrolled in the Framingham Offspring Study was significantly associated with smaller LDL particles (Campos et al. 1992), suggesting that a low saturated fatty acid intake confers greater atherosclerotic risk. However, saturated fatty acids are not as readily oxidized as unsaturated fatty acids (Gaster et al. 2005). Nevertheless, according to Rivellese et al. (2003), a high saturated fatty acid intake negatively influenced the cholesterol and TAG content of the LDL particle in 162 healthy Caucasian men and women. Increased saturated fatty acid intake has also been related to the development of insulin resistance in humans (Maron et al. 1991). One cross-sectional study found that fasting serum insulin concentration was positively associated with the percentage of saturated fatty acids in plasma phospholipids and inversely associated with the percentage of monounsaturated fatty acids (MUFA) in plasma phospholipids (Folsom et al. 1996). Thus, decreasing dietary saturated fatty acids may result in a reduced risk of insulin resistance. Trans fatty acids are also of public health concern because they raise the risk of type 2 diabetes (Salmeron et al. 2001). Some suggest that trans fatty acids decrease insulin sensitivity (Christiansen et al. 1997); however, Lovejoy et al. (2002) found that trans fatty acids did not have any effect on insulin sensitivity or secretion.

Unsaturated, or MUFA and polyunsaturated (PUFA) fatty acids, improve the lipoprotein profile (Binkoski et al. 2005), particularly if they partially replace saturated fatty acids. Replacing saturated fatty acids with PUFA has been shown to decrease LDL
cholesterol to a greater extent than MUFA (Mensink & Katan 1992). However, diets high in MUFA in comparison with PUFA have been shown to reduce the susceptibility of LDL to oxidative modification during the development of atherosclerosis (Bonanome et al. 1992). In healthy men and women, Rivellese et al. (2003) found that high MUFA diets had beneficial effects on LDL cholesterol and TAG. However, the high MUFA diet also increased lipoprotein (a) (Lp(a)), another marker of CVD risk. They suggested that this increase was insignificant, considering the other beneficial effects of a high MUFA diet. Rasmussen et al. (2006) found that a MUFA-rich diet, unlike a saturated fatty acid-rich diet, reduced the diastolic blood pressure in healthy, normotensive subjects.

A few researchers have studied the effects of PUFA on insulin sensitivity in type 2 diabetic subjects. For example, Kabir et al. (2007) studied the effect of 3 g/day of either fish oil (containing 1.8 g/day of n-3 PUFA) or a placebo (paraffin oil) using a double-blind two-month design in type 2 diabetic women. They found that the fish oil did not alter insulin sensitivity or plasma glucose, nor did it affect body weight. However, women in the active treatment group experienced a loss of adiposity, with a reduction in both abdominal (subcutaneous) fat mass as determined by computed tomography and adipocyte diameter (via a fat biopsy). In contrast, Mostad et al. (2006) studied two groups of normotriglyceridemic type 2 diabetics (men and women) who received either 17.6 mL fish oil (5.9 total g/day of n-3 PUFA as treatment) or 17.8 mL/d of corn oil (8.5 g/day 18:2 n-6 as control). The treatment group who received higher amounts of n-3 PUFAs experienced a moderate increase in blood glucose and decrease in insulin sensitivity. Taken together, these studies suggest that a modest or high intake of PUFA consumed as fish oil does not improve insulin sensitivity, but may possibly play a role in reducing adipocyte size and/or abdominal fat mass. Collectively, these results demonstrate the complexity of dietary fatty acid intake on atherosclerotic CVD risk.

**Protein**

Adequate dietary protein is required for normal growth and development. The Recommended Dietary Allowance (RDA) for adults is 0.8 g protein/kg of body mass (Food and Nutrition Board 2002). In Western cultures, it is currently estimated that dietary protein intake has increased in the past 50 years and exceeds the RDA by about 80% (Franz 2002), in part due to increased portion sizes and the popularity of high protein diets. High protein diets (e.g., Atkins) prompted researchers to study the effect of high protein (> 25% of dietary energy) diets on body composition and health. These
diets were (and still are) popular because individuals experience (at least initially) a large amount of weight loss. In a review by Halton & Hu (2004), it was demonstrated that increased dietary protein resulted in greater loss of body weight during the long-term (months or years), but not in the short-term. Further, they found consistent evidence that higher protein diets increased satiety. According to Baba et al. (1999), a high dietary protein diet (45% protein, 25% carbohydrate, 30% fat) allowed obese hyperinsulinemic individuals to lose a greater amount of weight than those on a high carbohydrate diet (12% protein, 58% carbohydrate, 30% fat). What is unclear is whether high protein diets reduce body weight by decreasing energy intake through satiety signals (e.g., ghrelin, cholecystokinin, or glucagon-like peptide), increasing energy expenditure, and/or increasing fluid loss that is reflected early on during the weight loss regimen (Blom et al. 2006). It has been found that a high protein intake suppressed circulating ghrelin, whereas it elevated cholecystokinin and glucagon-like peptide-1 in 72 healthy men in a randomized, crossover study of four preloads containing protein (soy, whey, gluten) or glucose (Bowen et al. 2006). Increased energy expenditure may be due to the higher thermic effect of protein as compared to carbohydrate or fat and/or upregulation of uncoupling proteins and facilitative thermogenesis (Halton & Hu 2004). Further, dietary protein is thought to be involved in the pathogenesis of insulin resistance by modulating glucose and energy homeostasis (Tremblay et al. 2007). Dietary protein may not only affect body composition, but also insulin secretion and action, thereby playing a role in regulating glucose. According to Linn et al. (2000), consumption of a moderately high protein diet (1.25-2.41 g/day) versus a low protein diet (0.57-0.80 g/day) for six months in healthy nonobese subjects, induced a higher-glucose stimulated insulin secretion, increased fasting glucose concentration, impaired suppression of hepatic glucose output by insulin, and enhanced gluconeogenesis.

Appetitive Hormones

In the past, adipose tissue has been thought of merely as a storage depot for fat. However, adipose tissue more recently has been examined as a metabolically active tissue playing a role in food intake regulation. Adipose tissue releases more than 20 substances into circulation (Guerre-Millo 2004) making it the largest endocrine organ. Adipose tissue releases cytokines, termed adipocytokines, such as tumor necrosis factor-alpha, adipsin, interleukin-6, resistin, adiponectin, leptin, angiotensinogen, and plasminogen activator inhibitor-1. The amount and frequency of food consumed is
mediated in part by the adipocytokines, adiponectin and leptin, the most intensively studied hormones released from adipose tissue. Further, hormones produced by the stomach and pancreas, ghrelin and insulin, respectively, play a significant role in energy homeostasis. Evidence suggests that dysregulation of adipocytokine production promotes the development of the metabolic and vascular diseases related to obesity (Funahashi et al. 1999). These hormones and their interactions are complex, but each plays a specific role in regulating energy homeostasis, as summarized in the following sections.

**Adiponectin**

The most abundant serum adipocytokine, adiponectin (also referred to as AdipoQ, ACRP30, apM1), was discovered in the mid 1990s by four different research groups (Scherer et al. 1995; Hu et al. 1996; Maeda et al. 1996; Nakano et al. 1996). This 30 kDa hormone is produced exclusively by mature adipocytes in visceral, subcutaneous, and bone marrow fat depots (Weyer et al. 2001). It has been reported that visceral adipose tissue produces the greater proportion of adiponectin on a gram-for-gram basis when compared with subcutaneous adipose tissue (Motoshima et al. 2002). Adiponectin is related to the complement C1q family containing a carboxyl-terminal globular domain and an amino-terminal collagenous domain (Scherer et al. 1995). Research from both animal and human studies have provided evidence that adiponectin increases insulin sensitivity (Yamauchi et al. 2002), exerts anti-atherogenic (Ouchi et al. 2001) and anti-inflammatory properties (Yokota et al. 2000), and may improve the lipoprotein profile (Rothenbacher et al. 2005). Fruebis et al. (2001) were the first researchers to provide evidence that adiponectin regulated lipid metabolism and body composition. Exogenous administration of adiponectin to mice caused a decrease in circulating concentrations of glucose, free fatty acids, and TAG (Fruebis et al. 2001; Yamauchi et al. 2001). Further, adiponectin decreased free fatty acids by promoting skeletal muscle fatty acid oxidation rather than storage by activating 5’AMP-activated protein kinase. This in turn activated a cascade that increased fatty acid transport into the mitochondria and hence stimulated fatty acid oxidation (Yamauchi et al. 2002; Jacobi et al. 2006). Activated AMP-kinase increased acetyl coenzyme A carboxylase phosphorylation, reduced malonyl coenzyme A production, increased carnitine palmitoyl transferase-1 activity, and thus increased mitochondrial fatty acid oxidation (Chen et al. 2005). Further, Kubota et al. (2007) recently found that adiponectin enhanced AMP-
kinase activity in the arcuate hypothalamus via an adiponectin receptor, to not only stimulate food intake in mice, but decrease energy expenditure.

Typically, obese or insulin-resistant individuals (Weyer et al. 2001), as well as those with coronary artery disease (Hotta et al. 2000), have a low adiponectin concentration. Low expression of adiponectin in skeletal muscle with obesity and diabetes in both humans (Civitarese et al. 2004) and rodents (Tsuchida et al. 2004) has been suggested to contribute to suppressed rates of fatty acid oxidation. In fact, Chen et al. (2005) studied cultured primary myotubes derived from skeletal muscle of lean, obese, and obese type 2 diabetic subjects and reported that in lean subjects, globular adiponectin stimulated skeletal muscle AMP-kinase, acetyl coenzyme A carboxylase phosphorylation, and fatty acid oxidation. In contrast, obese subjects had a low concentration of adiponectin, thereby blunting AMP-kinase signals to the myotubes. Obese type 2 diabetic subjects had an adiponectin concentration that ablated acetyl coenzyme A carboxylase phosphorylation and fatty acid oxidation. A study by Berg et al. (2001) suggested that adiponectin was a potent insulin enhancer by decreasing hepatic gluconeogenesis and muscle TAG concentration, suggesting that adiponectin sends signals from adipose tissue to the muscle and liver.

Adiponectin is increased with weight loss (Faraj et al. 2003) and individuals with high adiponectin are less likely to develop type 2 diabetes, whereas those with low adiponectin are more likely to develop this disease (Spranger et al. 2003). In this prospective study of apparently healthy adults aged 35-65, women had a higher mean concentration of adiponectin than men. In another study (Lindsay et al. 2002), low baseline adiponectin predicted the development of subsequent diabetes in humans. Adiponectin has been inversely associated with BMI (Hotta et al. 2000), central fat mass and overall body fat (Gavrila et al. 2003). In the face of adiponectin deficiency (hypoadiponectinemia), excess adipose tissue accumulates. Jurimae & Jurimae (2007) found that age, fasting insulin resistance index[^1], and leptin were independent contributors to adiponectin in middle-aged premenopausal, middle-aged postmenopausal, and older postmenopausal women (N=153). The adiponectin concentration of postmenopausal middle-aged and older women was significantly higher when compared with premenopausal middle-aged women. The researchers suggested this finding was probably due to the body composition changes as a result of

[^1]: Fasting insulin resistance index = [fasting glucose (mmol/L) X fasting insulin (μIU/ml) / 25]
menopause. As expected, the premenopausal women were leaner and had less fat than the postmenopausal women.

**Leptin**

In 1973 Coleman conducted rodent parabiosis studies on genetically obese \textit{ob/ob} and \textit{db/db} mice that provided evidence of a circulating factor that effected food intake (Coleman 1973). It appeared that the \textit{ob/ob} mice failed to produce this factor to inhibit food intake whereas, the \textit{db/db} mice produced this factor but did not respond with decreased food intake. This led to the discovery of leptin in 1994, a 16 kDa peptide of the \textit{ob} gene (Halaas et al. 1995). Leptin is produced mainly from subcutaneous adipose tissue (Faraj et al. 2003). This hormone attracted much attention as a possible therapeutic agent for obesity. Similar to adiponectin, leptin is secreted from adipose tissue, but its production is proportional to fat mass, especially the amount of subcutaneous fat, and also has an insulin-sensitizing effect by favoring tissue fatty-acid oxidation through activation of AMP-kinase (Guerre-Millo 2004). Leptin concentration is highly related to BMI (Lonnqvist et al. 1995; Considine et al. 1996) and leptin acts as a positive signal from energy reserves in adipose tissue to the brain. Leptin is transported into the brain via a saturable process (Banks et al. 1996). During an obese state, leptin concentration is elevated in most individuals (Matsubara et al. 2002; Rosicka et al. 2003). This observation lead to the speculation that elevated leptin caused obesity and later that obesity may be due to leptin resistance (Caro et al. 1996). Leptin resistance might result from either inadequate leptin production, lack of leptin receptors, or to insensitivity of the leptin receptors in the brain, all of which may contribute to obesity. However, during obesity, leptin production is elevated and thus leptin resistance is most likely due to insensitivity of brain leptin receptors rather than inadequate leptin production (Munzberg & Myers 2005).

The absence of leptin in the \textit{ob/ob} mouse leads to massive obesity, but administration of leptin reduced food intake and increased energy expenditure (Halaas et al. 1995; Pelleymounter et al. 1995). Similarly, this result has been shown in leptin deficient humans who are massively obese. Leptin administration to massively obese individuals has been shown to result in decreased food intake and mobilization of body fat such that body weight is normalized (Benoit et al. 2004). In a study of Pima Indians, those prone to a large weight gain had a lower leptin concentration than those who had never been obese (Ravussin et al. 1997). A low concentration of leptin is associated with
a decreased rate of fat oxidation (Filozof et al. 2000). Otero et al. (2005) also has shown that leptin suppressed appetite, increased energy expenditure, and decreased metabolic efficiency.

**Insulin**

Insulin has long been known as a key peripheral regulator of food intake and adiposity. There are two primary actions of insulin: homeostatic and anabolic. Insulin concentration is low in a fasted state in which the homeostatic role dominates, whereas insulin is stimulated after a meal in which the anabolic role dominates, particularly when carbohydrate is consumed, or with glucose infusion (Czech 1984). The beta cells of the pancreas synthesize insulin in response to local glucose, but also respond to amino acids (particularly arginine and leucine), oral hyperglycemic agents, calcium influx, gastric inhibitory peptide, and the parasympathetic nervous system. Insulin secretion is inhibited by hypoglycemia and the autonomic nervous system. Insulin signals cells to take up circulating glucose by signaling glucose transporters (particularly GLUT-4 receptors) to translocate to the cell membrane (Barnard & Youngren 1992). Ultimately, insulin regulates glucose utilization (homeostatic role) and/or storage (anabolic role) and also lipid utilization (homeostatic role) and storage (anabolic role). The insulin response to glucose only lasts as long as glucose is elevated postprandially; insulin is then rapidly cleared from the blood. Without sufficient insulin, as is the case in type 1 diabetes, cells are unable to take up glucose, causing a hyperglycemic condition. At the same time, adipocytes are unable to take up lipid for storage (Cryer & Polonsky 1998).

Plasma insulin concentration is directly related to adiposity (Woods et al. 1998), particularly visceral fat. Obese individuals have a higher concentration of insulin and secrete more insulin in response to a meal than do lean individuals (Bagdade et al. 1967). Further, obese individuals can become insulin resistant or develop type 2 diabetes when the insulin concentration is elevated over time. Individuals are considered insulin resistant when cells experience decreased sensitivity and/or responsiveness to the metabolic actions of insulin (Benoit et al. 2004). During an obese state, excess insulin is secreted to regulate the high concentration of circulating glucose. Excess insulin causes increased accumulation of fat in adipocytes and thus obesity is closely associated with type 2 diabetes. Evans et al. (1984) found that in healthy, premenopausal women, as WHR increased, plasma insulin concentration increased.
Further, obese women with androidal obesity have been found to have impaired glucose
tolerance, as well as hyperinsulinemia (Kissebah et al. 1982).

Insulin receptors are not only located on the membrane of cells, but also in the
brain, specifically in the regions of the central nervous system involved in the regulation
of food intake and body weight (Woods et al. 2003). In fact, exogenous administration of
insulin into the brain reduced food intake and increased energy expenditure (Air et al.
2002a). Similar to leptin, insulin enters the brain via a saturable transport process
(Hachiya et al. 1988). Insulin and leptin interact, providing information to the brain not
only about the size of fat mass, but also about adipose tissue distribution and important
recent changes in metabolic status (Benoit et al. 2004). While leptin is a long-term (days)
adiposity signal, insulin secretion assesses changes in energy storage within minutes
and hours.

Ghrelin

An orexigenic peptide, ghrelin, discovered in 1999 by Kojima et al. (1999), is a
hormone that has two independent physiologic roles. Ghrelin stimulates appetite and the
release of growth hormone from the pituitary gland. Ghrelin, a 3.3 kDa preprohormone,
must be proteolytically split into a 28-amino acid peptide to be activated. This peptide is
synthesized in epithelial cells of oxyntic glands lining the fundus of the stomach, with
smaller amounts synthesized from regions of the brain (pituitary and hypothalamus),
kidneys, bowel, and placenta (Guan et al. 1997). Prior to the discovery of ghrelin, its
receptor in the pituitary was characterized. The receptor located in the anterior pituitary
is known as the growth hormone secretagogue receptor because upon stimulation, it
secretes growth hormone. The growth hormone secretagogue receptor acts through a
G-protein-coupled response. After gastric production, ghrelin is secreted into the
bloodstream where it binds with receptors in the arcuate nucleus of the brain. The
appetite-stimulating effect of ghrelin is independent of growth hormone stimulation. The
location of ghrelin secretion in the stomach is ideal because it senses short-term
fluctuations in energy balance, thereby stimulating meal initiation. Evidence of this was
shown during administration of ghrelin peripherally to rodents. Ghrelin acted centrally to
stimulate food intake (Tschop et al. 2000), increasing adiposity by decreasing fat
oxidation. Administering ghrelin intravenously to humans also increased adiposity and
decreased fat utilization (Wren et al. 2001). Along with short-term regulation, ghrelin also
regulates body weight long-term through a complex central signaling network that regulates food intake and energy expenditure (Murray et al. 2003).

There are two active forms of ghrelin commonly found in tissue and plasma: \( n \)-octanoyl-modified and des-acyl ghrelin. The concentration of total ghrelin (\( n \)-octanoyl-modified plus des-acyl forms) in plasma is normally 100-150 fmol/mL. During times of fasting, plasma ghrelin concentrations are increased and then decreased within one hour after food consumption (Cummings et al. 2001), as demonstrated in the figure below. This study also found that the ghrelin concentration during a 24-hour period was reciprocal of insulin. Ghrelin rose sharply before meals while the insulin concentration was low; conversely, the ghrelin concentration declined post-meal, while insulin rose.

![Figure 4: 24-hour plasma ghrelin concentration](image)

Previous research has shown that plasma ghrelin concentration was lower in obese subjects than age-matched lean controls (Hansen et al. 2002), opposite than what one would expect. However, as obese patients lose body weight, ghrelin concentration increases (Cummings et al. 2002). Interestingly, in a study of obese patients receiving stomach-bypass surgery, the ghrelin concentration actually decreased suggesting that the size of the stomach may be directly related to ghrelin (Holdstock et al. 2003). In contrast, anorexia nervosa patients have higher plasma ghrelin, but as they gain weight, plasma ghrelin is decreased (Otto et al. 2001). In fact, ghrelin has been considered as a remedy to pathological anorexia that accompanies cancer, AIDS, tuberculosis, and aging by increasing food intake in these patients. A recent study by Garcia & Polvino (2007) studied the effects of ghrelin drug therapy (RC-1291) by providing ghrelin orally to
patients with cancer-associated anorexia/cachexia. These patients gained weight, suggesting that this may be an effective treatment for anorexia/cachexia. A ghrelin antagonist has been considered as a treatment for obesity by reducing the amount of food intake and/or reducing the sensation of hunger. To test this hypothesis, Zorrilla et al. (2006) used male rats and gave them a “vaccine” of ghrelin antagonist. They found that the immunized rats ate normally but gained less body weight and fat, suggesting that the rats reduced energy storage. Further testing is needed in animal models to ensure the safety and discover the exact mechanisms by which this “vaccine” may work.

**Inflammatory Markers**

Obesity is considered a state of low-grade inflammation, stemming from adipose tissue, resulting from chronic activation of the innate immune system. Long-term inflammation can lead to insulin resistance that, if left untreated, can develop into diabetes (Bastard et al. 2006). Adipocytes secrete many proinflammatory markers (e.g., tumor necrosis factor-α, interleukin-6, interleukin-1β, C-reactive protein, etc.) that contribute to the systemic inflammatory state (Gil et al. 2007). There is mounting evidence that these cytokines play a large role in the development of obesity-associated CVD. In fact, visceral obesity is associated with increased circulating concentrations of C-reactive protein and interleukin-6 in obese individuals (Panagiotakos et al. 2005). Further, central adiposity in postmenopausal women has been shown to be related to C-reactive protein and fibrinogen (Perry et al. 2008). Proinflammatory markers are increased during obesity, but also during inflammatory conditions. During an inflammatory condition, lymphocytes (e.g., white blood cells) migrate to the site of inflammation, thus acting as an acute responder to infection or inflammation. Although white blood cell count typically is thought of as an indicator of acute infection or inflammation, research has indicated that white blood cell count is also related to body fat in humans (Womack et al. 2007). Further research is warranted to elucidate the relationship between body fat and white blood cells, but it is clear that inflammation plays a role in obesity.

**OVERVIEW OF MENOPAUSE**

**Definitions**

Menopause marks the permanent end of menses and fertility (North American Menopause Society 2007), occurring on average by 51 years of age. According to the
WHO (1996), premenopause is any time in a woman’s life from puberty to her last menstrual period. Menopause is defined by 12 consecutive months of amenorrhea (North American Menopause Society 2007). Perimenopause means “around menopause” and is a time of irregular menstrual cycles before the final menses and lasts until one year after a woman’s last menses. The postmenopausal period begins at the time of the last menstrual period and extends through a woman’s life. The menopausal transition describes the time of increased menstrual cycle variability and may begin as early as the thirties for some women (Mitchell et al. 2000). These researchers suggest that during the menopausal transition, there are three key characteristics: changes in cycle length and flow, irregular menses, and missed periods. The first five years after the last menses is considered the “early” postmenopausal period, whereas the time thereafter is considered the “late” postmenopausal period. Determining the exact occurrence of menopause is difficult because reproductive hormone concentrations and menopausal symptoms are exceedingly variable among women and thus have been deemed unsuitable markers. Further, hormone values do not necessarily relate to menopausal symptoms or amenorrhea (Sherman 2005), thereby forcing clinicians to use the one year cessation of menses as the gold standard.

**Hormonal Changes During Menopause**

**Reproductive hormones**

During the transition through menopause, women experience physiologic and endocrinologic changes. With the menopausal transition, one physiologic change that occurs is that the menstrual cycle becomes irregular, representing a marked decrease in ovarian follicle number (Richardson et al. 1987) and change in hypothalamic-pituitary-ovarian axis function (Weiss et al. 2004). Cessation of follicular development decreases the production of estrogen, particularly $17\beta$-estradiol ($E_2$), from the ovaries starting about one year prior to the final menstrual period (Burger et al. 1999). In addition, progesterone production is decreased as a woman transitions through menopause. Progesterone is synthesized primarily in the adrenal glands and is the hormone that predominates during the luteal phase of the menstrual cycle and is also the primary hormone that is produced in high amounts during pregnancy. The gonadotropin hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), normally tend to increase with the occurrence of the final menstrual period (Rannevik et al. 1995), likely due to the decrease in estrogen. Postmenopausal concentrations of FSH and LH
range from 34-96 IU/L and 40-104 IU/L, respectively (Carr 1998). Estrogen provides a negative feedback signal to decrease LH, maintaining a low concentration of LH during the premenopausal period (Herbison 1998). According to Herbison (1998), estrogen also provides a positive feedback signal to increase LH because it induces the slight surge in LH concentration during ovulation. Thus, when estrogen production declines, the negative feedback signal is no longer present, allowing LH (and FSH) to increase. After the onset of menopause, there is a 10-15 fold increase in circulating FSH concentration, a 4-5 fold increase in LH, and more than a 90% decrease in circulating E2 (Burger 1999).

Lastly, the androgen hormone, testosterone, has been shown to change little across the menopausal transition. Two studies (Rozenberg et al. 1988; Bancroft & Cawood 1996) have compared cross-sectionally testosterone concentration between pre- and postmenopausal women. They found that postmenopausal women had a lower concentration than the premenopausal women, but postmenopausal women have higher testosterone in relation to estrogen concentrations. However, according to one longitudinal study (Burger et al. 2000), testosterone did not change across the menopausal transition. Further research is needed to clarify the role of testosterone during menopause.

Recently, the dimeric inhibins A and B have been described as playing a role during the menopausal transition. The inhibins and reproductive hormones are secreted from the granulosa cells lining the ovarian follicles. Thus, when follicle production begins to decline, there is a decrease in the concentrations of these hormones. In a longitudinal study of healthy women enrolled in the Melbourne Women’s Midlife Health Project (Burger et al. 1999), changes in inhibins A and B, as well as E2 and FSH, were measured in relation to the time of final menses. They found that FSH rose, whereas the concentrations of E2 and the inhibins fell at the time of final menses. They suggested that the decline in inhibins, along with E2, likely contributed to the rise of FSH. Another study by Burger et al. (2002) demonstrated that the earliest sign of hormonal change was reduced inhibin B concentration as women began the menopausal transition.

**Appetitive hormones**

Researchers have shown that appetitive hormones are related to body composition, but there are still many questions as to how the appetitive hormones affect body composition during the menopausal transition. Previous studies (Sowers et al. 2008; Mahabir et al. 2007; Gower et al. 2000; Gavrila et al. 2003; Purnell et al. 2003;
Lovegrove et al. 2002) have been designed to examine the appetitive hormones in healthy postmenopausal women. These researchers have studied appetitive hormones specifically in relation to central adiposity because of the change in body composition that most women experience during and after menopause. Since adiponectin and leptin are synthesized in adipocytes, do their concentrations affect the size and distribution of fat cells? Alternatively, is it that menopause affects the concentration of appetitive hormones because of the change in reproductive hormones? It is known that the adipocytokines and ghrelin are related to menopause (Sowers et al. 2008), but with the rise in obesity, especially for postmenopausal women, it is important to understand these relationships and the mechanisms that might alter disease risk during the menopausal transition.

**Adiponectin:** Few studies have found differences in adiponectin concentration between pre- and postmenopausal women. Although Gavrila et al. (2003) and Jurimae & Jurimae (2007) found that adiponectin was significantly lower in pre- versus postmenopausal women, Kanaley et al. (2001) and Hong et al. (2007) did not find a difference in adiponectin between these two groups. They attributed the lack of difference in adiponectin to a lack of dramatic difference in body fat between pre- and postmenopausal women. The finding that adiponectin was higher in postmenopausal women is hard to understand because as body fat increases, adiponectin concentration typically decreases. Thus, one would speculate that after menopause women might have a lower adiponectin concentration because of their greater fat mass. Perhaps this might be explained by the inverse relationship between adiponectin and estrogen, illustrating that estrogen deficient postmenopausal women may have a higher adiponectin concentration than their estrogen replete premenopausal counterparts. Gavrila et al. (2003) found that serum $E_2$ was a strong negative determinant of adiponectin in postmenopausal women; compared with premenopausal women, $E_2$ was lower while adiponectin was higher in postmenopausal women.

**Leptin:** Results are inconclusive regarding leptin concentration across the menopausal transition. According to Kanaley et al. (2001), similar to adiponectin, leptin concentration was not different between pre- and early postmenopausal women. However, according to Rosenbaum et al. (1996), the leptin concentration in postmenopausal women was lower than their premenopausal counterparts. Unlike these studies that compared the leptin concentration in women cross-sectionally, Sowers et al.
(2008) measured leptin sequentially at three time points (pre-, peri-, and postmenopause) and found in non-obese women that the leptin concentration increased throughout these menopausal stages, independent of FSH concentration. They suggested that leptin may play a direct role in affecting reproductive hormone concentration during the transition. This might be due to the ability of leptin to communicate with the brain at the hypothalamic-pituitary-ovarian axis, possibly allowing it to affect reproductive hormone action (Licinio et al. 1998), or conversely, reproductive hormones might also affect leptin action. Regardless, it is important to further investigate this relationship because of the important health-related consequences of obesity that may accompany menopause.

**Insulin:** The transition through menopause is marked by a decrease in insulin secretion and a parallel decrease in hepatic insulin clearance (Walton et al. 1993), thereby increasing the risk of elevated blood glucose concentration and diabetes. Postmenopausal status and aging both increase the risk of insulin resistance, as indicated by the statistic that ~44% of untreated (not taking hormone therapy) postmenopausal women are insulin resistant (Lindheim et al. 1993). Whether this decrease in insulin concentration and sensitivity is related to the decline in estrogen production has been suggested (Van Pelt et al. 2008), but is still unclear. However, it has been shown that estrogen plays a role in maintaining glucose homeostasis by affecting insulin secretion and clearance (Godsland 2005). Hormone therapy has been shown to increase insulin clearance and action, thereby increasing glucose disposal in postmenopausal women (Van Pelt et al. 2003) and hence decreasing the risk of insulin resistance and diabetes.

**Ghrelin:** Research investigating the relationship between ghrelin and menopause is lacking and the research that is presently available is conflicting. Sowers et al. (2008) found that ghrelin concentration was higher in both obese and non-obese perimenopausal women as compared with pre- and postmenopausal women. This increase in ghrelin during perimenopause coincidently occurs during increased cycle irregularity, accompanied by wide fluctuations in the circulating reproductive hormones. There is question as to whether ghrelin influences the reproductive hormones and/or body composition and thus further investigation is warranted.

**Summary:** Each of the appetitive hormones has been studied in postmenopausal women, although more research to determine their role throughout the
menopausal transition is needed. Further, it might be that a combination of these hormones impact reproductive hormones and body composition, thereby influencing the health of postmenopausal women. For example, it has been found that adiponectin is inversely related to leptin, which is consistent with some (Jurimae & Jurimae 2007; Matsubara et al. 2002) but not all studies (Gavrila et al. 2003). Both low adiponectin and high leptin have been related to an increased risk of insulin resistance (Matsubara et al. 2002). Hong et al. (2007) found that in obese postmenopausal women, an increase in leptin contributed to insulin resistance. Further investigation is certainly warranted.

**Long-Term Health Consequences of Menopause**

**Increased risk of obesity: Change in overall and regional adiposity**

Menopause-induced hormonal changes result in an increase in overall adiposity (Tchernof et al. 2004), but especially visceral adiposity (Lovejoy et al. 2008). This indicates that the change in reproductive hormone concentration during menopause may be linked to the change in body composition (Sowers et al. 2007). Premenopausal women who have a normal concentration of estrogen have a greater tendency to store fat in the gluteo-femoral (gynoidal) region; however, the loss of estrogen with menopause is associated with an increase in centrally-located (androidal region) fat. This is accompanied by an increase in disease risk, specifically the risk of insulin resistance (Lee et al. 2004), atherosclerotic CVD (Carr 2003), dyslipidemia (Schnatz & Schnatz 2006), hypertension (Reckelhoff & Fortepiani 2004), and breast cancer (Sellers et al. 1992). Although the mechanism responsible for the increase in central adiposity is unclear, the evidence presently available indicates that menopause is associated with a change in metabolic activity of the adipose tissue. Ferrara et al. (2002) has reported that adipose tissue lipoprotein lipase, a key enzyme in lipid metabolism, storage, and distribution, was significantly more active in gluteal and abdominal adipocytes from postmenopausal compared with perimenopausal women. This suggests that the adipocytes in the gluteal and abdominal area in postmenopausal women are more likely to store and distribute fat than in perimenopausal women, providing a possible link to the increased central adiposity that most postmenopausal women experience. Additionally, Lovejoy et al. (2008) performed a longitudinal study following 158 healthy premenopausal women for four years and suggested that the increase in central fat mass was related to fat oxidation and energy expenditure. They found that 24-hour fat oxidation declined in women who transitioned from pre- to postmenopause and
suggested that this may predispose postmenopausal women to gain body fat, particularly in the central region. They also found that energy expenditure (assessed by an activity monitor) decreased in women who transitioned into menopause compared to women who remained premenopausal during the course of the study. Some attribute changes in body composition to changes in reproductive hormone status during the menopausal transition. The Study of Women’s Health Across the Nation (SWAN), a survey of ~13,000 multi-ethnic women in the U.S., indicated that in 543 pre- and perimenopausal Caucasian and African-American women, a change in circulating FSH (increase) was positively related to change in fat mass (increase) during six years of follow-up (Sowers et al. 2007). They concluded that ovarian aging plays a role in body composition changes during the menopausal transition. More research is necessary to study women through the menopausal transition to determine the mechanisms of the postmenopausal “shift” of fat from the gynoidal to the androidal region.

**Increased risk of chronic disease**

Postmenopausal women are at a greater risk of osteoporosis (Siris et al. 2006) and atherosclerotic CVD (Stangl et al. 2002), specifically coronary artery disease, than premenopausal women, most likely related to the loss of estrogen protection (Barrett-Conner & Bush 1991). Along with increased body weight, many unfavorable consequences typically occur after menopause, including changes in lipid and lipoprotein metabolism (Stevenson et al. 1993), glucose and insulin metabolism (Walton et al. 1993), and coagulation and fibrinolysis (Winkler 1992). Stevenson et al. (1993) found that postmenopausal women (independent of age) had significantly higher circulating concentrations of total cholesterol, TAG, and LDL cholesterol, but lower HDL cholesterol. Additionally, pancreatic insulin secretion was markedly lower in postmenopausal versus premenopausal women (Walton et al. 1993). Dyslipidemia and insulin resistance may develop from highly lipolytic visceral adipocytes that increase circulating free fatty acids, which in turn interfere with hepatic clearance of insulin and enhance hepatic synthesis of TAG-rich lipoproteins (Tchernof et al. 2000). Taken together, these studies provide valuable information as to why postmenopausal women are at an increased risk of atherosclerotic CVD. However, the relationship between alterations in reproductive hormone concentrations at menopause and CVD is still uncertain. Many menopausal women are searching for treatment options other than hormones to decrease their risk of chronic diseases.
Treatment of Menopause

Common hormonal therapies

Postmenopausal women are often prescribed HT to relieve menopause-related symptoms, specifically vasomotor symptoms (hot flushes), memory loss, and loss of sleep. Additionally, many women are prescribed HT to prevent bone loss, and for many, HT has been shown to beneficially affect body composition by preventing an increase in body weight. A study with postmenopausal women found that after five years of HT, the postmenopausal increment of fat mass, especially in the trunk region, was attenuated by 60% (Kristensen et al. 1999).

There are two common types of HT available, estrogen and estrogen plus progesterone, with choices of route of delivery (oral, transdermal, vaginal, nasal) for each formulation, dependent upon individual preference. Various HT formulations are prescribed for different indications, but all carry contraindications in particular circumstances. In addition, side effects have been observed with all types of therapies, as illustrated and summarized by the Writing Group of the Women’s Health Initiative Trial (Rossouw et al. 2002). Since results of this trial have been published, physicians have tended to prescribe lower doses of both estrogen and progesterone to exert similar benefits, but with less risk of adverse effects. “Natural” hormones appear to be more widely accepted by women than synthetic hormones (Fournier et al. 2008), but likely carry similar risks.

**Estrogen:** Estrogen therapy is typically used to treat vasomotor symptoms, such as hot flushes and/or nights sweats. Also, estrogen therapy has been shown to help prevent osteoporosis (Greendale et al. 1998) in postmenopausal women who have a relative estrogen deficiency after menopause. Women who have undergone a hysterectomy are typically prescribed estrogen alone, whereas women with an intact uterus taking estrogen alone are at an increased risk for developing endometrial cancer due to over-stimulation of the endometrial tissue. This could lead to continuous glandular cell proliferation (Sitruk-Ware 2007), increasing the likelihood of endometrial cancer. Currently, both oral and non-oral (e.g. transdermal, vaginal, and nasal) estrogen therapies are available. There is growing evidence that non-oral estrogen is better than oral because non-oral estrogen avoids metabolism through the gastrointestinal tract and liver, known as the “first pass”. Thus, non-oral administration does not stimulate hepatic synthesis, and hence does not promote dyslipidemia. Also, non-oral administration of
estrogen allows the serum concentration to more accurately reflect the dose delivered and absorbed (Sitruk-Ware 2007). Vaginal delivery of estrogen can be via a silastic ring or cream, both of which have been shown to be effective methods for delivery of estrogen. Nash et al. (1999) used vaginal rings to deliver 60-140 ug E$_2$/day for six months and effectively relieved menopausal symptoms in 35 women who had undergone a hysterectomy. Additionally, nasal administration of HT (300 ug/day of E$_2$) has been shown to be an effective method to reduce menopausal symptoms for postmenopausal women. (Studd et al. 1999). This method was (and is) well tolerated and accepted by women.

**Estrogen plus progesterone:** Combined HT, estrogen plus progesterone (or synthetic progestin), is a commonly used formulation. This type of therapy is recommended for women with an intact uterus because estrogen alone stimulates the endometrium, whereas the addition of progesterone exerts a protective effect on the endometrium. However, the estrogen-progesterone preparations have been challenged because they are thought to impact other target tissues, such as breast cells (Beral 2003). Progesterone therapy may be delivered vaginally via a gel or ring, or via an intrauterine device, although transdermal gels or patches are also available. Both oral and non-oral estrogen plus progesterone are available, whereas non-oral delivery avoids the first pass effect, thereby limiting systemic action.

**Alternative therapy: Soy isoflavones**

In 2002, the Women’s Health Initiative Trial demonstrated that HT may carry greater risks than benefits (Rossouw et al. 2002). Some of these risks include breast and uterine cancer, stroke, and thromboembolic disease. Since these studies became available, HT use has declined (Ettinger et al. 2003) and women are searching for alternative therapies to relieve menopausal symptoms. Further, the rising health concerns that surround central adiposity after menopause have driven women to search for ways to either maintain or lose weight. Finding effective therapies to ameliorate the rising prevalence of obesity among postmenopausal women is crucial. Globally, some of the lowest disease rates are in Asia where soy food consumption is high. A typical Asian diet contains about 20-80 mg isoflavones/day (Somekawa et al. 2001). The low disease rates among Asians have led health professionals to question whether soy foods (containing isoflavones) might affect body composition. Further, the risks of HT outweigh the benefits for some women and thus many are interested in a more “natural” approach.
Soy isoflavones, with their estrogen-like properties, are thought by some to be an ideal alternative to HT because of perceived health benefits (Messina 2002). However, we have sparse information on the effect of soy isoflavones on body composition in humans.

**Food sources, health benefits:** Soy isoflavones have been touted as a natural and safe alternative to HT, in part because Asian women report fewer menopausal symptoms (Boulet et al. 1994) and they have lower rates of obesity (Stevens & Nowicki 2003). Soy isoflavones are thought to relieve menopausal symptoms (Upmalis et al. 2000), similar to estrogen, but further research is needed to understand the underlying mechanisms. Isoflavones are found naturally in soybeans and products made from soybeans, as well as red clover. Soybeans provide both soy protein and soy isoflavones. Soy protein is thought to exert a number of health benefits and, in fact, the Food and Drug Administration in 1999 issued a health claim stating that 25 grams of soy protein/day may reduce the risk of coronary heart disease (Food and Drug Administration 1999). However, this amount may be too high for most consumers to achieve and they did not provide a recommendation about soy isoflavone intake. Messina (2002) recommended 15 grams of soy protein and 50 mg of isoflavones per day for a healthy adult population, which may exert hypocholesterolemic effects while providing protection from diseases, such as CVD, cancer, and osteoporosis. A diet rich in soy isoflavones has been shown to decrease the risk of breast cancer (Linseisen et al. 2004), prostate cancer (Ozasa et al. 2004), and CVD (Tikkanen et al. 1998), as well as reduce bone loss (Alekel et al. 2000). Soy isoflavone metabolites have also been shown to exert potent antioxidant effects (Arora et al. 1998). Goodman-Gruen and Kritz-Silverstein (2001) found that mean BMI and waist circumference were significantly smaller in postmenopausal women who reported consuming ≥1.0 mg/day of isoflavone genistein compared with women who did not consume any isoflavones. However, this cross-sectional study was limited by inadequate measures of body composition, self-reported food intake, small amount of isoflavone intake, and failure to account for dietary intake (other than energy) or physical activity that might have influenced the results.

**Structure, metabolism:** Isoflavones (Figure 5) are composed primarily of the β-glycoside forms genistin, daidzin, glycitin, and the aglycone forms genistein, daidzein, and glycine. Although isoflavones are primarily in the glycosidic form in foods, this form has not been detected in plasma or urine; thus, it is thought that the isoflavones are
metabolized by glucosidases prior to absorption, converting the isoflavones to a more bioavailable form (Hendrich & Murphy 2007). The aglycone form of isoflavones typically diffuses across the intestinal brush border, making their absorption more rapid than the glycosidic forms. A greater percentage of ingested daidzein is excreted in urine than genistein (Bloeden et al. 2002), suggesting that daidzein is more bioavailable than genistein. Isoflavones are affected by many factors that impact bioavailability, such as solubility, absorption mechanisms, metabolism, microbial biotransformation, and interaction with other dietary compounds (Hendrich & Murphy 2007).

Urinary excretion of isoflavone metabolites is highly variable because of inter-individual variation, thus making it difficult to detect consistent trends in absorption. One trend has been shown to be consistent: those individuals who are considered “high excreters” of urinary isoflavones also have greater fecal excretion of isoflavones (Xu et al. 1995). Because plasma concentrations are much lower than in urine, plasma concentrations are more difficult to detect. The isoflavones are readily hydrolyzed by intestinal bacteria (Setchell et al. 1984), perhaps accounting for the variability of isoflavone excretion. The microbial biotransformation of daidzein forms equol (Atkinson et al. 2005), which is of interest because humans who produce equol may derive greater benefit from soy foods. About one-third to one-half of human subjects produce equol (Hendrich & Murphy 2007). Case-control studies have shown that those who produce equol are also at a reduced risk for cancer.

Isoflavones may be especially of interest to women because their structure is similar to E2 (Figure 6) (Kuiper et al. 1998). Isoflavones from plant sources can bind to human estrogen receptors, ERβ and ERα, and are thus called phytoestrogens. The structure of daidzein is most similar to the endogenous hormone E2. Isoflavones are also considered selective estrogen receptor modulators (SERMs) because of their ability to induce both estrogen agonistic and antagonistic effects (Bhathena & Velasquez 2002). Typically, genistein binds more strongly to ERβ, although both genistein and daidzein have a lesser affinity to the receptors than estrogen. ERβ receptors are widely distributed in tissues, and because soy isoflavones act as weak estrogens, it is important to understand the underlying mechanism of this relationship. Because many postmenopausal women are seeking alternative therapies to HT, further investigation is warranted.
Effects on regional fat and lean tissue distribution: Few human epidemiologic studies have investigated the relationship between soy isoflavones and regional fat and lean tissue distribution. Animal studies suggest that overall adipose tissue is affected by isoflavones, but much less is known about changes in regional adipose tissue. In 2003, it was discovered by Naaz et al. (2003) that genistein injections into ovariectomized mice decreased overall adipose tissue. Arjmandi and colleagues (1996) reported that an isoflavone-rich soy protein diet suppressed weight gain, specifically abdominal fat deposition, in ovariectomized (i.e., estrogen-deficient) rats. Interestingly, Hanson et al. (1999) found that male hamsters fed a casein diet supplemented with the single isoflavone daidzein were significantly leaner and lighter at the end of a 10-week feeding period compared with male hamsters fed a soy protein diet containing a mixture of isoflavones. Further, Wagner et al. (1997) found that ovariectomized monkeys fed
Isoflavone-rich soy protein tended to have less total body mass, abdominal fat mass, and a lower subscapular-to-triceps skinfold thickness ratio compared with monkeys fed casein. Few studies, especially long-term, have been conducted in humans, specifically postmenopausal women, examining this relationship. Wu et al. (2006) found that one year of isoflavone intake significantly reduced fat mass in the trunk region in Japanese women. However, this study did not control for soy consumption outside of the study protocol, making conclusions difficult. Finally, data from the Alekel laboratory (Moeller et al., 2003) showed that perimenopausal women who consumed isoflavone-rich (80 mg/day) soy protein for six months had slightly but significantly lower thigh fat mass and greater hip lean mass compared with women who consumed either isoflavone-poor (4.4 mg/day) soy protein or whey protein powder. Thus, limited evidence derived primarily from animal studies suggests that isoflavones may favorably alter body composition. Although few data are available from human studies, results are intriguing. Hence, a well-designed, long-term prospective clinical trial is necessary to determine whether isoflavones have a physiologically important impact on body composition in humans. Although soy isoflavones, as a natural alternative to HT, may play a role in preventing fat mass deposition, we must further elucidate the mechanisms that may lead to changes in body composition.

**Effects on appetitive hormones:** Currently there is limited research studying the effects of soy isoflavones on appetitive hormones: adiponectin, leptin, insulin, and ghrelin. Many studies have been conducted using soy protein, which contains soy isoflavones, but the use of soy protein makes it impossible to determine which soy protein-related factors may be causing the effects. Further, most studies do not isolate one isoflavone, thereby making it impossible to differentiate the effects due to genistein, daidzein, and/or glycitein.

Research on the effect of soy isoflavone consumption on adiponectin is very limited, whereas a small study (Phipps et al. 2001) reported the effect of isoflavones on leptin. Researchers hypothesized that a high soy isoflavone (either 65 or 130 mg/day) intake would result in a higher leptin concentration in postmenopausal women, possibly by directly signaling adipocytes to produce leptin. However, they found that isoflavones had no effect on leptin concentration in either pre- or postmenopausal women and concluded that soy isoflavones did not directly act on adipocytes to increase leptin production, although they did not directly measure isoflavone action on adipocytes.
Further, Goodman-Gruen & Kritz-Silverstein (2001) reported that consumption of isoflavones reduced fasting insulin concentrations in postmenopausal women. In fact, Jayagopal et al. (2002) demonstrated that, in response to 30 g of isolated soy protein with 132 mg of isoflavones per day for 12 weeks, postmenopausal women with type 2 diabetes experienced an 8% decrease in fasting insulin and a 6.5% reduction in insulin resistance. Further, dietary supplementation with soy isoflavones also favorably altered serum lipoproteins, thereby improving their CVD risk profile. More recently, Crisafulli et al. (2005) observed that genistein (54 mg/day) provided to 60 healthy postmenopausal women resulted in lower fasting serum glucose, insulin, and HOMA-IR compared to the placebo. Lastly, Weickert et al. (2006) found that soy isoflavone treatment did not affect ghrelin concentration in 34 healthy postmenopausal women.

In conclusion, we have a paucity of data on the effect of isoflavones on the appetitive hormones. Further studies need to be conducted to determine whether soy isoflavones affect the appetitive hormones either directly or indirectly, and to determine whether this purported effect will beneficially influence body composition in postmenopausal women. It may be that the reproductive hormones play a role in regulating body composition by affecting the appetitive hormones, although the data to date do not allow these conclusions.

SUMMARY

In summary, obesity is a major public health concern because of the increased risk of mortality from chronic disease. The location of body fat is critical in terms of chronic disease risk, with central adiposity conferring greater health consequences. In the past, adipose tissue has been thought of merely as a storage depot for fat. However, more recently adipose tissue has been appreciated as a metabolically active tissue playing a role in food intake regulation. The amount and frequency of food consumed is mediated in part by the adipocytokines, adiponectin and leptin. Insulin and ghrelin each play a role in appetite regulation, with insulin working in concert with short-term signals and leptin to limit food intake. Evidence suggests that dysregulation of the appetitive hormones promotes the development of the metabolic and CVD related to obesity.

During the transition through menopause, women experience cessation of follicular development that decreases the production of estrogen, particularly 17β-estradiol. The loss of estrogen with menopause is associated with an increase in overall
and central adiposity. This is accompanied by an increase in disease risk, specifically the risk of insulin resistance, atherosclerotic CVD, dyslipidemia, hypertension, and breast cancer. The rising health concerns that surround HT and central adiposity after menopause have driven women to search for ways to either maintain or lose weight and decrease their risk of chronic disease. Soy isoflavones, with their estrogen-like properties, are thought by some to be an ideal alternative to HT because of perceived health benefits. However, we have sparse information on the effect of soy isoflavones on body composition in humans, especially related to the appetitive hormones. Further studies need to be conducted to determine whether soy isoflavones affect the appetitive hormones either directly or indirectly, and to determine whether this purported effect will beneficially influence body composition in postmenopausal women.

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Centrally-Located Body Fat is Related to Appetitive Hormones in Healthy Postmenopausal Women

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Objective: Body composition and energy homeostasis are thought to affect the appetitive hormones: adiponectin, leptin, insulin, and ghrelin. This study examined whether centrally-located fat and/or overall adiposity were related to these appetitive hormones in healthy postmenopausal women.

Design: Overall and regional body composition was assessed by dual-energy X-ray absorptiometry in relation to plasma adiponectin, serum leptin, serum insulin, and plasma ghrelin in 242 postmenopausal women.

Results: Regression analyses revealed that the androidal-to-gynoidal fat mass ratio (18.0%), age (3.2%), and white blood cell count (1.8%) accounted for 28% of the variability in adiponectin (F=22.2; \( P \leq 0.0001 \)); androidal (waist + hip) fat mass (66.0%), (androidal fat mass)^2 (6.2%), whole body lean mass (2.2%), and age (0.8%) accounted for 69% of the variability in leptin (F=102.5; \( P \leq 0.0001 \)). Regression analyses revealed that sagittal abdominal diameter (8.4%), glucose (5.4%), white blood cell count (2.6%), and dietary omega-3 fatty acids (2.0%) accounted for 32% of the variability in insulin (F=20.8; \( P \leq 0.0001 \)); waist circumference (12.7%), hip lean mass (2.0%), and white blood cell count (1.9%) accounted for 26% of the variability in ghrelin (F=20.7; \( P \leq 0.0001 \)). Our results indicated that centralized fat mass was the primary contributor to these appetitive hormones in healthy postmenopausal women.

Conclusion: Since central adiposity in postmenopausal women was related to appetitive hormones, minimizing weight gain during the menopausal transition may optimize appetitive hormones, thereby facilitating appetite control and weight maintenance.

Keywords: Adiponectin, leptin, ghrelin, androidal fat mass, postmenopausal women
INTRODUCTION

Body composition changes become evident as women transition through menopause. These changes include an increase in overall (total body) and central adiposity (androidal region), especially visceral adipose tissue (1), and a decrease in total and central lean tissue mass (2). In particular, central adiposity in postmenopausal women is a major risk factor for developing insulin resistance (3), atherosclerotic cardiovascular disease (CVD; 4), dyslipidemia (5), hypertension (6), and breast cancer (7).

Adipose tissue is the largest endocrine organ, releasing more than 20 substances into circulation that are termed adipocytokines (also known as adipokines), such as adiponectin and leptin. Adiponectin is produced exclusively by mature adipocytes in visceral, subcutaneous, and bone marrow fat depots (8), whereas leptin is produced mainly in subcutaneous adipose tissue (9). Typically, obese or insulin-resistant individuals (8), as well as those with coronary artery disease (10), have low adiponectin but elevated leptin concentrations (11). Adiponectin increases insulin sensitivity (12), exerts anti-inflammatory properties (13), and may improve the lipoprotein profile (14). Leptin signals the brain to suppress appetite, increase energy expenditure, and decrease metabolic efficiency (15). Further, ghrelin and insulin, produced by the stomach and pancreas, respectively, play a significant role in regulating food intake and energy homeostasis. Obese individuals who are fasting typically have low ghrelin (16), whereas insulin concentration is elevated (17) in obesity. Ghrelin, an orexigenic hormone, rises sharply before meals while insulin is low; conversely, ghrelin declines after meals while insulin rises (18). Collectively, these adipocytokines, ghrelin, and insulin are termed appetitive hormones because they affect food intake, energy homeostasis, and body composition.

Centrally-located fat can be assessed using waist circumference, waist-to-hip ratio, and sagittal abdominal diameter measurements, although these methods do not distinguish between subcutaneous and visceral fat, the metabolically active fat that is related to disease risk. Computed tomography is considered the gold standard in assessing centrally-located fat, whereas dual-energy X-ray absorptiometry (DXA) provides an accurate and reliable estimate of overall and regional body composition (19) with minimal radiation exposure. However, unlike computed tomography, DXA cannot differentiate between subcutaneous and visceral adipose tissue because measurements
are only two-dimensional. Nevertheless, soft tissue analyses of DXA scans may be used to estimate visceral adipose tissue (20), as well as regional lean mass (21), by dividing the whole body scan into subregions of interest (19).

Appetitive hormones have been studied independently for potential mechanisms, but it is important to examine these hormones collectively because they interact to regulate energy homeostasis. The hypothesis of this study was that abdominal fat would be directly related to serum leptin and serum insulin, but indirectly related to plasma adiponectin and plasma ghrelin in healthy postmenopausal women. The specific aim of this study was to determine abdominal fat (waist + hip) tissue mass using DXA and relate abdominal fat to plasma adiponectin, serum leptin, serum insulin, and plasma ghrelin concentrations in 242 healthy postmenopausal women.

SUBJECTS AND METHODS

Study Design

Healthy postmenopausal women (45.8-65.0 years of age) were enrolled as part of a randomized, double-blind, placebo-controlled multi-center (Iowa State University [ISU], Ames, IA and University of California at Davis [UC-Davis], Davis, CA) NIH-funded clinical trial. This ongoing parent study (Soy Isoflavones for Reducing Bone Loss; SIRBL) was designed to examine the effect of two doses of isoflavones extracted from soybeans on bone loss during three years in at-risk postmenopausal women. Eligible participants (non-osteoporotic, without diseases or conditions, not taking hormones or medications) were enrolled in the ongoing parent trial starting in 2003. This ancillary project is focused on overall and regional body composition using DXA in which we report only baseline data for 242 women. We excluded 13 women at UC-Davis from this analysis because they did not meet the entry criteria (11 due to a thickened endometrium, 1 with breast cancer, 1 without a blood sample at baseline).

Subject Screening, Selection, and Characteristics

For the parent SIRBL project, we recruited subjects throughout the state of Iowa and the Sacramento region in California primarily through direct mailing lists, stories in local newspapers, local/regional radio advertisements, as well as other recruiting avenues. We screened women who responded (N=5,255) to outreach materials initially via a telephone questionnaire to identify healthy women who went through a natural menopause (cessation of menses nine months to 10 years), were not experiencing
excessive vasomotor symptoms, were ≤ 65 years of age, nonsmokers, and had a body mass index (BMI, kg/m²) ranging from 18.5 through 29.9 (inclusive) to exclude women at the extremes of adiposity. We excluded vegans and high alcohol consumers (>7 servings/week) because alcohol interferes with isoflavone metabolism. The parent SIRBL project established the inclusion/exclusion criteria; thus, we also excluded women diagnosed with chronic disease and those who had a first-degree relative with breast cancer. We also excluded women who chronically used medications, such as cholesterol-lowering or anti-hypertensive medications. Use of oral hormone or estrogen therapy, selective estrogen receptor modulators, or other hormones within the last 12 months, use of estrogen or progestogen creams or calcitonin within the last six months, use of antibiotics within the last three months, and/or any previous use of bisphosphonates were grounds for exclusion.

Women who met the initial criteria via telephone (N=677) attended a pre-baseline appointment to determine eligibility for additional entry criteria. We measured height and weight to confirm BMI status and used DXA to assess bone mineral density (BMD) to establish eligibility. The SIRBL project is focused on prevention rather than treatment of disease; thus, we excluded women with osteopenia or osteoporosis based on lumbar spine and/or proximal femur BMD (using >1.5 SD below the young adult mean as cut-off), with evidence of previous or existing spinal fractures, or with a high BMD (>1.0 SD above the mean). Once a woman qualified based on her BMD, our phlebotomist drew blood for a chemistry profile. We excluded women if their fasted blood values indicated diabetes mellitus (fasting blood glucose ≥126 mg/dl), abnormal renal (elevated creatinine), liver (elevated enzymes), and/or thyroid function, or elevated lipid profile (low density lipoprotein-cholesterol >160 mg/dl; triacylglycerol >200 mg/dl). For this ancillary project, we included 242 women who met our entry criteria (Figure 1).

The respective Institutional Review Boards (IRB) at ISU (ID# 02-199) and at UC-Davis (ID# 200210884-2) approved our study protocol, consent form, and subject-related materials. Approvals for the DXA procedures were obtained from each institution’s IRB and State Department of Public Health in Iowa and California. We obtained informed consent from all women at the start of pre-baseline screening.
Data Collection

Questionnaires

At the pre-baseline visit, trained interviewers administered three questionnaires to ensure the health status of participants: health and medical history (modified from citation 22), reproductive history (modified from citation 23), and nutrition history (22). Subjects were asked to cease taking herbal therapies and/or dietary supplements prior to baseline testing. At baseline, we assessed dietary intake using a semi-quantitative food frequency questionnaire from Block Dietary Data Systems (Berkeley, CA).

Body Size and Composition Measurements

A trained anthropometrist measured standing and sitting heights (Model S100; Ayrton Corp., Prior Lake, MN), weight (abco Health-o-meter; Bridgeview, IL), waist circumference, and sagittal abdominal diameter (Holtain-Kahn abdominal caliper; Crosswell, Crymych Dyfed, U.K.). Sagittal abdominal diameter was measured at the narrowest section between the small of the back and navel, with subjects relaxed in the supine position with knees bent. Body composition measurements were obtained using (Delphi W Hologic Inc; Waltham, MA) matching DXA instruments at each site and daily calibration to ensure that the instruments provided comparable results. One certified DXA operator at ISU and one at UC-Davis performed all DXA scans, with cross-training between sites to ensure comparable quality control. We standardized subject placement for the scans and adhered to the manufacturer recommendations. One operator assessed overall adiposity from the whole body DXA scans. To assess central adiposity, one evaluator sectioned each whole body DXA scan into waist, hip, and thigh regions based on bone landmarks (Figure 2) (19, 24) and these regions were analyzed using special software (Discovery Version 12.3:7). The waist region included the first lumbar through the fourth lumbar vertebrae. The hip region began below the fourth lumbar vertebrae and extended to the tip of the greater trochanter of the femur. Lastly, the thigh region extended superiorly from the greater trochanter to the approximate midpoint between the edge of the thigh region and lateral condyle of the femur. The lateral edge of each region was extended distally to encompass all tissue. This analysis provided an estimate of the fat and lean mass within each of these three regions. The androidal-to-gynoidal fat mass ratio was calculated for each subject: waist + hip fat mass / thigh fat mass.
Laboratory Measurements

Phlebotomists collected fasted (9 hours) blood samples between 0700 h and 0800 h. We separated serum and plasma from whole blood by centrifuging for 15 minutes (4°C) at 1000 x g and stored aliquots at -80°C until analyses. Certified clinical laboratories (LabCorp; Kansas City, KS at the ISU site and the UC-Davis Medical Center Laboratory; Sacramento, CA at UC-Davis site) analyzed our blood samples, including a complete blood count (CBC) with differential, general chemistry panel, and thyroid screen. Adiponectin (heparnized plasma), leptin (serum), insulin (serum), and ghrelin (total) (EDTA-treated plasma) concentrations were determined in duplicate with radioimmunoassay (RIA) kits (Linco Research; St. Charles, MO) using a Cobra II series auto-gamma counting system (Packard Instrument Company; Meriden, CT). We used manufacturer-provided quality controls and in-house quality control sera/plasma for calculating intra- and inter-assay coefficient of variation (CV). The intra-assay and inter-assay CVs (%) for adiponectin, leptin, insulin, and ghrelin, respectively, were 1.6 and 1.5, 3.0 and 2.7, 2.3 and 4.0, and 3.4 and 3.1.

Statistical Analyses

Statistical analyses were performed using SAS (version 9.1; Cary, NC) with results considered statistically significant at $P \leq 0.05$. The exception was that to account for numerous tests of normality, we considered data to be normally distributed if $P > 0.0001$. Descriptive statistics included means ± standard deviation (SD) for normally distributed data and medians for data that were not normally distributed, with range provided for each variable. Ghrelin and leptin were log-transformed prior to the regression analyses because the residual analysis indicated nonconstant error variance, thus violating model assumptions. We did not log-transform adiponectin since its residual plot did not indicate any violations of the linear regression model assumptions. Based on residual analysis, adding a quadratic term (androidal fat mass$^2$) to the leptin model improved the residual plot, but adding a higher-order term to the other models was not beneficial. Classes of variables in modeling the outcomes of interest included independent variables that were biologically plausible and/or significantly related using Pearson correlation analysis. We used stepwise regression analyses to assess the combined contribution of independent variables to adiponectin, leptin, insulin, and ghrelin. Classes of variables in modeling each of these four outcomes included: age or years since menopause, overall adiposity (whole body fat mass or weight), indices of
centralized fat mass (waist circumference, sagittal abdominal diameter, waist fat mass, hip fat mass, androidal [waist + hip] fat mass, or androidal/gynoidal fat mass ratio), whole body lean mass, indices of centralized lean mass (waist lean mass, hip lean mass, thigh lean mass, hip circumference), likelihood of concomitant infection (reflected in white blood cell count), physical activity, energy intake-related factors (total saturated fatty acids, total oleic fatty acids, total omega-3 fatty acids), and total dietary protein. Each model included site as an obligatory variable to account for potential study site differences. In modeling each outcome, we removed variables that exhibited multicollinearity as indicated by the variance inflation factor. The variance inflation factor measures the impact of collinearity among the independent variables in a regression model and the degree to which multicollinearity degrades the precision estimate. A value exceeding 10 is of concern, but in weaker regression models, a value exceeding 2.5 may be cause for concern (25).

RESULTS

Subject Characteristics

Two hundred and forty-two healthy, postmenopausal women were included in this analysis. The baseline characteristics of women are presented in Table 1. Women ranged from 45.9 to 65.5 years of age and from 0.8 to 10.0 years since menopause. We enrolled three African American (1%), one Native Hawaiian (<1%), one Native American (<1%), three Asians (1%), seven women of more than one race (3%), two of unknown race (<1%), and two who chose not to report race (<1%); the remaining women were Caucasian (92%). Women had a wide range (17.8-32.7) of BMI values because the UC-Davis site enrolled nine women beyond our BMI inclusion criteria. Approximately half of the women had a BMI <25.0 kg/m². Overall and regional body composition as assessed by DXA (Table 1) indicated wide variability in both overall and regional body fat measures among these women. The median values for dietary intake are listed in Table 2, illustrating wide variability of these nutrients. Values for circulating analytes are presented in Table 3, demonstrating that mean or median values were within the range reported in the literature.

Correlation Analyses

Pearson correlation analysis indicated a negative association between adiponectin and leptin (r=-0.35, P≤0.0001) and insulin (r=-0.33, P≤0.0001), but a positive
association between adiponectin and ghrelin ($r=0.29, P\leq0.0001$). As expected, we confirmed a positive association between leptin and insulin ($r=0.44, P\leq0.0001$) and glucose ($r=0.23, P=0.0004$), but a negative association between leptin and ghrelin ($r=-0.34, P\leq0.0001$). There was a negative relationship between ghrelin and insulin ($r=-0.24, P=0.0002$) and glucose ($r=-0.15, P=0.02$), but a positive association between insulin and glucose ($r=0.38, P\leq0.0001$).

**Regression Analyses**

We performed regression analyses to examine the independent factors contributing to the variability in adiponectin, leptin, insulin, and ghrelin as our primary outcomes (Table 4). No notable multicollinearities emerged among the independent variables, as indicated by the low (<2) variance inflation factors in all regression models. Residual analyses indicated that the model assumptions of normality of error terms and homogeneity of residual variance were satisfied for the final regression models. Geographic site was significant in the adiponectin ($P=0.0024$), insulin ($P=0.017$), and ghrelin ($P=0.0003$) models, but not for leptin ($P=0.59$). After variable elimination was completed, multiple regression analyses revealed that the androidal-to-gynoidal fat mass ratio (18.0%), age (3.2%), and white blood cell count (1.8%) accounted for 28% of the variability in adiponectin ($F=22.2, P\leq0.0001$). Regression analyses revealed that androidal (waist+hip) fat mass (66.0%), (androidal fat mass)$^2$ (6.2%), whole body lean mass (2.2%), and age (0.8%) accounted for 69% of the variability in leptin ($F=102.5, P\leq0.0001$). Regression analyses revealed that sagittal abdominal diameter (8.4%), glucose (5.4%), white blood cell count (2.6%), and omega-3 fatty acids (2.0%) accounted for 32% of the variability in insulin ($F=20.8, P\leq0.0001$). Regression analyses indicated that waist circumference (12.7%), hip lean mass (2.0%), and white blood cell count (1.9%) accounted for 26% of the variability in ghrelin ($F=20.7, P\leq0.0001$).

**DISCUSSION**

This study is unique in that we used a regional analysis of whole body DXA scans to examine central fat and lean mass, rather than the standard DXA analysis to estimate overall fat and lean mass. Our results indicated that centralized body fat (androidal-to-gynoidal fat mass ratio, androidal fat mass, sagittal abdominal diameter, or waist circumference, respectively) was the largest contributor to each circulating appetitive hormone: adiponectin, leptin, insulin, or ghrelin in healthy postmenopausal
women. As expected, the relationship of centralized body fat to leptin and insulin was positive, but negative to adiponectin and ghrelin. We confirmed previous findings (21, 26-28) of a relationship between measures of central adiposity and adiponectin, leptin, and insulin in healthy postmenopausal women. However, unlike previous studies, we found a strong negative relationship between central adiposity (reflected by waist circumference) and ghrelin in these women. Recent research (29) has reported a strong negative relationship between waist circumference and ghrelin in younger (<30 years), but not in mid-life (aged 30-56 years) women. Further, in a study of 79 adult opposite-sex twin pairs, Makovey et al. (30) found a weak relationship between abdominal fat mass assessed by DXA and ghrelin in women, whereas this relationship in men was strong. Further research is needed to understand the response of ghrelin to central adiposity, particularly in postmenopausal women. We also confirmed the findings of other studies that postmenopausal women with a higher level of adiposity have higher concentrations of both leptin (21, 31) and insulin (28), but a lower adiponectin concentration (8).

Although lean tissue is typically not considered in central body composition assessment, it is important to assess because it is metabolically active. Limited data comparing pre- versus postmenopausal women suggest a decline in both overall and centralized lean tissue with menopause (2). However, few studies have examined lean tissue in relation to appetitive hormones. Because a higher body weight requires greater muscle mass for movement, a higher fat mass has been associated with higher lean mass, mainly localized in the legs, but with a decrease in overall lean/fat ratio (32). We found that leptin and ghrelin were the two appetitive hormones related to lean mass in the regression models, with a significant negative relationship between leptin and whole body lean mass and positive relationship between ghrelin and hip lean mass. However, we hypothesized that because of the direct relationship between overall fat and lean mass, leptin, ghrelin, and insulin would be directly related to lean mass in the thigh region, whereas adiponectin would be indirectly related. Although the Pearson correlation analysis indicated that leptin was positively related to whole body lean mass, the direction of the relationship was altered once we took other factors into account. In probing a possible explanation, we found that in the presence of androidal fat mass, the direction of the relationship between whole body lean mass and leptin may have changed because the simple correlation (positive) between lean mass and leptin was
fairly weak \((r=0.14, P=0.028)\), whereas the correlation (positive) between androidal fat mass and leptin was strong \((r=0.77, P\leq 0.0001)\). It seemed that androidal fat mass thereby exerted a dominant effect in the regression model. Similar to our findings using correlation analysis, Mahabir et al. (21) recently found that a higher lean mass was associated with a higher leptin concentration, but they did not take other factors into account. Their participants were older (age range 49.2-78.8 years), tended toward the higher end of adiposity (ranged from underweight to morbidly obese status with BMI range 17.7-42.5 kg/m²), and were further from menopause (up to 38 years postmenopausal) than the women in our study. Research is needed to examine the relationship between lean mass and appetitive hormones.

Interestingly, our results suggest that white blood cell count was related to adiponectin, ghrelin, and insulin. White blood cell count is typically used as an indicator of infection or inflammation. To our knowledge, none of our women had an infection during baseline testing, and no women exhibited an elevated white blood cell count \((>11 \times 10^9/L)\). Research has indicated that white blood cell count is related to body fat in humans (33), suggesting that adipocytokines may be involved in the adipocyte-induced inflammatory response. Further, Vozarova et al. (34) found that with a high white blood cell count, insulin sensitivity declined in non-diabetic Pima Indians. Although we did not assess insulin sensitivity, our study found a positive relationship between white blood cell count and circulating insulin in the regression model. Collectively, these studies suggest that white blood cells may reflect an obesity-induced inflammatory state that is also mirrored by the appetitive hormones. The context of our study is important because the majority of these women, except for 8 women at UC-Davis whose BMI was \(\geq 30.0\), were not considered obese based upon BMI (ranged from 17.8 to 32.7). This suggested that modestly elevated (but still within normal range) white blood cell count, elevated insulin, and low adiponectin is an unfavorable metabolic profile in overweight postmenopausal women. In our study, we had two women who had adiponectin concentrations below the reference range \((5.0-30.5 \mu g/mL)\) for healthy postmenopausal women (35) and six women who had a higher insulin concentration than the upper limit of the reference range \((5.0-24.0 \mu U/mL; 3)\). However, none of our women were beyond the reference range for white blood cell count.

Our study suggests that age influenced adiponectin and leptin, but not ghrelin or insulin, concentrations in healthy postmenopausal women. Based on the regression
models, we noted a positive relationship between age and adiponectin, confirming recently reported results (36) in similarly aged (45-62 years) subjects. In contrast, but similar to our findings, Ostlund et al. (31) noted an inverse relationship between age and leptin, attributing this finding to decreased leptin production from adipose tissue and/or increased leptin clearance with increasing age. As expected, fasting glucose was an important (positive) contributor to insulin, second to sagittal abdominal diameter. Interestingly, omega-3 fatty acid concentration was the only dietary factor that emerged as significant in any of the regression models. Lombardo et al. (37) have suggested that in a rat model, dietary polyunsaturated fatty acids may enhance insulin sensitivity, thereby improving the lipoprotein profile and decreasing CVD risk. Increased CVD risk is related to insulin resistance because it contributes to dyslipidemia (38). We also noted relationships among the appetitive hormones. Adiponectin was inversely related to leptin, which is consistent with some (11, 36) but not all studies (26). A low concentration of adiponectin but a high concentration of leptin has been related to an increased risk of insulin resistance (11). Likewise, we found that leptin was significantly and positively related to insulin in these non-diabetic healthy postmenopausal women, prior to emergence of disease. Further, similar to Purnell et al. (27), we confirmed that ghrelin was negatively associated with insulin. The associations among these appetitive hormones are not fully understood, but adiponectin, leptin, and ghrelin may be early indicators of insulin resistance in overweight but healthy postmenopausal women.

Study site was a significant factor in the adiponectin, insulin, and ghrelin models, also evidenced by statistical differences ($P \leq 0.0001$) in mean values between study sites, possibly related to the somewhat greater ($P = 0.077$) variability in whole body fat mass in the women at UC-Davis (8.05-47.77 kg) compared with those at ISU (8.43-37.01 kg). Our entry criteria was designed to exclude women with a BMI $\geq 30.0$, although eight women at UC-Davis did not meet this criterion but had a BMI $> 29.9$. Despite no difference ($P = 0.97$) in the mean value for BMI between sites, we noted a lack of homogeneity of variance ($P = 0.0030$) in BMI with respect to site, likely due to the women at UC-Davis whose BMI ranged from 30 to 32.7. We suspect that this wider range in body size and adiposity at UC-Davis likely contributed to the significant site difference in adiponectin, insulin, and ghrelin in these regression models.

This study was hypothesis-generating and could not determine cause and effect because it was cross-sectional. In addition, the participants in this study were healthy
postmenopausal women, primarily of Caucasian descent. Thus, our results cannot be
generalized to all women across ethnic groups. Since central adiposity in
postmenopausal women was related to appetitive hormones, despite the apparent
health of these women, minimizing weight gain during the menopausal transition may
optimize appetitive hormones, thereby facilitating appetite control and weight
maintenance. Additional studies are needed to determine at what level central adiposity
should be maintained to optimally affect these appetitive hormones, thus potentially
preventing further gain in centralized fat with menopause.

ACKNOWLEDGMENTS
We would like to thank each participant who volunteered for this study. We would
also like to thank our phlebotomists, Cindy Kruckenberg, Marilyn Chrusciel, and Shirley
Nelson, as well as our undergraduate research assistants who assisted with many
aspects of this project.

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Table 1
Characteristics of Subjects<sup>a</sup> at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>54.6 ± 3.4</td>
<td>45.9 – 65.5</td>
</tr>
<tr>
<td><strong>Years Since Menopause</strong></td>
<td>3.5 ± 2.0</td>
<td>0.8 – 10.0</td>
</tr>
<tr>
<td><strong>Body Size</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.7 ± 9.3</td>
<td>43.7 – 94.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.7 ± 6.3</td>
<td>146.3 – 182.2</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>24.9 ± 3.1</td>
<td>17.8 – 32.7</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77.9 ± 8.0</td>
<td>59.1 – 100.6</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>100.3 ± 6.8</td>
<td>80.9 – 118.2</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.8 ± 0.1</td>
<td>0.6 – 0.9</td>
</tr>
<tr>
<td>Sagittal abdominal diameter (cm)</td>
<td>18.6 ± 2.3</td>
<td>11.0 – 25.2</td>
</tr>
<tr>
<td><strong>Overall Body Composition&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>23.26 ± 6.35</td>
<td>8.05 – 47.78</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>34.5 ± 5.7</td>
<td>18.1 – 55.9</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>43.18 ± 4.60</td>
<td>29.49 – 55.59</td>
</tr>
<tr>
<td><strong>Regional Body Composition&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist fat (kg)</td>
<td>2.37 ± 1.10</td>
<td>0.39 – 5.59</td>
</tr>
<tr>
<td>Waist fat (%)</td>
<td>28.6 ± 8.7</td>
<td>7.1 – 52.5</td>
</tr>
<tr>
<td>Hip fat (kg)</td>
<td>3.38 ± 1.05</td>
<td>0.72 – 6.41</td>
</tr>
<tr>
<td>Hip fat (%)</td>
<td>32.9 ± 5.9</td>
<td>12.1 – 51.6</td>
</tr>
<tr>
<td>Thigh fat (kg)</td>
<td>5.28 ± 1.34</td>
<td>2.12 – 10.85</td>
</tr>
<tr>
<td>Thigh fat (%)</td>
<td>38.7 ± 5.0</td>
<td>24.2 – 59.7</td>
</tr>
<tr>
<td>Androidal (waist + hip) fat (kg)</td>
<td>5.75 ± 2.06</td>
<td>1.12 – 11.78</td>
</tr>
<tr>
<td>Androidal-to-gynoidal fat mass ratio</td>
<td>1.09 ± 0.30</td>
<td>0.46 – 2.21</td>
</tr>
<tr>
<td>Waist lean (kg)</td>
<td>5.53 ± 0.72</td>
<td>3.50 – 7.52</td>
</tr>
<tr>
<td>Hip lean (kg)</td>
<td>6.71 ± 0.93</td>
<td>4.13 – 9.23</td>
</tr>
<tr>
<td>Thigh lean (kg)</td>
<td>8.20 ± 1.04</td>
<td>4.85 – 11.42</td>
</tr>
</tbody>
</table>
Number of subjects = 242 for all variables, except sagittal abdominal diameter (N=237) because 5 of these values were missing due to instrument malfunction.

Assessed by DXA (dual-energy x-ray absorptiometry).
### Table 2
Dietary Intake of Subjects\textsuperscript{a} at Baseline

<table>
<thead>
<tr>
<th>Nutrient Intake from Food\textsuperscript{b,c}</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Energy (kJ)</td>
<td>6455</td>
<td>1772 – 19,096</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>175</td>
<td>27 – 476</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>61</td>
<td>15 – 168</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>65</td>
<td>17 – 247</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>19</td>
<td>5 – 65</td>
</tr>
<tr>
<td>Trans Saturated Fat (g)</td>
<td>5</td>
<td>0.8 – 25</td>
</tr>
<tr>
<td>Oleic Fatty Acid\textsuperscript{d} (g)</td>
<td>25</td>
<td>6 – 97</td>
</tr>
<tr>
<td>Linoleic Fatty Acid\textsuperscript{d} (g)</td>
<td>16</td>
<td>3 – 70</td>
</tr>
<tr>
<td>Total Omega-3 Fatty Acid (g)</td>
<td>1.4</td>
<td>0.3 – 5</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>16</td>
<td>4 – 49</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The number of subjects was 242 for all variables.

\textsuperscript{b} Reported from Semi-Quantitative Food Frequency Questionnaire.

\textsuperscript{c} Distributions for these variables were not normal and thus median values are reported.

\textsuperscript{d} Represents monounsaturated and polyunsaturated fatty acids, respectively.
### Table 3
Circulating Analytes of Subjects\(^a\) at Baseline

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Adiponectin (μg/mL)(^b)</td>
<td>17.3</td>
<td>3.2 – 37.1</td>
<td>5.0 – 30.5(^d)</td>
<td></td>
</tr>
<tr>
<td>Serum Leptin (ng/mL)(^b)</td>
<td>12.8</td>
<td>2.6 – 34.9</td>
<td>2.0 – 54.0(^d)</td>
<td></td>
</tr>
<tr>
<td>Plasma Ghrelin (pg/mL)(^b)</td>
<td>938</td>
<td>345 – 2383</td>
<td>[713 – 1198](^e)</td>
<td>995 – 1794(^e)</td>
</tr>
<tr>
<td>Serum Insulin (μU/mL)(^c)</td>
<td>11.6 ± 5.4</td>
<td>0.1 – 36.1</td>
<td>5.0 – 24.0(^f)</td>
<td></td>
</tr>
<tr>
<td>Serum Glucose (mg/dL)(^c)</td>
<td>85.5 ± 8.8</td>
<td>57.0 – 117.0</td>
<td>82.0 – 116.0(^f)</td>
<td></td>
</tr>
<tr>
<td>White Blood Cell Count (x10(^9)/L)(^a,c)</td>
<td>5.07 ± 1.03</td>
<td>2.3 – 8.4</td>
<td>4.5 – 11.0(^g)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Number of subjects = 242 for the adipocytokines, ghrelin, insulin, and glucose; number of subjects = 237 for white blood cell count.

\(^b\) Distributions were not normal; thus, median values are reported.

\(^c\) Distributions were normal; thus, mean ± SD are reported.

\(^d\) Reference range reported for healthy postmenopausal women (35).

\(^e\) Interquartile range for study participants in comparison with interquartile range reported for postmenopausal women (39).

\(^f\) Reference range reported for healthy postmenopausal women (3).

\(^g\) Reference range reported for adults (40).
Table 4
Regression Analyses: Contributors to Appetitive Hormones

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>Percentage Variance</th>
<th>$P$ value</th>
<th>Variance Inflation Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adiponectin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Model $R^2=27.7%$ (Adj $R^2=26.4%$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[F=22.19; df=(4, 232)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P≤0.0001$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>16.93727</td>
<td>0.0026</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study site</td>
<td>-2.10013</td>
<td>2.9</td>
<td>0.0024</td>
<td>1.07</td>
</tr>
<tr>
<td>And/Gyn Fat Mass</td>
<td>-8.80741</td>
<td>18.0</td>
<td>$≤0.0001$</td>
<td>1.10</td>
</tr>
<tr>
<td>Age</td>
<td>0.31884</td>
<td>3.2</td>
<td>0.0017</td>
<td>1.04</td>
</tr>
<tr>
<td>White Blood Cell Count</td>
<td>-0.81601</td>
<td>1.8</td>
<td>0.018</td>
<td>1.13</td>
</tr>
</tbody>
</table>

| **Leptin**                 |                    |                     |           |                          |
| Overall Model $R^2=68.5\%$ (Adj $R^2=67.8\%$)       |  |  |  |  |
| [F=102.51; df=(5, 236)]     |  |  |  |  |
| $P≤0.0001$                  |  |  |  |  |
| Intercept                  | 2.84103            | ≤ 0.0001            |           |                          |
| Study site                 | -0.02111           | 0.04                | 0.5929    | 1.01                     |
| Androidal Fat Mass         | 0.00023957         | 66.0                | $≤0.0001$ | 1.28                     |
| (Androidal Fat Mass)$^2$   | -2.47059E-8        | 6.2                 | $≤0.0001$ | 1.13                     |
| Whole Body Lean Mass       | -0.00001834        | 2.2                 | $≤0.0001$ | 1.13                     |
| Age                        | -0.01434           | 0.8                 | 0.0147    | 1.03                     |
### Insulin\(^c\)

Overall Model $R^2=31.5\%$ (Adj $R^2=30.0\%$)  
$$[F=20.82; \text{ df}=(5, 226)]$$

$P \leq 0.0001$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>Percentage Variance(^d)</th>
<th>$P$ value(^e)</th>
<th>Variance Inflation Factor(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-15.70447</td>
<td></td>
<td>$\leq 0.0001$</td>
<td></td>
</tr>
<tr>
<td>Study site</td>
<td>-1.50451</td>
<td>1.7</td>
<td>0.017</td>
<td>1.09</td>
</tr>
<tr>
<td>Sagittal Abdominal Diameter</td>
<td>0.71995</td>
<td>8.4</td>
<td>$\leq 0.0001$</td>
<td>1.14</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.15127</td>
<td>5.4</td>
<td>$\leq 0.0001$</td>
<td>1.14</td>
</tr>
<tr>
<td>White Blood Cell Count</td>
<td>0.93610</td>
<td>2.6</td>
<td>0.0038</td>
<td>1.22</td>
</tr>
<tr>
<td>Omega-3 Fatty acids</td>
<td>-0.94795</td>
<td>2.0</td>
<td>0.011</td>
<td>1.05</td>
</tr>
</tbody>
</table>

### Ghrelin\(^a\)^\(^g\)

Overall Model $R^2=26.3\%$ (Adj $R^2=25.0\%$)  
$$[F=20.67; \text{ df}=(4, 232)]$$

$P \leq 0.0001$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>Percentage Variance(^d)</th>
<th>$P$ value(^e)</th>
<th>Variance Inflation Factor(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>8.64936</td>
<td></td>
<td>$\leq 0.0001$</td>
<td></td>
</tr>
<tr>
<td>Study site</td>
<td>-0.17405</td>
<td>4.3</td>
<td>0.0003</td>
<td>1.13</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>-0.02269</td>
<td>12.7</td>
<td>$\leq 0.0001$</td>
<td>1.61</td>
</tr>
<tr>
<td>Hip Lean Mass</td>
<td>0.00007417</td>
<td>2.0</td>
<td>0.013</td>
<td>1.50</td>
</tr>
<tr>
<td>White Blood Cell Count</td>
<td>-0.05657</td>
<td>1.9</td>
<td>0.015</td>
<td>1.13</td>
</tr>
</tbody>
</table>

\(^a\) N = 237 for the adiponectin and ghrelin models because 5 women were missing CBC values.

\(^b\) N = 242 for the leptin model.
N = 232 for the insulin model because 5 women were missing CBC values and 5 women were missing sagittal abdominal diameter measurements.

Squared semi-partial Type II correlation coefficient; accounts for shared variance among variables.

Variables (except site) left in the model were significant at $P \leq 0.10$ level.

Measures the impact of collinearity among the independent variables in a regression equation and the degree to which multicollinearity degrades the precision estimate.

Leptin and ghrelin were log transformed because they were not normally distributed.
Figure 1: Subject screening and enrollment flow chart

- Completed Telephone screening = 5,225
- Completed pre-baseline screening = 677
- Excluded = 422
  - Did not meet inclusion criteria = 335
  - Did not want to participate = 87
- Randomized to treatment = 255
- Included in this analysis = 242

*a We excluded 13 women at UC-Davis from this analysis because they did not meet entry criteria (11 due to a thickened endometrium, 1 with breast cancer, 1 without a baseline blood sample)
Figure 2: Whole body DXA scan with regional soft tissue analysis
GENERAL CONCLUSIONS

In summary, the results from our primary objective of this ancillary study suggest that centralized body fat (androidal-to-gynoidal fat mass ratio, androidal fat mass, sagittal abdominal diameter, or waist circumference) was the largest contributor to each circulating appetitive hormone: adiponectin, leptin, insulin, or ghrelin, respectively, in healthy postmenopausal women. As expected, the relationship of centralized body fat to leptin and insulin was positive, but negative to adiponectin and ghrelin. This study is unique in that we used a regional analysis of the whole body DXA scan to examine central fat and lean mass, rather than the standard DXA analysis to estimate overall fat and lean mass. Few studies have examined lean tissue in relation to appetitive hormones; however, we found that lean mass in the regression models was related to leptin and ghrelin, with a significant negative relationship between leptin and whole body lean mass and a positive relationship between ghrelin and hip lean mass. Future studies should further investigate how these appetitive hormones affect lean mass and what factors might influence these appetitive hormones to optimally affect lean mass.

The results from our first objective revealed that the androidal-to-gynoidal fat mass ratio, age, and white blood cell count accounted for 28% of the variability in adiponectin \( (P<0.0001) \); androidal (waist + hip) fat mass, androidal fat mass\(^2\), whole body lean mass, and age accounted for 69% of the variability in leptin \( (P<0.0001) \). Regression analyses revealed that sagittal abdominal diameter, glucose, white blood cell count, and dietary omega-3 fatty acids accounted for 32% of the variability in insulin \( (P<0.0001) \); waist circumference, hip lean mass, and white blood cell count accounted for 26% of the variability in ghrelin \( (P<0.0001) \). Additional studies are needed to determine at what level central adiposity should be maintained to optimally affect these appetitive hormones, thus potentially preventing further gain in centralized fat with menopause.

Data analyses will be conducted in the near future to fulfill the second and third objectives. We will determine the effect of two doses of soy isoflavones (80 and 120 mg/day) on change in central adiposity and thigh lean mass, as mediated by circulating adiponectin, leptin, insulin, and ghrelin in healthy postmenopausal women.